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GENERAL EDITOR

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VOLUME VIII
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WITH 48 PLATES, 1 TEXT FIGURE, 2 MAPS,
5 SKIAGRAMS, AND 7 CHARTS

MANILA
BUREAU OF PRINTING
1913
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QUANTITATIVE DETERMINATION OF THE BALANTIDICIDAL ACTIVITY OF CERTAIN DRUGS AND CHEMICALS AS A BASIS FOR TREATMENT OF INFECTIONS WITH BALANTIDIUM COLI

By Ernest Linwood Walker

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Infections with Balantidium coli are much more prevalent in the Philippine Islands than has been generally recognized. The first case reported was that by Strong (1901). In Bilibid Prison 10 cases were found in the routine examination of stools during 1911, and 11 cases in 1912. At the Philippine General Hospital 8 cases have been observed during 1911 and 1912.

Balantidiasis is characterized by the frequency of latent infections, infections in which the patient may show no clinical symptoms, or only occasional attacks of diarrhoea, over long periods of time. Of the 29 cases observed in Bilibid Prison and at the Philippine General Hospital during 1911 and 1912 only 5 have exhibited diarrhoeal or dysenteric symptoms. Bowman (1909 and 1911) published a description of 3 fatal cases in Manila which came to necropsy. A further characteristic of these infections is that the parasites only appear in the stools of the patient at irregular intervals. On account of these peculiarities of this disease and the parasite, infections are probably frequently overlooked in the routine examination of stools.

The medical importance of Balantidium coli, notwithstanding these conditions, consists in the facts that persons parasitized...
with this protozoön are likely sooner or later to develop balan-
tidial dysentery; that, once the dysenteric condition is estab-
lished, it is apt to run a fatal course; and in that no efficient
treatment is known for this disease.

Various drugs and chemicals given by mouth or used as
enemata have been employed in the treatment of balantidial
infections. Authors have reported the disappearance of the
balantidia from the stools and the improvement of their patient
following treatment with certain of these substances; but the
specific therapeutic action of them has in each case been ren-
dered doubtful by the fact that other authors have found the
same treatments worthless. The explanation of these inco-
sistent results is to be found in the latency characteristic of
the disease and in the tendency of the parasites to disappear
spontaneously from the stools of infected persons for variable
lengths of time. As a result of these peculiarities of the in-
fection, the determination of the efficiency of any treatment of
balantidiasis by clinical observation is extremely fallacious
unless the patient be kept under observation for a long time.

It has, therefore, seemed possible that the probable value of
different drugs and chemicals in the treatment of this infection
could be more quickly and accurately determined by laboratory
tests of the balantidicidal action of them in vitro.

The practical value of tests in vitro of the action of drugs
and chemicals on parasitic protozoa has been demonstrated by
the investigations of Vedder (1911 and 1912) and Rogers (1912a
and 1912b) in the application of ipecac and its alkaloid, emetine,
to the treatment of entamoebic dysentery. Vedder found that
cultures of amœbæ were killed by solutions of different fluid
extracts of ipecac in dilutions of 1 in 10,000 to 1 in 50,000 and
that the active amœbicidal principle of ipecac was probably
emetine, since a solution of this latter substance killed the
amœbe in dilutions of 1 in 100,000. These tests established
a scientific basis for the empiric treatment of amœbic dysentery
with ipecac. Very recently Rogers, undoubtedly influenced by
Vedder’s investigation, has tested in vitro the entamœbicidial
properties of the soluble salts of emetine. He found that Enta-
mœba histolytica was killed by dilutions of 1 in 100,000 of this
alkaloid. Applying these laboratory results to clinical medicine,
Rogers has reported results which are so striking in the treat-
ment of entamœbic dysentery and liver abscesses that, if sub-
stantiated, they will prove that we have a specific for this disease.
The conditions for testing the balantidicidal action of drugs and chemicals in vitro are extremely favorable. *Balantidium coli*, under favorable conditions, remains alive and active in the faeces for several days. Moreover, we have, in the active locomotion and the persistent activity of the oral cilia during life, and in the disintegration of the protozoön at death, the most delicate and at the same time the most striking indicators of injury to, and death of, the parasite. The active movements of *Balantidium coli* usually persist up to, and the movement of the oral cilia even after, the beginning of disintegration of the protozoön. Death of the parasite is usually accompanied or closely followed by disintegration. This disintegration takes place by the extrusion of the protoplasm through the oral, and sometimes also the anal, orifice of the protozoön. The process of protoplasmic extrusion is closely correlated with the balantidicidal activity of the substance employed. In slightly toxic solutions the balantidia exhibit only the protrusion of small buds of protoplasm, the organism still maintaining its active movements. In more toxic solutions the extrusion of the protoplasm becomes progressively greater until the protozoön collapses. And in eminently toxic solutions this disintegration becomes explosive in character, the ectosarc being ruptured and the whole contents liberated at once. An exception to this disintegration of balantidium occurs in the presence of certain chemicals, such as the salts of mercury, which act as a fixative. In this case the protozoön is killed and fixed with cilia extended.

There exist records of a few balantidicidal tests performed in vitro, but these tests have been limited to acids, alkalies, alcohol, and a few common salts, and definite quantitative determinations of the balantidicidal limits of dilution of the substances have not been made. Thus Glaessner (1908) made the following crude tests of the action of a few chemicals on *Balantidium coli*:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Result after 6 hours</th>
</tr>
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<tbody>
<tr>
<td>1. Ten grams of faeces were added to 40 cubic centimeters of water at 40° C.</td>
<td>Balantidia mostly dead, a few were living.</td>
</tr>
<tr>
<td>2. Ten grams of faeces were added to 40 cubic centimeters of 0.6 per cent NaCl.</td>
<td>Some of the balantidia dead.</td>
</tr>
<tr>
<td>3. Ten grams of faeces were added to 40 cubic centimeters of 1 per cent alcohol.</td>
<td>Balantidia dead.</td>
</tr>
<tr>
<td>4. Ten grams of faeces were added to 40 cubic centimeters of 5 per cent alcohol.</td>
<td>Balantidia dead.</td>
</tr>
<tr>
<td>5. Ten grams of faeces were added to 40 cubic centimeters of 0.3 per cent NaCl.</td>
<td>Balantidia dead.</td>
</tr>
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Ortman (1891) observed the action of certain chemicals on balantidia in hanging-drop preparations as follows:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Strength of solution</th>
<th>Time of exposure</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1/3 per cent</td>
<td>45 H. mi.</td>
<td>Balantidia dead.</td>
</tr>
<tr>
<td>Karlsbader water</td>
<td>1:3,000</td>
<td>30</td>
<td>Negative.</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>2:2,000</td>
<td>20</td>
<td>Balantidia dead.</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>1:3,000</td>
<td>13</td>
<td>Do.</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>1:1,000</td>
<td>13</td>
<td>Some of the balantidia living.</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>1:400</td>
<td>25</td>
<td>Do.</td>
</tr>
<tr>
<td>Quinine sulphate</td>
<td>1:2,000</td>
<td>11</td>
<td>Balantidia dead.</td>
</tr>
<tr>
<td>Do</td>
<td>1:1,000</td>
<td>3 Do.</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>1:100</td>
<td>5</td>
<td>Do.</td>
</tr>
</tbody>
</table>

In the experiments described in this paper, an attempt has been made to determine quantitatively the balantidicidal action; first, of those drugs and chemicals which have been more or less successfully employed in the treatment of other protozoan infections, and, if these failed to be active; secondly, to seek for some efficient balantidicidal drug or chemical that could be used in the treatment of balantidial dysentery. The first of these purposes has been limited to some extent by my inability to procure in Manila all of the drugs and chemicals that have been employed in the treatment of protozoan diseases. However, a certain number of them have been obtainable which represent the chief groups of these substances, as the aniline dyes, the arsenic and the antimony compounds, ipecac and its alkaloid, emetine, and the salts of quinine. The second purpose, to seek some new drugs or chemicals having a specific action on *Balantidium coli*, has also proceeded according to a definite plan which will be apparent later.
Balantidium coli has been found to be extremely sensitive to changes in the tonicity of the medium in which it is placed. It was, therefore, necessary to determine the most favorable tonicity of the fluid to be employed in making the dilutions of the drugs and chemicals in order not to introduce a source of error from this factor in the tests. It was found that 0.85 per cent normal physiological salt solution was slightly hypertonic, while distilled water was slightly hypotonic, for Balantidium coli. Therefore, comparative tests were made with different strengths of sodium chloride solutions, and it was decided that 0.5 per cent solution represented about the optimum tonicity for this organism. A departure from the use of 0.5 per cent sodium chloride for making the dilutions was found to be necessary in certain cases. Thus, in the case of mercuric iodide, which is not soluble in water or physiological salt solution, a 2 per cent solution of potassium iodide in water was employed for dissolving and making the first dilution of 1 in 50; the subsequent dilutions were then made with 0.5 per cent sodium chloride solution. In this case the first and consequently the subsequent dilutions contained the same percentage of potassium iodide as of mercuric iodide. A series was then run with potassium iodide as a control, which was found to be inert. Again, in testing some of the compounds of silver, which are precipitated by weak solutions of sodium chloride, the dilutions were made with distilled water and a control run with distilled water.

In the preliminary tests of each substance dilutions of 1 in 50, 1 in 500, 1 in 5,000, and 1 in 50,000 were made. These, when mixed with equal parts of the fluid faeces containing the balantidia, gave final dilutions of 1 in 100, 1 in 1,000, 1 in 10,000, and 1 in 100,000. The limits of the balantidical action of the substance between any two of these dilutions having been determined, the more precise limit of its action was then determined by making intermediate dilutions between the highest positive and the lowest negative dilutions.

Dilutions of the substances to be tested were made in the following manner. If, as in most cases, the substance was a solid, 0.2 gram was weighed out with an analytical balance in a 10 cubic centimeters volumetric flask. The substance was then dissolved with 0.5 per cent sodium chloride solution and the flask filled to the graduation mark. This gave a dilution of 1 in 50. If, as was rarely the case, the substance was a
liquid, the first dilution was made by measuring 0.2 cubic centimeter of it and making up to 10 cubic centimeters with the salt solution in the volumetric flask as before. This small quantity of the drug or chemical was used throughout in making the primary dilutions because some of the substances were obtainable only in small quantities. The succeeding dilutions were made in 50 cubic centimeters volumetric flasks by measuring appropriate amounts from each preceding dilution of the substance with a graduated pipette and filling the flask to the graduation mark with the diluting liquid.

The balantidia used in making these tests were in part derived from men, in part from domesticated pigs, and in part from a monkey infected from a pig. *Balantidium coli suis* is generally considered to be identical with *Balantidium coli hominis*; it has been found possible to infect monkeys with the balantidia from the pig; and comparative tests of the same drug or chemical made with the pig and the human balantidia have given uniform results. Balantidia from the pig and monkey have been employed in part in these tests, because human cases of balantidiasis which showed a sufficient number of the organisms in the stools could not at all times be obtained. The material used has consisted of fresh fæces containing living and actively motile balantidia. Unless the fæces was sufficiently fluid, a portion of it was rubbed up in 0.5 per cent sodium chloride solution to fluid consistency.

A large platinum loopful of the fluid fæces containing the balantidia was placed on a microscope slide. Beside it was then placed a drop of equal size of the dilution of the substance to be tested. The two drops were then thoroughly mixed and a cover-glass placed upon it. The edges of the cover-glass were then sealed with vaseline to prevent evaporation. Similar preparations were made of all of the dilutions of the drug or chemical being tested and of a control, consisting of a drop of the fæces and a drop of 0.5 per cent sodium chloride or, in case distilled water had been used as a diluting fluid, distilled water. These preparations were observed with low magnification under the microscope at frequent intervals, and the effect on the balantidia noted. The intervals of observation were usually one, two, five, ten, fifteen, thirty, and sixty minutes. In most cases the observations did not extend beyond one hour, since it was considered that a substance at a given dilution is of little value if it did not kill the organisms within that
period of time. Indeed, if the balantidicidal action was not instantaneous or did not take place within a few minutes, the substance at the given dilution has not been considered to be of practical value.

As an example of these tests the following one of silver nitrate is quoted from my notes:

**TESTS OF THE BALANTIDICIDAL ACTION OF SILVER NITRATE ON "BALANTIDIUM COLI SUIS"**

Control preparation made at 9.15 a.m. Balantidia active. 10.15, balantidia active.

Dilution of 1:100 made at 9.17 a.m. Balantidia killed and fixed immediately.

Dilution of 1:1000 made at 9.20 a.m. Locomotion and movement of cilia of the balantidia lost immediately. 9.21, extrusion of the contents of the balantidia.

Dilution of 1:10,000 made at 9.35 a.m. Balantidia more or less actively motile; 9.35, locomotion of all balantidia have ceased; 9.36, balantidia have collapsed and contents extruded.

Dilution of 1:20,000 made at 10.09 a.m. Balantidia sluggishly motile; 10.09, balantidia motionless, oral cilia motile; 10.10, the same; 10.11, some of the balantidia show extrusion of small drops of protoplasm from the cytostome; 10.12, more pronounced and widespread extrusion of the protoplasm from both the cytostome and cytopyge of the balantidia; 10.15, progressive extrusion of the protoplasm of the balantidia; 10.16, the same; 10.20, the same; 10.30, all balantidia have disintegrated.

Dilution of 1:25,000 made at 10.30 ½ a.m., balantidia actively motile; 10.42, the same; 10.44, some of the balantidia are nearly motionless with drops of protoplasm extruding from the cytostome and cytopyge; 10.48, balantidia sluggishly motile with large buds of protoplasm extruding from the oral and anal orifices; 10.53, the same; 11.06, some of the balantidia still motile; 11.33, the same.

Dilution of 1:100,000 made at 9.43 ½ a.m. 9.43, balantidia actively motile; 9.45, the same; 9.52, the same; 10.00, the same; 10.34 the same; 11.10, the same.

From these tests it may be concluded that silver nitrate is balantidicidal to a dilution lying between 1 in 20,000 and 1 in 25,000.

The results of the first series of tests of the balantidicidal action of drugs and chemicals are given in Table I. In this table are not included all of the experiments made nor all of the dilutions tested in many of the experiments; only one experiment with each substance and the critical dilutions which determine quantitatively the balantidicidal activity of the substance are given.
### Table I.—The balantidicidal action of various chemicals.

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<tr>
<th>Substance tested</th>
<th>Balantidicidal action</th>
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<tbody>
<tr>
<td></td>
<td>Dilution</td>
</tr>
<tr>
<td></td>
<td>1:100.</td>
</tr>
<tr>
<td>Atoxyl</td>
<td>0</td>
</tr>
<tr>
<td>Sodium arsenate</td>
<td>0</td>
</tr>
<tr>
<td>Antimonyl potassium tartrate</td>
<td>0</td>
</tr>
<tr>
<td>Trypan red</td>
<td>0</td>
</tr>
<tr>
<td>Methylene blue (medicinal)</td>
<td>±</td>
</tr>
<tr>
<td>Fluid extract of ipecac</td>
<td>±</td>
</tr>
<tr>
<td>Emetine hydrochloride</td>
<td>±</td>
</tr>
<tr>
<td>Quinine hydrochlorate</td>
<td>+</td>
</tr>
<tr>
<td>(5-10 min.) (10-15 min.) (60 min.)</td>
<td></td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>+</td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td>+</td>
</tr>
<tr>
<td>Mercuric iodide</td>
<td>+</td>
</tr>
<tr>
<td>Silver nitrate</td>
<td>+</td>
</tr>
</tbody>
</table>

This series of tests presents some interesting and unexpected results.

In the first place, it is noteworthy that the compounds of arsenic and antimony and the aniline dyes, which have been so extensively employed with more or less success in the treatment of other protozoan diseases, especially the trypanosomiasis, have proved to possess little or no balantidicidal action. Atoxyl, sodium arsenate, antimonyl potassium tartrate, and trypan red are absolutely inert in the low dilution of 1 part in 100 after acting for one hour on *Balantidium coli*.

Medicinal methylene blue is scarcely more active, a part only of the balantidia being killed after exposure for one hour to a dilution of 1 part in 100 of this dye.

1 However, the fact should not be overlooked that some of these substances may be more balantidicidal in *vivo* than in *vitro*. Such has been found to be the case with certain arsenic compounds in the treatment of trypanosomiasis.
The second surprising result is the feeble balantidicidal action of ipecac and its alkaloid, emetine, which are used so successfully in the treatment of entamoebic dysentery. In the low dilutions of 1 part in 100 and 1 part in 200, ipecac shows only feeble balantidicidal action, more or less but not all of the protozoa being killed after exposure to its action for one hour. Emetine hydrochloride, which has been proved by Vedder and Rogers to possess specific entamoebacidal properties to an eminent degree, proves to be scarcely as balantidicidal as ipecac.

Quinine, which is a specific for malaria and which has been frequently employed in the treatment of balantidiasis, is somewhat more active. The hydrochloride has killed all of the balantidia in the dilution of 1 part in 100 in five to ten minutes, in the dilution of 1 part in 300 in fifteen to thirty minutes, in the dilution of 1 part in 400 in thirty to sixty minutes, and in the dilutions of 1 part in 500 and 1 part in 600 in sixty minutes. In dilutions of 1 part in 800 and higher, it is inactive. The relatively feeble balantidicidal action of this drug does not promise much success in its application to the treatment of balantidiasis.

These well-known protozoicidal substances having been proved to possess feeble or no balantidicidal action, attention was turned to the salts of the heavy metals.

Copper sulphate, as is well known, displays a profound toxic action toward certain of the lower organisms. It is said to kill fresh-water algae in the remarkably high dilution of 1 part in 1,000,000, and it has consequently been widely recommended for the purification of water supplies. Its balantidicidal strength, however, has proved to be only moderate. In the dilution of 1 part in 1,000 it killed all of the balantidia in thirty minutes. In higher dilutions it was inert.

The salts of mercury possess eminent germicidal properties, and they have been extensively employed not only as antiseptics and disinfectants, but in the treatment of spirochæte and protozoan infections. The experiments with the chloride and the iodide of mercury have demonstrated that they are also strongly balantidicidal. In dilution as high as 1 part in 20,000 they kill all of the balantidia within one minute.

Silver is said to be one of the most toxic metals for bacteria and protozoa, but unlike mercury to be comparatively innocuous for the mammalian organism. Tests of the balantid-
bicidal action of silver nitrate show it to be an equally efficient balantidicide as the more poisonous mercurial salts. It does not act quite so quickly at the dilution of 1 part in 20,000, but its balantidicidal activity extends to slightly higher dilutions, a part of the balantidia being killed at the dilution of 1 part in 25,000.

The salts of mercury and silver are, therefore, much more balantidicidal than any of the other drugs or chemicals that have been tested; but their application to the treatment of balantidiasis is subject to certain limitations. The salts of mercury and silver nitrate are precipitated on coming in contact with albumin, and the albumin is coagulated, forming an impervious layer. Therefore, when employed for local treatment they are quickly rendered inert and possess little power of penetrating the tissues.

A search was, therefore, instituted for some compound of these metals which possess to some degree the eminent balantidicidal activity, which are not precipitated by albumin, and which might at the same time be less toxic than the ordinary inorganic salts. Substances satisfying these requirements to a greater or lesser degree appear to exist in the organic compounds of silver. Some 15 or 20 of these compounds of silver are known. They are said not to be precipitated by albumin or, if precipitated, to form compounds soluble in an excess of albumin. And it is claimed for many of them that they are as strongly germicidal as, and much less toxic for man than, the nitrate of silver. Accordingly, as many of the more promising of them as could be obtained have been tested for their balantidicidal value. The name, composition, percentage of metallic silver, and notes on the toxicity for mammals of the compounds, and the results of the tests of their balantidicidal action are given in Table II. Corresponding data of silver nitrate are included for comparison.

It is evident from this table that these organic compounds of silver are extremely variable in their balantidicidal action. In general, the larger the percentage of metallic silver, the more actively balantidicidal is the compound. But this does not invariably hold good; for argyrol, which contains about one-third as much silver, is only one two-hundredth as balantidicidal as silver nitrate. It is probable that a number of factors influence the balantidicidal action of these compounds. Of these, the silver content is perhaps the chief; but the action on albumin
and the readiness with which the compound is reduced to metallic silver probably play a part in the process. Among these silver compounds several, especially ichtargan and actol, look promising for the treatment of balantidiasis. These compounds are relatively nontoxic; while they are precipitated by albumin, they are said to form soluble compounds; and they possess a balantidicidal activity which is as great, in proportion to the silver contained, as silver nitrate.

In the application of the organic compounds of silver to the treatment of balantidiasis, there are four ways in which they might be employed, namely: first, by subcutaneous or intravenous injections; second, by high rectal enemata; third, by mouth; and, fourth, by appendicostomy and colon irrigations. The first of these methods, by subcutaneous or intravenous injections, has a precedent in Roger's treatment of entamoebic dysentery by subcutaneous injections of the soluble salts of emetine. In such treatment, 1 part of the silver compound to the number of parts of the body weight which are equivalent to the highest efficient balantidicidal dilution of the compound would have to be administered. Whether such a dose could be safely given would have to be determined experimentally. It is noteworthy in this connection that for several of these compounds such a dose has been experimentally determined to be harmless for guinea pigs (Table II). The chief objection to rectal enemata is that they cannot be made high enough to reach all of the infected gut. Nevertheless, it is one of the most convenient methods of treatment, and, if the large intestine be first emptied of faecal matter as completely as possible, should be efficient so far as the infected areas can be reached. For this method of treatment, solutions of the compounds of silver of a strength at least equivalent to the highest efficient balantidicidal dilution should be employed. Capsules coated with creatin, salol, or other substance that would be dissolved only just before discharge into the large intestine would probably be an efficient method of treatment, since by this means the whole length of the large intestine could be reached with the balantidicidal agent. In cases of balantidial infection in which dysenteric symptoms are established and which yield to none of the other methods of treatment, appendicostomy and the flushing out of the whole large intestine with the balantidicidal solution would undoubtedly be the most efficient method of treatment and would under the circumstances be advisable.
<table>
<thead>
<tr>
<th>Silver compound</th>
<th>Composition</th>
<th>Percentage of silver contained in compound</th>
<th>Reaction with albumin</th>
<th>Toxicity for mammals</th>
<th>Balantidical action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argyrol</td>
<td>Silver and vitellin (20 to 25)</td>
<td>...</td>
<td>1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.</td>
<td>+ (10 min)</td>
<td>1:100. 1:200. 1:500. 1:1000. 1:10,000. 1:20,000. 1:25,000. 1:100,000. Control.</td>
</tr>
<tr>
<td>Collargol</td>
<td>Colloidal silver</td>
<td>...</td>
<td>do</td>
<td>+ (4 min)</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Argentamin</td>
<td>Silver phosphate diethylen diamin (6 ±)</td>
<td>Not precipitated by albumin.</td>
<td>1 part to 1,000 of body weight given subcutaneously is harmless to guinea pig.</td>
<td>+ (immediately)</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Protargol</td>
<td>Silver and albumose (8)</td>
<td>Precipitated by albumin. Albumin not coagulated.</td>
<td>1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.</td>
<td>+ (1-3 min) (1-3 min.)</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Argonin</td>
<td>Silver and casein (4)</td>
<td>Not precipitated by albumin.</td>
<td>1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.</td>
<td>+ (1-3 min) (20 min)</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Drug</td>
<td>Description</td>
<td>Effect</td>
<td>Reaction Time</td>
<td>Activity</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>---------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>Nargol</td>
<td>Silver and nucleic acid</td>
<td>Not precipitated by albumin. Albumin not coagulated.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albargin</td>
<td>Silver and gelatose</td>
<td>Affects albumin slowly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ichthargan</td>
<td>Silver-thio-sulphate</td>
<td>Precipitated by albumin, but precipitate is redissolved in excess of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actol</td>
<td>Silver lactate</td>
<td>Decomposed by proteids, but said to form soluble compounds. Albumin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silver nitrate</td>
<td>Silver nitrate</td>
<td>Precipitated by albumin. Albumin coagulated.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 part to 1,000 of body weight given subcutaneously causes local necrosis only in guinea pig.
1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.
Less toxic to man than silver nitrate (Aufrecht and Wood). 1 part to 5,000 of body weight subcutaneously is harmless to guinea pigs.
1 gram can be given to man subcutaneously without serious symptom (Wood). 1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.
Average therapeutic dose for man is 0.01 gram (U.S.P.).
SUMMARY AND CONCLUSIONS

1. These tests have determined quantitatively within narrow limits the balantidicidal action of the substances tested.

2. It has been demonstrated that the compounds of arsenic and antimony, the aniline dyes, ipecac and its alkaloid emetine, and quinine, substances which are employed more or less successfully in the treatment of other protozoan diseases, possess little or no balantidicidal value.

3. The salts of the heavy metals, especially mercury and silver, have been found to be eminently balantidicidal.

4. It is possible that some of the inorganic salts of mercury or silver, administered by mouth, or by subcutaneous or intravenous injection, might be efficient in the treatment of balantidiasis. The salts of mercury are successfully employed in the treatment of certain spirochaete infections, and when given internally are eliminated in part by the mucosa of the large intestine; consequently, the mercury would be brought in direct contact with the infected tissues in balantidiasis.

5. The application of these inorganic salts of mercury and silver to the local treatment of balantidiasis is rendered impracticable by the facts that they are precipitated by albumin, and consequently possess little power of penetrating the tissues, and that they are relatively toxic for man.

6. The organic compounds of silver are not precipitated by albumin or, if precipitated, form soluble compounds that should be capable of penetrating the tissues, and they are relatively nontoxic for man.

7. Quantitative tests have demonstrated that certain of these organic compounds of silver possess a balantidicidal activity as great, in proportion to the amount of silver contained, as silver nitrate.

8. The practical value of these organic compounds of silver in the treatment of balantidiasis can be determined only through clinical experience.

LITERATURE CITED


THE RELATIONSHIP OF VARIOLA AND VACCINIA

By P. M. Ashburn, E. B. Vedder, and E. R. Gentry

(From the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands)

From Jenner's day until the present the relationship of variola and vaccinia has been a subject of frequent, often violent, and always unsatisfactory discussion, and no explanation of the relationship has yet received general acceptance. We think that an explanation that is simple, complete, and satisfactory is here presented. The prolonged absence of smallpox from Manila has hitherto prevented our undertaking certain experiments that we think might afford definite proof of our view. Therefore, we advance the view, supported by facts already known, and hope that others may be able to do the experimental work, although we shall undertake it in case we can obtain smallpox cases.

1. BASIC FACTS

The following basic facts as to the smallpox-vaccinia relationship merit first mention.

1. Smallpox contagion or inoculation gives rise in man to smallpox, a highly contagious, generalized disease of considerable mortality, characterized ordinarily by a preeruptive stage, and other stages related to the appearance, development, and subsidence of the eruption.

2. Passed through monkeys and cattle for a few generations and brought back to man, the virus gives rise to vaccinia, a localized, noncontagious, mild disease, that in itself causes no mortality, although septic complications may cause some.

3. Having, by passage, once lost its power to produce smallpox, the virus never regains it, even though passed from person to person (proper hosts for variola virus) for thirty-five (1) or one hundred (2) (3) years.

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II. EXPLANATION OF BASIC FACTS

We can think of two possible, complete, and satisfactory explanations for the above facts.

1. The germ of smallpox by passage through certain lower animals loses (acquires) certain properties, and it transmits its altered condition to its offspring forever, a more striking instance of hereditary transmission of acquired characteristics than has ever before (so far as we know) been cited.

2. Smallpox is due to a dual and divisible virus, one part of which causes vaccinia and the specific smallpox eruption, the other part being necessary for the production of the contagious, generalized, mortal disease with a distinct pre-eruptive stage and initial rashes.

We are unable to imagine a third explanation that does not ignore facts now known; of the two set forth above we favor the second for two reasons:

A. Without desiring to enter into the discussion of the hereditary transmission of acquired characteristics and without professing to have made a thorough study of the subject, we

Kelsch and his coworkers apparently hold the view that variola virus and vaccinia virus are distinct entities, but are derived from a common stem. This view ignores, and Kelsch appears to deny, the possibility of transformation of smallpox to vaccinia by animal passages, a fact that we consider clearly established.

Meirelles advances the view that smallpox is flea-borne and argues at length in favor of his belief. Under this hypothesis it would be understandable that the smallpox virus undergoes a sexual stage of development in the flea and that vaccinia is milder because it lacks that stage. But we think this disproved by several facts; namely:

1. Inoculated smallpox never changes in man to vaccinia.
2. Variola may be transmitted to man after 3 or even more animal passages that have all been made by inoculation.
3. Vaccinia has never been known to start a smallpox epidemic, even in Meirelles' own flea-ridden country.
4. Strains of vaccine have been kept pure as such, by human passages, since Jenner's day, probably too long a period for an asexual phase of a normally sexual organism to live.

Supple says that the vaccine germ remains localized and only leads to a local manifestation and reproduction. The immunity is purely histogenic and is limited to the epithelial layers that are physiologically closely related to the inoculation site. The nature of the attenuation of variola to vaccine lies in the fact that the growth intensity of the germ is lowered by calf passage. The immune bodies are developed and appear soon enough to prevent generalization of the locally inserted vaccine germs. This hypothesis ignores the fact that vaccinia never regains the power to produce smallpox, even though it become generalized at times.
incline to the opinion that nontransmission of acquired characters by heredity is a general law.

B. We think that already known facts, to be set forth in this paper, point rather to the correctness of the dual virus hypothesis.

III. EVIDENCE SUPPORTING THE HYPOTHESIS

1. MORE OF LESS ANALOGOUS CLOSE ASSOCIATION OF VIRUSES

Hog cholera was long ascribed to B. suistifer, the disease was stated to be produced by its inoculation, and extensive vaccination was practiced as a preventive measure. (4)

Yet now it is considered to be due to a filterable virus, and possibly other diseases (distemper of dogs, guinea-pig epizootic of Petrie and O'Brien, and scarlet fever) present a typical disease picture only as a result of the combined action of filterable virus and bacteria. (8) Prowazek and Baurepaire (9) think that smallpox virus acts in symbiosis with streptococcus.

The original street virus of hydrophobia, uniformly fatal to man, may be changed by numerous passages through rabbits to virus fixe, which causes a much shorter incubation and earlier death in rabbits, yet this fixed virus may be used to immunize man. Högye's method of using fresh virus diluted has had extensive trial; (10) and it is even stated (11) that the entire brains of rabbits dying from inoculations with virus fixe may be and have been injected into men without harm resulting, and that the use of considerable doses of fresh virus fixe has given excellent results in 92 cases in the Allegheny General Hospital. (12)

This indicates a close analogy between smallpox and hydrophobia, and suggests to us that hydrophobia may be due to a dual and divisible virus.

2. ANIMAL POXES. VARIOLATION OF ANIMALS

There are a number of animal poxes, the most important being smallpox, cow-pox, horse-pox (grease), sheep-pox, and swine-pox.

We know, from trial and personal observation, that man, ox, horse, sheep, and swine, the respective natural hosts of these diseases, are all subject to vaccinia.

Immermann (3) says that man is occasionally subject to ovination, the result being a single pock, and says further:

We have already mentioned and considered in detail the fact that cattle are readily inoculated, not only with humanized vaccinia and human
variola, but also with sheep-pox and horse-pox. The reaction to all these procedures is, without exception, the production of a local pustular eruption, which, when inoculated back into the original animal, likewise causes nothing but a local affection capable of further cultivation throughout the species. Finally, the temporary or permanent protection conferred upon individuals of any species (human or animal) by successful inoculation with any of these varieties of virus extends to all forms of smallpox, whether they be animal or human.

It would appear from this that the pock-causing element is common to these diseases and that they are mutually protective one against the other; yet that smallpox and sheep-pox, both highly contagious and highly mortal diseases in their respective natural hosts, are not the same is indicated by the fact that man does not get sheep-pox and sheep do not get smallpox. It is further indicated by the pathologic findings in sheep-pox.\(^{(13)}\)\(^{(14)}\)

While touching the relationship of animal poxes we may here mention, on the authority of Doctor Ruediger, in charge of the serum laboratory, Bureau of Science, Manila, that the strain of vaccine virus now used by that Bureau and by the Government of the Philippine Islands, a strain with which almost 7,000,000 vaccinations have been made with great success, was derived from a fatal case of human smallpox in 1908, having been passed first through monkeys, and from the second monkey to a heifer, and from the fifth heifer to man.

Gauducheau\(^{(15)}\) reports a similar experience from French Indo-China.

For many years the common origin of smallpox and vaccinia was disputed, largely because numerous able investigators were unable to inoculate cattle satisfactorily, or at all, with variolous lymph.

Kelsch\(^{(5)}\) reports 20 failures in 1909 and 1910.

Copeman\(^{(16)}\) gives a good account of the early work.

At present it is conceded that direct inoculation from variolous man to cattle is often, if not usually, unsuccessful, and the more satisfactory method of obtaining variola-vaccine is first to inoculate from man to monkey and later from monkey to cattle, although Freyer\(^{(17)}\) reports success from the use of rabbits instead of monkeys. From this it might be presumed that the bovine host is normally less susceptible and more resistant to smallpox virus than is the monkey, and the same is probably true of vaccine virus, as in our experience, with 16 monkeys and 9 cattle, the vaccine pustules are uniformly much larger in the monkey, as they also are in man.
The heifer being more resistant to vaccine virus, it follows that virus growing on the heifer must be able to withstand the greater resistance there offered, only the more vigorous and counter-resistant virus will survive, and by that process of selection the virulence of the strain becomes exalted, a conclusion supported by the following observations:

Brinckerhoff and Tyzzer(18) found that vaccinia more thoroughly protected monkeys from subsequent vaccination than did a previous attack of variola inoculata, although both protected against later variola inoculata. They quote Roger and Wiel as having made similar observations.

Dupont(19) found 22 per cent of 2,601 Sudanese, who had had variola vera or variola inoculata, still susceptible to vaccinia, although he says that the immunity of these people to smallpox is usually complete and permanent.

Schamberg(20) quotes Martin as having obtained 35 per cent of successful vaccinations with old, long humanized virus, and 80 per cent with animal virus and early human removes.

It is stated(2) that strains of vaccine passed from man to man, in China, for a hundred years, are almost inert. However, Immermann says that bovine lymph also deteriorates when passed exclusively from bovine to bovine host. Still, that does not affect the fact that the pock-forming element of smallpox virus first becomes exalted by passage through cattle.

Under our hypothesis there is another element in that virus, apparently one without which it cannot produce a contagious, generalized, mortal disease with a distinct preeruptive phase and initial rashes. This element dies or is otherwise eliminated by the animal passages, but such elimination does not always occur on the first passage.

Copeman and Immermann both discuss this matter rather fully, and agree that smallpox occasionally may be transmitted from cattle to man after two or three passages.

Brinckerhoff and Magrath(21) state that they carried a strain of variola virus from man to monkey, thence through 4 generations on rabbits' corneas, and then back to a monkey, causing smallpox, with the formation of a propustule and a secondary eruption. We know of no instance of smallpox production at a later remove than this from the human case. A single bovine passage may suffice to change the virus to vaccine.

It may be inferred, we think, that although the whole disease, smallpox, does not occur in cattle, the whole virus may live in them for a time, the separation of elements not always occurring suddenly.
This gradual separation of elements of a virus may be cited as an instance of selection, rather than one of either hereditary transmission or double virus. To this we reply that a virus offering so wide a field for selection is, in our view, dual and divisible.

We may here mention that a recent report by Simpson (22) of the apparent simultaneous occurrence of rinderpest and smallpox in an Indian buffalo, taken in connection with the case of Private Vann, to be mentioned later, caused us to think that possibly rinderpest might so weaken the general resistance of cattle as to allow vaccinia to become generalized on them.

Thanks to the cooperation of the Bureau of Agriculture of these Islands, we have been able to vaccinate 9 Batan or Luzon cattle, either before, at the time of, or shortly after their inoculation with rinderpest. We have obtained localized vaccinia and rinderpest, but no generalization of vaccinia. With a piece of scab the size of a grain of wheat, taken from the vaccination site of one of these animals, triturated in water, and inoculated into numerous small skin incisions, we have produced good, localized vaccinia and fatal rinderpest in another, but no generalization of vaccinia.

We hope to experiment soon with a buffalo.

3. CLINICAL OBSERVATIONS ON SMALLPOX

In 1899 Private Vann of the Twenty-third Infantry was admitted to the military hospital at Cebu, P. I., in a very low state from sprue. Death appeared imminent, and although the man was awaiting transportation to the United States it was feared that he would not live long enough to reach there. Another soldier, suffering from fever, vomiting, headache, and backache was admitted to the ward, and placed in the bed adjoining Vann's. He died of purpura variolosa in a few days. The condition having been suspected to be variola the day following the admission, all men in the hospital were at once vaccinated. Within a time now thought to have been not more than a week (the notes made in 1899 are not available), Vann showed a generalized eruption resembling discrete smallpox. Concomitantly he expressed himself as feeling better and asked for solid food which he ate without discomfort or injury. The improvement begun then continued, and a complete recovery from the sprue followed rapidly. The man was still in the service late in 1911 as a sergeant of the Hospital Corps.

This case was interpreted at the time and since as one of generalized vaccinia, the generalization occurring because of
the patient’s low resistance, a consequence of the extreme state of debility resulting from the sprue.

The evidential value of recollections of thirteen years ago is recognized as slight, but the facts are essentially as stated, and the case is reported as being of interest, at least.

There are three irregular forms of smallpox that are characterized by a total lack of pock formation or by a very brief and atypical pock stage. They are (1) purpura variolosa, always fatal, (2) varioloid or modified smallpox, and (3) variola sine eruptione.

Variolous purpura is described by practically all writers on smallpox, and there is general agreement that it proves fatal before any eruption appears. It is not to be confounded with that form of hæmorrhagic smallpox in which the hæmorrhages occur into the pocks (hæmorrhagic pustular smallpox). The investigation of a large number of writings does not show unanimity of opinion as to the protective value of vaccination against this form of the disease, but, as the form itself is rare and as a few positive observations outweigh many negative ones, we think it safe to say that vaccination does not protect against it.

Bancroft (23) had 12 cases among 1,200 of smallpox. Of these, 3 were unvaccinated; 7 had been vaccinated in childhood, of whom 3 had good foveated scars; and in 2 vaccination had been attempted, without success, two weeks prior to the onset of the disease.

Armstrong (24) says:

A history of previous vaccination, unless recent, does not play apparently a very important rôle in this variety of the disease, as the infection is of such severity that all resistance to immunity is overcome.

He reports one case in the person of a discharged soldier, who, because a soldier, had presumably been successfully vaccinated, though the fact is not mentioned.

MacCombie (25) says:

I have not met with a case in any one who had one-third of a square inch of well foveated vaccination cicatrix, and who had been successfully revaccinated.

Osler (26) reports 27 cases, of whom 13 had been vaccinated, but none revaccinated.

Kaposi (27) says:

Vaccination does not appear to offer the slightest protection against this form of the disease.
Meirelles(6) reports 2 cases in persons successfully vaccinated within five years. Without desiring to discuss at present this writer's hypothesis that smallpox is flea-borne, we quote the following statements as valuable because based on very extensive clinical observation:

The evolution of the blood phase of variola is similar in vaccinated and unvaccinated.

The eruptive phase is benign, almost absent, or rapid and slightly pustulous in the vaccinated, even when confluence of macules and papules promises confluent pustulation; it is grave, on the contrary, in the greater part of the nonvaccinated.

He then reports a case that we may include here as one of severe varioloid, or possibly hemorrhagic smallpox modified by vaccination.

A German, aged 45 years, vaccinated in his own country on his entrance to school and revaccinated later on entering military service, was admitted during my service.

He had fever of 40°C. with intense headache and backache, pains in all the body, vomiting, delirium, etc., like other variola cases. The third or fourth day all his body was covered with macules and papules of smallpox, so confluent that there was not a patch of sound skin the size of a pinhead. The diagnosis of confluent smallpox was necessary. * * * Notwithstanding the enormous confluence of macules and papules that enabled one to foresee confluent and abundant pustulation, a half dozen only, on the face and chest, became pustules of the size of a pinhead, at the center of the papule; all the others disappeared; their red color darkened progressively to black, while the macules diminished in size, so that toward the end of the disease the German had his body covered with black points.

One sees that the hematic phase of smallpox in this patient, vaccinated and revaccinated, was developed with the same intensity, with the same symptoms as in the nonvaccinated; the eruptive phase, above all the pustular, which in the nonvaccinated is usually grave and abundantly purulent, was nothing, or insignificant in him. * * * I could cite still other cases of variola similar to this, all vaccinated, where the hematic phase was intense and where the confluence of macules and papules indicated a grave infection, that nevertheless terminated with no or insignificant pustulation. I do not recollect seeing a single similar case in a nonvaccinated individual.

Vaughan(28) asks:

Why is it that in a protected case suffering from an affection that is practically nearly universal, and almost confluent everywhere on the trunk, one not infrequently finds practically no secondary fever, whereas a case with a similar rash in an unprotected subject would give an abundant secondary fever and would prove not by any means a matter for congratulation, nor would it offer grounds for a prognosis such as may amply be justified in a case protected by vaccination?
Concerning mild smallpox (varioloid) Councilman says:

The initial period may be typical and severe or mild. The symptoms suddenly abate and are followed by an eruption that may consist of only a few pocks. Welch and Schamberg report a case in which but a single pock appeared. The pocks are usually small and superficial and may be readily overlooked or their nature unsuspected. * * * Cases of confluent and purpuric smallpox are just as apt to follow infection by these mild cases as from any other form. * * * Such cases are now rarely found except in vaccinated individuals.

Immermann says:

Fever, delirium, and other combinations of initial symptoms are often as violent as could be wished. * * * The differences between mild and grave variola (varioloid and variola) become more decided with the eruption of the smallpox exanthem, that is, in general from the end of the third day of the disease—and from that on the differences are found at least as much in the general as in the local symptoms.

The condition of the body temperature and the general condition are pathognomonic for decided cases of varioloid. Immediately with the first appearance of the smallpox exanthem on the skin the fever begins to abate, and the fall of the temperature is generally so rapid and so complete that on the fourth day of the disease complete apyrexia has already made its appearance.

Bancroft (23) says:

In some instances an initial fever of the most severe type was present, accompanied by delirium and unconsciousness, and continuing for four or five days.

*Variola sine eruptione* is recognized by practically all writers. Councilman (29) says:

It appears as an illness of an indefinite character, occurring chiefly in hospital attendants on the twelfth day after exposure to smallpox. The symptoms consist in headache, pain in the back, fever and nausea. They may be so slight that the individual pursues his ordinary vocations, or they may approach in severity an ordinary initial stage. The symptoms last two or three days and then suddenly abate. The condition was well marked in one of the physicians investigating the Boston epidemic in 1901. Characteristic initial rashes may appear during the attack. One patient, a pregnant woman, remembered having a headache about two weeks after exposure to the disease, but was not otherwise affected. Her child showed a typical eruption when two days old. A group of three cases which appeared in one of the large hospitals in Boston, the onset in whom was nearly simultaneous, was traced to a ward tender who had an attack of what was supposed to be grip.

There were 12 of these cases among Bancroft's 1,200. Osler (26) says:

They seem to have been not uncommon in recent epidemics.
MacCombie (25) says:

I have only seen such cases in vaccinated, sometimes in revaccinated, subjects.

Wilson (30) says:

Variola sine eruptione occurs in young persons who have been well vaccinated.

We think that the above quoted clinical observations indicate that vaccination protects against the eruptive, and especially against the pustular stage of smallpox, rather than, or to a greater degree than, against the whole disease, smallpox.

This, if true, would afford strong support for our hypothesis.

The statement does not in any way imply that the value of prophylactic vaccination is less than has been thought, but does explain some, if not most, of the apparent failures, and also explains the successes resulting from its use; for, with the exception of variolous purpura, smallpox principally kills by, in, or as a result of, its pock stage.

We do not at this time desire to discuss the conclusions of Councilman, Calkins, (31) Prowazek, Casagrandi, (32) (33) (34) and others as to the cause of smallpox and the nature of the germ. It may be noted, however, that Calkins and Councilman cause Cytoryctes variola to transmit acquired (loss of) characters, in that by bovine passage it forever loses the power it once possessed of entering the nuclei of ectodermal cells and of undergoing a sexual cycle.

Prowazek’s “initial bodies” (Chlamydozoa) (35) are endowed by him with the power to transmit acquired characters.

Whether or not the work of any one of these or of any other investigator of the cause of smallpox be correct, it may be so only in so far as it relates to the common element in vaccinia and variola.

That there is such a common element is shown by the mutual protection afforded by inoculation of the two viruses, by the common histologic and other microscopic findings, by the mutual deviation of complement, (36) (37) and probably by allergic reactions. (38)

Thus far we know of no work and no observations that necessarily controvert our hypothesis. Should this hypothesis prove to be a truth, it might be found applicable to many diseases, and it would certainly provide a viewpoint from which they should, at least, be considered.
POSTSCRIPT

Only since writing the above article have we been able to obtain Ricketts and Bayles’ Diagnosis of Smallpox. These authors, while not entertaining our views as to the dual and divisible nature of smallpox virus, and holding strongly that vaccinia protects against the whole of smallpox, nevertheless do consider and speak of smallpox as a “dual disease,” and say, “The fever proper of smallpox is that of the septicæmia, and the local rash and the secondary fever bear the same relation to it as the pneumonia to measles, or the adenitis to scarlet fever.” Inasmuch as the pneumonia of measles and the adenitis of scarlet fever are probably always due to invasion of bacteria distinct from the viruses of the specific diseases, we regard the analogy as fairly good. It would be better could the pneumonia of measles or the adenitis of scarlet fever be shown to be due always to one specific virus (as is the pock of variola), and could that virus, by cultivation or by growth in animals, be obtained in a relatively pure and harmless condition and used to immunize healthy infants against the entire disease, or the more serious part of the disease, measles, or scarlet fever.

Using Ricketts and Bayles’ nomenclature in explaining our view, we may say that smallpox is due to virus AB. Of these (or the parts of this), A is mainly responsible for the “toxic fever” and “toxic rashes,” although possibly the combination AB is necessary before A can manifest itself; B is responsible for the “focal eruption” and for vaccinia. A’s action is first manifested, B’s follows, and it may be followed in turn by a third cause of injury, for instance, a streptococcus or staphylococcus infection. Complete immunity to B should constitute at least partial and possibly complete immunity to AB, although, should A be capable of acting alone, immunity to B would not affect it.

REFERENCES

(3) IMMERMANN. Nothnagel’s Practice. Amer. ed. (1906).
(4) STERNBERG. Text Book of Bacteriology (1901).
(6) MEIRELLES. Bull. Soc. path. exotique (1910).
(7) SÜPFLE. Arch. f. Hyg. (1908), 48, 237.
(10) Ravenel. Osler's Modern Medicine (1907), 3, 42.
(14) Bosc. Centralbl. f. Bakt. etc., Orig. (1903), 34, 413, 517.
(21) Baingærhoff and Magrath. Journ. Med. Research (1904), n. s. 6, 446.
(26) Osler. Practice (1906).
(30) Wilson's Hand Book of Medical Diagnosis. 3rd ed. (1911).
(37) Sugai. Ibid. (1909), 49, 650.
THE BIONOMICS OF STOMOXYs CALCITRANS LINNAEUS; A PRELIMINARY ACCOUNT

By M. Bruin Mitzmain

(From the Veterinary Division,^ Bureau of Agriculture, Manila P. I.)

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SUMMARY.

INTRODUCTION

I have had unusual opportunity to study the bionomics of Stomoxys calcitrans in connection with a large number of experiments on the transmission of surra, where thousands of flies of this species were captured or bred. It has been my aim not to duplicate the information presented by Newstead in his excellent treatise on this subject, and the present paper may be considered as supplementary to it. I have attempted to present the subject in as nontechnical a manner as is feasible.

OVIPosition

Age at which the fly lays eggs.—This was definitely determined in two instances in flies bred in the laboratory. In both the time was the same. The females were found with 2 males in copulation, April 11, when they were removed from the

1 Archibald R. Ward, chief.
breeding jar and kept in individual test tubes. Two days later, April 13, eggs were found in both tubes. These flies had emerged April 4 and had been fed daily on a monkey. Their actual age at the time of egg laying was 9 days. Another fly which emerged from its puparium on March 21 laid its batch of eggs, 81 in all, on March 30, when 9 days old.

Number of eggs laid by a single Stomoxys.—Under normal conditions the eggs of Stomoxys calcitrans are laid in the manure of its host. The eggs have been found in the faeces of the horse, the carabao, the bullock, and no doubt are to be looked for in the faeces of all domesticated animals. Under laboratory conditions guinea-pig manure offers the best medium for egg laying and subsequent development. In the laboratory, under artificial conditions, this insect deposits its eggs in ordinary glass tubes and on filter paper under glass. These ova hatch in due time if moisture be furnished them.

In order to determine accurately the number of eggs laid by a single female, not much reliance could be placed on observations under field conditions; therefore, it was necessary to resort to experimental procedure. At first captive gravid flies were used for this investigation; then an attempt was made to verify the findings with flies bred and mated in the laboratory.

The flies collected in the open were usually taken from healthy work animals and placed at once in test tubes plugged with cotton. By using numbers of females of various ages, it was hoped to obtain approximately the number in one which had not yet made an initial oviposition. From these eggs flies were reared for the purpose of checking the total oviposition in the wild flies.

Under these artificial conditions the females laid eggs in the glass tubes beginning as early as the second day of their captivity. The greatest number of eggs laid at one deposition was 94, while 5 flies laid respectively 82, 86, 91, 91, and 94 eggs. In every instance the eggs laid proved fertile, and the larvae from them were transferred at once to breeding jars with suitable food.

The flies used for this study were kept in the dark at a uniform temperature not exceeding 22° C. They were transferred daily to clean vessels and fed on monkeys and guinea pigs. Seven of the number which survived beyond ten days furnish the following data: Egg deposition extended over a period which, in 3 instances, comprised the entire life of the flies during captivity; in 2 others, to within three and four days of the death of the flies. Two flies escaped after having laid, respectively, 446 and 438 eggs.
In two cases recorded, as many as 20 batches of eggs were laid by a single fly, and the greatest number of eggs deposited by an individual was 632. In this instance oviposition occurred one day preceding the fly's death, which took place on the sixty-fifth day of its laboratory history.

Table I.—Data on oviposition.

<table>
<thead>
<tr>
<th>Length of time fly was kept</th>
<th>Number of depositions made</th>
<th>Total number of eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>168</td>
</tr>
<tr>
<td>34</td>
<td>9</td>
<td>182</td>
</tr>
<tr>
<td>60</td>
<td>20</td>
<td>438</td>
</tr>
<tr>
<td>72</td>
<td>13</td>
<td>455</td>
</tr>
<tr>
<td>65</td>
<td>20</td>
<td>632</td>
</tr>
<tr>
<td>70</td>
<td>11</td>
<td>318</td>
</tr>
<tr>
<td>64</td>
<td>15</td>
<td>446</td>
</tr>
</tbody>
</table>

In addition to the eggs laid, there were present after death a substantial number in each of the females dissected. In three of these the contents of the egg chambers in the dissected ovarian tubes were counted. In order to simplify the count, the chains of immature ova were not taken into consideration. It was found that the contents of the ovaries of the fly which had laid 632 eggs consisted of 90 ripe eggs and 98 partially developed eggs, making a total of 820 for 1 female. This number, 820, may be fairly accepted, I think, as the maximum number of eggs produced by a female Stomoxys. Twelve bred flies were employed for enumeration in oviposition. In these cases the eggs produced were sterile. As the result of these counts, no information can be added to that obtained previously. The total number of eggs deposited by any laboratory-bred female did not approach the maximum of the depositions made by the captive flies.

If the contents of the ovarian tubes can be accepted as a criterion of the possible maximum of egg production, one instance among the new flies would appear to establish the record in this regard. A fly emerging April 1 laid 106 eggs previous to its death, which occurred May 14. The dissected contents of the ovaries showed 71 ripe eggs, 112 half-developed eggs, and 840 immature eggs in various stages of development; a total of 1,123 from 1 female. However, until further investigation, this number will not be accepted as the possible maximum number of eggs that would be deposited by a Stomoxys. I prefer to consider 820 as the more authentic number.
The egg is creamy white and of the ordinary muscid type, with its convex side adhering to the place of attachment when laid. The grooved concave side through which the larva makes its exit is conveniently considered the dorsal side. The length of the egg averages 1 millimeter. There is no appreciable change either in color or form during incubation, which under ordinary conditions takes from twenty to twenty-six hours.

THE PROCESS OF HATCHING

In observing the hatching microscopically, it is quite necessary for the best results to suspend the eggs in a moist medium, preferably, physiological salt solution. When fertile eggs are placed in the air on a dry slide for as long as one hour, the hatching which has begun is inhibited and the embryo dies within the egg.

A certain sign of sterility, which can be applied as a test for eggs of this species, is the absence of embryonic movement within two days. There is not a distinctive dark polar spot, and the color changes to a dull pink, then rose, followed by brown after two days. If some moisture is present, the sterile egg ordinarily does not shrivel.

The movements of the embryo usually cannot be observed until the last four hours of incubation, when the cephalic area becomes ash-gray and gradually darkens as the movements become more active. The usual dilatation and contraction of the chorion take place accompanied by the occasional movement of the amniotic fluid.

Two pharyngeal spines, which appear under the cuticle, curved slightly posteriorly on the pharyngeal apophyses assist the embryo to escape from the egg. Prior to the process of hatching, the embryo lies curled up, and as the body gradually straightens out the head capsule extends from its sinus bringing the hatching spines into contact with both slits of the micropyle canal, the cephalic end of which is neatly carved out, laying open a flap of the chorion of the egg. By means of pressure from the posterior end and a constant prodding of the head appendages, the micropyle canal is forced open and the larva glides through the opening.

The ova are very sensitive to changes of temperature, light, and humidity. Incubation can be lengthened to fully double its normal length by lowering the temperature or by withdrawing the moisture from the medium in which eggs are laid. Exposure to light also influences the metamorphosis of this fly. At a room temperature of 30° to 31°C., eggs hatch in
from twenty to twenty-six hours. At a temperature of 20°
to 22° C., eggs hatch in from forty-eight to sixty hours, depend-
ing on the humidity. Eggs kept in a darkened closet hatch
four to six hours sooner than individuals of the same brood
kept at the same temperature in a room exposed to light from
windows.

The influence of moisture on the incubation period may be
illustrated by the following observation. A fly was placed in
a test tube while in the act of laying eggs. Forty eggs were
deposited, distributed along the sides of the tube. The first
egg was observed to be laid at 11.40 a. m., and the remainder
during thirty minutes. A piece of filter paper half the length
of the tube was soaked with salt solution and placed with the
eggs. Note was taken where the first egg of the batch was
laid, which was about 3 centimeters from the end of the mois-
tened paper and close to the end of the cotton plug. At 1 p. m.
of the following day the eggs at the bottom of the tube, where
the moisture accumulated, commenced hatching. All of the
eggs, except the first one laid, hatched before 2.30 p. m. of the
same day. This egg hatched upon the day following at 11.20
a. m., nearly one day later than the eggs in the moistened end
of the tube. The larva from this egg appeared quite normal;
it was observed to crawl immediately toward the moisture at
the bottom of the tube. This phenomenon appears to be in
keeping with notes made of field conditions, and when flies
are reared in glass vessels in the laboratory.

LARVAL LIFE

The young larva loses no time in consuming whatever de-
sirable food may be present. The feeding seems to be con-
tinuous, broken only by the short period of seclusion just before
the formation of the pupa. Food seeking is relatively a passive
process with this insect; the mother provides for the life of
its progeny, in laying the eggs only where there is an abundant
food supply.

Very little time is consumed by the larva in adapting itself
to food conditions. The bloody excreta of the mother, if pres-
ent, is first consumed, then the more inviting portion of the
animal manure is devoured. The color of the insect, at first
waxy or creamy white, rapidly assumes the color of the ingested
food. This is pale green at first, changing, from the posterior
end to the anterior portion, to pale brown. The larval cuticle
does not become darker before six to seven days.

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The larvae thrive on many kinds of food. The following materials were found experimentally to produce healthy adult flies: Manure from the horse, cow, carabao, and guinea pig; guinea grass; bran; bran and horse manure; corn-meal and horse manure; and horse manure saturated with blood from the horse and monkey.

It was found that ordinary filter paper served as food for the omnivorous larvae. The paper was invariably placed over the manure in the jar to assist in regulating the moisture content, and it was noticed that within three days the paper was full of holes and jagged along the edges. In about five days the filter paper was represented by a few scattered strands which disappeared usually before the larvae matured. It was demonstrated that larvae could develop on filter paper soaked in manure decoction, provided the latter was supplied fresh daily.

The development of molds in the breeding jars interferes with the growth of the larvae, but the appearance of some fungi does not usually produce any effect. Indeed, the spore heads of certain fungi, which are commonly a part of the flora of this manure, seem to be greatly relished by larvae of *Stomoxys*.

It was found to be advantageous to boil all of the ingredients with the exception of the blood in the culture jars, in order to destroy the animal and vegetable life, especially the mites found commonly in manure, and various species of mold, imical to the fly's development.

**Cannibalism**

The parasitic tendencies of this fly are developed at an early age. The first manifestation is shown in the young maggots which when confined to a test tube will invariably remove the moisture from each other's bodies. When the food in the breeding jar is allowed to become dry, the larvae clump together and lick the moisture from each other's bodies. This is only a step toward the stage where portions of the body are removed and a state of cannibalism results.

Two instances were observed of larvae feeding upon other injured larvae. On one occasion 2 nearly full-grown larvae were seen feeding on the juices exuding from a large jagged hole torn in the side of an injured, but still active maggot. It was not ascertained if the 2 feeders were responsible for the injury, but the injured larva was soon helpless and became an easy prey to its fellows.
Stomoxys calcitrans remains in the larval stage under ordinary laboratory conditions for a period averaging twelve days. Between the third and fourth days the larva makes its greatest growth in length. By the sixth day the larva has reached its maximum thickness.

Table II.—Development of a typical larval Stomoxys.

<table>
<thead>
<tr>
<th>Date</th>
<th>Period of development</th>
<th>Length</th>
<th>Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 9</td>
<td>After hatching</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>April 12</td>
<td>Third day</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>April 13</td>
<td>Fourth day</td>
<td>6.0</td>
<td>0.75</td>
</tr>
<tr>
<td>April 15</td>
<td>Sixth day</td>
<td>7.0</td>
<td>1.5</td>
</tr>
<tr>
<td>April 17</td>
<td>Eighth day</td>
<td>9.0</td>
<td>1.5</td>
</tr>
<tr>
<td>April 21</td>
<td>Twelfth day</td>
<td>10.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>

THE FORMATION OF THE PUPARIUM

The puparium is formed two days after the larva has attained its maximum size, which under ideal conditions is from the eighth to the twelfth day. The puparium is constructed without any apparent sloughing or shedding of the larval skin, the process being one of simple contraction. A larva measuring 10 millimeters is reformed slightly, and contracts to 5 millimeters. The body is thickened from 1.5 millimeters to 2 millimeters. There is an invagination of the cephalic end bearing the head capsule, and this and the anal end become broadly rounded.

The full-grown larva is coated with a glossy, slimy cuticle which is pale chrome yellow in color. It lies inert at the beginning of the somnus which lasts until the puparium is formed. The viscera wrinkle and disintegrate and assume the yellowish color of the Malpighian tubules and the cecal glands. Soon the color of the cuticle blends with that of the internal structures, becoming pale clay yellow. The only structures now visible are the lines of the trachea and the dark brown anal stigmata. During the changes indicated the mouth cavity is constantly kept in slight action accompanied by a barely perceptible general telescopic movement. Upon the following day these activities cease, the barrel-like puparium is completed, and no internal organs are visible. With the absence of movement, the buccal cavity has become invaginated and is covered by the cap of the
puparium. This end of the insect is red-tipped, while the remainder is decided golden in color. The color of the whole puparium changes rapidly to burnt sienna.

The encapsulated puparium is usually from 5 to 9 millimeters in length, depending on the nourishment and care the larva has received. The female has a larger puparium than the male fly, and the female is as a rule 0.5 millimeter longer than the male from the same lot.

The male fly takes less time than the female to pass the nymph stage. In more than 40 instances recorded, first emergences were marked by the appearance of males. The male fly usually precedes the female by two days.

**INFLUENCES OF ENVIRONMENT ON THE DEVELOPMENT OF THE NYMPH**

Certain artificial conditions have been found to affect the developing nymph. When kept in water during the whole of this stage, 30 pupae failed to develop; nor did they develop when removed from the water after seven days and placed in a dry glass after drying on filter paper. The lowering of the temperature from that of the room at an average of 29° C. to that of the cold room at 21° C. retarded emergence two to four days, but seemed to increase the percentage of emergences. An exposure to the outdoor light and sunshine at a maximum temperature of 43° C. killed the developing nymphs, while the light of the room at the same temperature of the darkened cabinet inhibited the development to the extent of two to three days.

It can be inferred from these experiences that the optimum conditions for pupal development include a dark, cool, moist medium; and these conditions prevail where the puparium occurs in nature.

The length of time spent in the puparium is fairly constant. It is usually never greater than five and one-half days under natural conditions, but when modified by the artificial environment of the laboratory this period may be extended to six or even to ten or twelve days.

**EMERGENCE OF THE FLY**

The pupa gives no indication of movement of any sort, such as occurs in some orthoraphous flies, which might be interpreted as premonitory of emergence. The only sign of activity, to inform the observer of what is occurring within, precedes emergence only by a minute or two, and consists of a barely perceptible rising and falling of the operculum or cap. Immediately
there follows a splitting of the cleavage lines at the cephalic end of the pupal envelope. A slit appears in the fourth segment encircling the puparium, isolating an apical section of a cone which is also divided by a median line. Usually one-half of this raised cap serves as a lid which, opening, allows the fly to escape.

The subsequent development prior to flight is divided into 3 stages which are so graphically described by Newstead that I refrain from repetition, and refer the reader to his paper in which this phase of the life history as well as many others are treated with his usual faithfulness and clearness of description.

In emerging, sometimes the imago is held at the anal segment by a tissue which at first sight appears to be the lining of the puparium. This is the exuvia of "the final ecdysis" (Newstead) which the fly attempts to part with when it leaves the puparium. In some cases the emergence is effected without the effete skin intact, when it is plastered to the anal end of the fly and remains attached even after the insect takes its initial flight.

It has been observed in a few cases that if at this stage, prior to the unfolding and hardening of the wings, the fly be immersed in water for two minutes or more, development ceases.

The sexes are readily distinguished upon emergence. The female is invariably the larger and the lighter in color. It emerges with its long tapering ovipositor projected until the body dries thoroughly and flight is begun.

To show the time required before a fly is able to take care of itself after emerging, the following chronological note is appended:

Morning of April 30, 1912.

9.35: The operculum has been split and the fly, a male, released from the puparium.

9.40: Length, 5 millimeters.

9.42: The wings unroll and separate from the body, where they are held while drying and hardening. Since emerging, the proboscis is held against the notum between the 2 interlocking processes of the mesotrochanters.

9.52: The labellum of the proboscis is seen to change from the ultra ash gray to brown, then to black.

9.55: The entire labium becomes brown.

9.58: The proboscis has been detached from the thoracic clasp through movements of the legs. When released it gradually drops and swings in place through traction by the longitudinal muscles. This organ then assumes the normal position under, and anterior to, the head.

*Lec cit.*
10.00: The length of the fly, including the projection of the labium, has increased to 5.5 millimeters.
10.03: The fly makes its initial flight inside of the flask.
10.10: The proboscis is now jet black and fairly hard.

From these notes it can be seen that it requires one-half hour of drying before the insect is able to fly and also that it would be impossible for the fly to apply its proboscis in feeding for an equally long time after emergence.

The difference in the time of emergence of flies from the same lot of eggs is usually out of all proportion to the difference of time of deposition of the eggs. The larvae from a lot of eggs laid by a collection of females on June 24 to 26 commenced pupating July 2 and emerged July 15. Emergence continued daily up to July 26, fully eleven days after the first appearance of flies. It was noticed that the flies appearing last were fully 1 millimeter smaller than those appearing first. This was due perhaps to the gradual drying of the food medium which was less suitable for nourishing the larvae hatching last.

This difference in size is seen also in flies of precisely the same age. This was noted in 2 females emerging April 4, fed daily upon the same animal. When 20 days old they measured, respectively, 5.5 millimeters by 2.5 millimeters, and 7 millimeters by 3 millimeters.

**FEEDING HABITS OF STOMOXYS CALCITRANS**

In my experience the adult fly, male and female, can be kept alive only by feeding on the blood of animals. Drawn blood, although accepted, apparently does not answer the requirements of the fly, even when renewed daily. Flies nourished in this manner usually do not survive longer than flies kept without food.

Under laboratory conditions flies of this species will feed for the first time six to eight hours after leaving the puparium, but I do not doubt that, in nature, blood is taken as early as one hour after emergence. Several laboratory-bred flies have been seen feeding in an apparently half-hearted fashion for a few minutes within one hour after emerging.

Judging from observations made under experimental conditions, *Stomoxys* is essentially a blood feeder; it has never been observed to take plant juices, although it sips water when confined in jars and test tubes.

Under conditions obtaining in these Islands, the fly will feed readily on man, although it rarely attacks him in the presence of...
domesticated animals. It is rarely found to annoy man to the extent that prevails in temperate climates. Its attacks upon man generally take place shortly after the atmosphere has been cooled by a rain shower and at certain seasons of the year when this species is unusually abundant. The following notes are added to show the extent of the attacks of these flies on man when they are abundant.

At 8.50 a.m. August 27, 1912, a female *Stomoxys calcitrans* flew into the laboratory after a rain shower, alighted on my exposed arm, and in a few seconds commenced to scrape the skin with its labellum. Within ten seconds sharp pain was felt. The probing continued for two minutes when apparently a satisfactory insertion of the proboscis was effected. At this time the distention of the abdomen of the fly became apparent. The aspirating process caused only a dull pain. Although the blood gushed into the stomach of the parasite the labium was inserted to only one-third its length. The base of the labium was not inserted nor was the labium buried in the skin to the bulbous portion as is usual when this fly is feeding on other mammals.

The fly under observation fed for three minutes and thirty seconds. A blood drop the size of a pinhead was left at the site of feeding, and one hour later a very slight pain was felt, while a minute hæmorrhagic spot marked the place of feeding. The bitten area on the arm was marked with a blue paraffin pencil, and within an hour another fly settled within the boundaries of the blue mark. It fed one minute and a few seconds, during which time a third fly appeared and made a bite only 4 millimeters distant from the blue mark, and fed for two minutes.

While I was jotting down these notes, another fly, the fourth parasite, visited the bitten arm and selected a spot within the marked area bitten by its fellows. All four of the foregoing parasites were males. Less than ten minutes elapsed when a fifth *Stomoxys* appeared on the bared arm, and commenced operations within a centimeter of the area bitten by the last fly. In this instance the parasite was a female, and, from appearances, one which had been subjected to a long fast. This fly required nearly two minutes to aspirate any blood. In six minutes the labium was inserted to the maximum depth, that is, to the bulb, and the fleshy portion of the labium leading into the
pharynx kept up a constant titilation. At no time was the labium held still, but there was a continual piston-like movement. The fly bit for twelve minutes and forty seconds, defecating at intervals of from thirty to forty seconds, and at each deposition the fluid voided was bloody. The first evacuation took place synchronously with the first dilatation of the abdomen.

The termination of the biting was followed by a quick withdrawal of the labium to its middle, then the rest was slowly withdrawn and cleaned on the fore tarsi. A blood drop flowed to the surface of the skin at the site of feeding. No pain was felt when the labium was withdrawn. All of the bites occurred in an area of 3 centimeters on the fleshy part of the left forearm. I do not attempt to explain why these parasites showed such a marked predilection for a restricted area of my arm. The same phenomenon occurred again subsequently.

*Stomoxys calcitrans* appears to attack all animals with equal avidity. Sick animals especially are marked for their attacks. It has been observed by many investigators that infected animals, particularly horses, are more susceptible to the attacks of *Stomoxys* than are healthy horses. During an outbreak of surra, two years ago, it was noticed in a public corral that 3 horses among a large number of work animals contracted surra, probably several days before the malady was diagnosed. The attention of the veterinarian in charge was attracted, primarily, not by the clinical symptoms, but by the large number of biting flies present on one of the sick animals. This horse did not attempt to dislodge the parasites, the flies feeding until engorged, then flying to the nearest fence to rest. Three horses were examined for blood parasites, and many trypanosomes were found. The weakest horse showed the greatest number, and concomitantly the predominance of the ectoparasites. Incidentally, when the 3 sick horses were removed, the infection among the remainder of the animals was checked.

Experimentally *Stomoxys* will feed on any animal offered for this purpose. Twenty-four specimens of *Stomoxys calcitrans* were taken while observed in the act of biting a horse. They were placed in 24 glass tubes and applied each day, as long as they survived, to a different species of animal. In every instance the flies bit and fed upon the blood of the animal on which they were placed. The following table shows the number of flies which survived after each meal and the average number of minutes each fly fed on the various hosts.
TABLE III.—Showing the feeding of Stomoxys on various animals.

<table>
<thead>
<tr>
<th>Date</th>
<th>Animal used</th>
<th>Number of flies fed</th>
<th>Length of time fed (average per fly)</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 16...</td>
<td>Horse</td>
<td>24</td>
<td>3.5</td>
</tr>
<tr>
<td>October 17...</td>
<td>Monkey</td>
<td>23</td>
<td>4.0</td>
</tr>
<tr>
<td>October 18...</td>
<td>Carabao</td>
<td>20</td>
<td>3.0</td>
</tr>
<tr>
<td>October 19...</td>
<td>Bullock</td>
<td>18</td>
<td>4.0</td>
</tr>
<tr>
<td>October 20...</td>
<td>Goat</td>
<td>15</td>
<td>2.5</td>
</tr>
<tr>
<td>October 21...</td>
<td>Sheep</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>October 22...</td>
<td>Guinea pig</td>
<td>11</td>
<td>3.5</td>
</tr>
<tr>
<td>October 23...</td>
<td>Pig</td>
<td>11</td>
<td>2.5</td>
</tr>
<tr>
<td>October 24...</td>
<td>Cat</td>
<td>11</td>
<td>4.5</td>
</tr>
<tr>
<td>October 25...</td>
<td>Deer</td>
<td>10</td>
<td>2.0</td>
</tr>
<tr>
<td>October 26...</td>
<td>Dog</td>
<td>10</td>
<td>3.0</td>
</tr>
<tr>
<td>October 27...</td>
<td>Rabbit</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>October 28...</td>
<td>Chicken</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>October 29...</td>
<td>Bat</td>
<td>8</td>
<td>2.5</td>
</tr>
<tr>
<td>October 30...</td>
<td>Rat</td>
<td>7</td>
<td>4.5</td>
</tr>
<tr>
<td>October 31...</td>
<td>Lizard (gecko)</td>
<td>4</td>
<td>8.5</td>
</tr>
<tr>
<td>November 1...</td>
<td>Man (Filipino)</td>
<td>4</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The table shows that at least 4 of the flies fed on 17 species of animals in as many days.

The only fact of practical value suggested by these experiments is that Stomoxys calcitrans will accept any host which will submit to its attacks. Therefore, a sick animal would be most exposed to the bites of Stomoxys or other insects of similar habits. The inference to be drawn relative to epidemiology is obvious.

THE FEEDING RELATION OF NONBITING FLIES TO STOMOXYS

A peculiar feeding relation has been observed to exist between Stomoxys and certain nonbiting flies. I was curious to learn why such large numbers of nonbiting flies were generally found in collecting insects from domesticated animals. Moreover, in an examination of extensive collections made with a net swung over the backs of the animals, the majority of the nonbiting flies were found to have blood-engorged abdomens. When these were dissected and examined microscopically, mammalian blood was found to be the principal food constituent.

A quiet bullock was selected for closer observation. On this animal some 150 to 200 flies, mostly muscids, were seen. Many hundred dung flies, including house flies, were scattered about on the floor of the stall, and occasionally one of these joined the blood-sucking flies on the body of the bullock.
My attention was attracted by the peculiar grouping of the ectoparasites; groups of from 2 to 5 predominated. On closer inspection the group was found to consist almost invariably of more than 1 species, a Stomoxys usually being the central figure. Where a Stomoxys was lacking, it was found that the group fed from a common area with the heads of the individuals in close contact. The food of these flies was found to be a droplet of freshly exuded blood, and among the blood imbibers often not an individual belonged to a species with a piercing mouth; they consisted principally of house flies. Other groups of flies surrounding a Stomoxys attracted attention by the fact that while it was feeding the rest waited. The latter gave evidence of great impatience and eagerness in the movements of nudging one another and colliding with the Stomoxys, apparently making efforts to dislodge it. The Stomoxys having been satisfied, the other flies pounced upon the feeding spot where trickled a well-rounded blood drop. These flies collected around the puncture, and lapped the blood as it oozed from the wound. In a moment the group disbanded with abdomens more or less reddened and distended, the individuals either flying off the host to rest or to join another biting Stomoxys. In many instances the Stomoxys was accompanied by a single fly which hovered above it until the Stomoxys was fully engorged and left the exuding blood to the disposal of the second passive parasite.

THE OCCURRENCE OF STOMOXYS CALCITRANS IN NATURE AND ITS RESTING HABITS

Stomoxys calcitrans is essentially a parasite of live stock, and the natural occurrence of the fly is related to these domesticated animals and their environs. The resting habits of the stable fly are quite characteristic and related in a measure to the feeding habits. It may be found at almost any time of the year in these localities feeding several hours before 8 o'clock in the morning by which time it in many instances has engorged itself. At about this time many may be found at rest either upon a part of the animal inaccessible to the host, or more commonly upon trees and fences bordering corrals and sheds.

I have found that in this locality the most frequent resting place is the interwoven cross-wire fence, a small section of which will accommodate from 4 to 6 flies. The fly is perched usually away from the sun under the wire with its head directed toward the shaded side. All the feet clasp the wire, and the proboscis is slightly drooped. So immobile are they that when the fence is violently shaken few flies are disturbed. They are so sluggish
that they become easy victims of spiders, and the cautious observer, after a little practice, may easily capture the fly with the thumb and forefinger. This method of collecting leaves the fingers stained with considerable blood from the engorged parasite. Feeding is usually resumed when the heat of the day has subsided, and after the second daily engorgement another long rest follows.

The occurrence of *Stomoxys* under natural surroundings has been carefully noted. The following observations will throw some light on the habits of this fly.

On April 3 at 5 a. m. (in bright moonlight) the cattle shed and the stock in the open woods near the laboratory were inspected. At this time there was not an indication of a fly. A few mosquitoes buzzed about, but not enough to cause any appreciable stir among the resting animals. I waited in the woods with the resting cattle and carabaos until 5.20 a. m., when a remarkable awakening took place. Every animal, almost simultaneously, was on its feet switching its tail and squirming. A small band of flies had made their appearance, and at 5.30 a. m. the flies had attacked the cattle and carabaos in swarms. Dense black patches of them were seen on white cattle. The same phenomenon was noted at the shed quartering the work animals. Flies which at 5.15 a. m. were found resting on the boards of mangers and stalls were actively infesting the awakening cattle at 5.30 a. m. This invasion continued until sunrise at 6.40 a. m. when the hordes of flies gradually diminished. At this time of the year the greatest infestation from these flies is the hour preceding 6.30 a. m. Flies of this species were attacking stock in the sheds at 6 a. m., even as late in the year as October 24. The animals were terrorized by the quick sharp pricks of the stinging flies, and seemed unusually restless, moving both laterally and backward and forward to escape attack. Some actually reared into the feed mangers. At this time of the day it was very dark and sultry.

**LONGEVITY**

An attempt was made to determine the longevity of this species, both with flies collected in their natural state and flies bred in the laboratory. In either case only an approximation is obtained, since artificial conditions were present, which might have increased or diminished the natural length of life.

Guinea pigs and monkeys were utilized to provide food for the flies which were kept separately in suitable glass tubes. When not being fed, the flies were kept in the dark at a tem-
perature of 20° C. to 23° C. No special provision was made for moisture in the tubes. Under these conditions the maximum period of life was seventy-two days. This was a female.

In the tests with laboratory-bred flies, which emerged about the middle of July, 63 flies were employed. Twenty-four males had an average longevity of twenty-eight days, while 39 female flies lived for an average of thirty-two days. Six of the females showed an average longevity of fifty-four days, and 6 of the males averaged thirty-nine days. One female of this series lived seventy-two days, while 1 male lived fully ninety-four days.

Perhaps a fairer method to determine the natural longevity would be to mark thousands of bred flies and set them free, and from time to time, through systematic collecting, attempt to recover them.

MATING

The mating of *Stomoxys calcitrans* was never observed under field conditions except in one instance where a pair was observed in copulation while the female was attempting to feed. The process was observed in bred flies in 3 instances, occurring in 2 pairs upon the seventh day after emerging from the puparium. As has been noted previously, two days after the copulatory act, fertile eggs were laid. These flies had been kept in company with many others in a large bottle which was daily applied to a monkey for feeding purposes.

In the act of mating the male assumes the active rôle, flying off after a period of ten minutes. In these flies the male is above, almost at right angles, adhering only by the genitalia and front legs, the other legs being suspended at each side. The clasping organ of the male exerts a pressure upward and forward, the female genitalia respond in a backward and downward movement, resulting in a quick, decided telescoping of the parts. The male withdraws the intromittent organ by a downward pull at the conclusion of the process. It releases its hind legs and flies away directly while the female remains for a few minutes. During this time a reflex telescopic action takes place until the invaginated parts, which have been depressed, are extended to their normal length.

METHODS EMPLOYED IN KEEPING AND FEEDING FLIES FOR LABORATORY PURPOSES

The methods employed for keeping and feeding *Stomoxys* in captivity will serve for other species of blood-sucking flies; for example, species of *Lyperosia* and of the Hippoboscidae. The
greatest difficulty has been encountered in attempting to keep flies in a common enclosure.

**Screened stable.**—In a screened stable, aside from the artificial conditions of confinement, the difficulties are chiefly from the presence of natural enemies, and do what one may it is well nigh impossible to wholly eradicate them. Particular reference is made to the common insectivorous lizard and the ubiquitous spider. Spraying with pure creoline was partially, but not wholly effective.

**Glass vessels.**—Large bottles and museum jars of a minimum capacity of 3 liters were used when it was desired to confine and to feed at one time a considerable number of flies. Thirty days was the longest time that flies were kept in these containers. In this instance, it was found necessary, for the preservation of life during the last ten days, to transfer the flies to individual test tubes after feeding.

In the use of large glass vessels, untimely death resulted from mite infestation, cannibalism, and excess of moisture.

An unknown mite, not restricted to these flies, was found to be parasitic, both in the hypopial stage and in the adult form, upon the flies. The first of these stages did not prove a menace unless present in great numbers either on the body, which precluded proper functioning of the spiracles, or on the proboscis, which prevented the insertion of the labium in feeding. When the mite was present as a true parasite, in the adult form, an occasional one or two did not seriously affect the fly, but when present in large numbers they were sufficient to enfeeble it.

Cannibalism was encountered in the experiments to an unusual degree. Often the disability of an individual fly attracted the attention of another more active member which promptly attempted, and usually succeeded, in puncturing the helpless fly's abdomen. This disability might result from engorgement, infirmities resulting from broken labium, or from the wings adhering to the glass due to an excess of moisture. I have found numerous cases of flies actually fracturing the labium in attempting to penetrate the host's epidermis, and it may result from the fly pricking at the glass in attempting to sip moisture from the container. Such a condition, of course, makes feeding impossible as the proboscis is not rigid enough to puncture the skin, and as a result the fly dies from starvation.

Where a large number of flies are quartered it is difficult to avoid an excess of moisture even though a bibilous filter paper is employed. The condition is probably the result of excretory
contamination and the condensation of the moisture in the air in the bottle, when kept at a temperature of from 20° to 28° C. The excess of moisture causes the flies to become stuck by their wings to the sides of the bottle where they soon die.

The use of individual glass tubes.—This method has proved the most successful for keeping Stomoxys in captivity. The fly can be observed at all times, and its longevity is increased to nearly the normal. Ninety-four days was found to be the maximum life of adult flies kept individually in glass tubes. A test tube of 24-millimeter bore, plugged with cotton, was found the most convenient sort. A piece of white filter paper conforming to the size of the tube was found ideal to regulate the moisture, and this was changed at least every two to three days. It was found advantageous to change the fly to a fresh tube not oftener than twice each week. In feeding it was not found necessary to screen the mouth of the tube. The filter paper was first removed, the base of the tube was directed toward the window light and the tube was inverted immediately over the animal's body. The fly after feeding was induced to release its hold upon the skin of its host by gently tapping the tube and gradually inclining the latter toward the light, after which the filter paper was restored and the tube stopped with a cotton plug.

The flies when not fed were kept in the dark at a temperature between 20° and 28° C.

METHOD OF APPLYING THE FLIES TO THE HOST IN FEEDING

Monkeys.—The following method was pursued in applying large numbers of flies in a bottle. The monkey was strapped, abdomen down, to an improvised stock by means of surgical gauze or twine. The wrists and ankles, which were bandaged previously to prevent chafing, were first secured; then the tail was closely cropped, bound to a stout wire with straps of gauze, and thrust into a narrow-necked bottle which contained the flies to be fed. The other end of the wire was kept at a convenient distance from the mouth of the bottle to facilitate manipulation. Wiring the tail was necessary to prevent the animal from switching that appendage against the glass and crushing numerous flies.

In feeding the flies from test tubes, the tubes were inverted over the thigh or other convenient part of the monkey. At least 2 flies could be fed at once in this manner.

Guinea pigs.—When this animal was subjected to fly bites in a large museum jar it was found to be of advantage to im-
mobilize it by strapping to a frame of brass wire. This was done in order that movements of the animal would not interfere with the biting of the flies or with the observation of the flies throughout feeding. Cropping the hair of this host was found to facilitate the feeding of the flies. It was necessary to hold the museum jar horizontally with the bottom toward the light. Here the majority of flies assemble when not feeding, and the light reactions of the fly are taken advantage of in withdrawing and introducing the host. If desired, ether can be used to advantage in the transfer of animals. It should be applied at the screened end of the jar, lightly enough to prevent flight, but not sufficiently to stupefy the insects. The flies can also be fed individually in test tubes to guinea pigs strapped to stocks, being applied to some convenient part of the body, preferably the side of the abdomen.

Horses.—The method more commonly employed by investigators is to enclose both the flies and the horse in a screened stall or shed. Here it is not possible to make close and accurate observations, and despite the fact that many thousands of flies could be kept at once they did not, in my experience, live longer than eight days, and usually died in five days even when food was constantly available.

By strapping the horse to an operating table, accurate data of the feeding process can be obtained. This method supplanted the crude one of throwing the horse to the ground and feeding flies from inverted bottles. The violent struggling of the horse under these conditions is not conducive to making accurate observations.

In all of the methods of feeding the flies, the hair of the host was closely cropped with scissors. It was found advantageous, also, to slightly dampen the skin of the host to make the animal odor more attractive to the insect and to arouse its blood-drawing instincts.

**SUMMARY**

1. The age at which *Stomoxys calcitrans* begins egg laying has been determined in bred flies to be nine days.

2. The maximum number of eggs produced by a single *Stomoxys* may be stated as at least 632 and possibly 820. As many as 20 depositions may be made in the lifetime of a female.

3. The incubation period for these eggs is from twenty to twenty-six hours at a temperature of from 30° to 31° C.

4. The larval stage under optimum conditions is usually from seven to eight days.
5. The imago emerges from the puparium generally in five days.

6. The fly of either sex takes its initial bite in from six to eight hours after emergence. Flies of this species have fed experimentally on 17 species of vertebrates including mammals, reptiles, and birds.

7. In feeding on live stock, *Stomoxys calcitrans* makes a wound with its labium from which nonbiting flies suck blood.

8. The female may live at least seventy-two days and the male ninety-four days.

9. The development of *Stomoxys calcitrans*, as shown by Table IV, varies considerably, depending upon the environment. Under optimum conditions, it is twelve days.

**Table IV.**—Life history of *Stomoxys calcitrans* at various periods under various conditions.

<table>
<thead>
<tr>
<th>Date of oviposition.</th>
<th>Incubation period.</th>
<th>Larval stage.</th>
<th>Pupal stage.</th>
<th>Life cycle.</th>
<th>Conditions of development.</th>
</tr>
</thead>
<tbody>
<tr>
<td>February 7.</td>
<td>2 Days.</td>
<td>25 Days.</td>
<td>6 Days.</td>
<td>35 Days.</td>
<td>Medium of dry horse manure left in light of the room.</td>
</tr>
<tr>
<td>February 17.</td>
<td>2 Days.</td>
<td>14 Days.</td>
<td>7 Days.</td>
<td>23 Days.</td>
<td>Do.</td>
</tr>
<tr>
<td>February 23.</td>
<td>2 Days.</td>
<td>11 Days.</td>
<td>6 Days.</td>
<td>19 Days.</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>1 Days.</td>
<td>13 Days.</td>
<td>5 Days.</td>
<td>19 Days.</td>
<td>Do.</td>
</tr>
<tr>
<td>April 7</td>
<td>2 Days.</td>
<td>14 Days.</td>
<td>6 Days.</td>
<td>22 Days.</td>
<td>Do.</td>
</tr>
<tr>
<td>June 14</td>
<td>1 Days.</td>
<td>8 Days.</td>
<td>5 Days.</td>
<td>14 Days.</td>
<td>Medium of moist horse manure and corn meal.</td>
</tr>
<tr>
<td>August 10</td>
<td>1 Days.</td>
<td>9 Days.</td>
<td>5 Days.</td>
<td>15.5 Days.</td>
<td>Medium of moist horse manure and brand.</td>
</tr>
<tr>
<td>October 1</td>
<td>1 Days.</td>
<td>7 Days.</td>
<td>5 Days.</td>
<td>12 Days.</td>
<td>Medium of moist guinea-pig manure mixed with chopped guinea grass.</td>
</tr>
<tr>
<td>October 12</td>
<td>1 Days.</td>
<td>9 Days.</td>
<td>5 Days.</td>
<td>15 Days.</td>
<td>Medium of moist horse manure and layers of filter paper.</td>
</tr>
<tr>
<td>October 23</td>
<td>1 Days.</td>
<td>6 Days.</td>
<td>5 Days.</td>
<td>12 Days.</td>
<td>Medium of carabao and horse manure placed in a barrel shaded at all hours.</td>
</tr>
</tbody>
</table>

* These 5 cultures were developed in open jars in an airy closet darkened at all hours.

* From this brood several flies emerged one month after egg laying.
GENERAL CONDITIONS AFFECTING THE PUBLIC HEALTH AND DISEASES PREVALENT IN THE BATANES ISLANDS, P. I.¹

By(David G. Willets

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

The following report is based upon observations made in the Batanes Islands, chiefly in the town of Santo Domingo de Basco, Batan Island, from April 2 to May 7 inclusive, 1912. Much of my information was received from the padres, of whom there are seven in the province, and from the provincial cirujano administrante.²

The Batanes are located about 240 kilometers north of the Island of Luzon in the typhoon belt, and scarcely a year passes without considerable damage being done to homes and live stock for this reason. Several very strong currents about the islands render communication between the various members of the group not infrequently extremely dangerous and at times impossible. Lives are lost almost yearly in these currents.

The population is approximately 8,000, distributed as follows: Batan Island, 5,200; Sabtang Island, 1,300; and Itbayat Island, 1,500. It is said that at one time the total was about 20,000, but that emigration to various parts of Luzon is responsible for the great reduction.

The occupation of the inhabitants is cattle raising; a few devote their time to fishing. Cattle raising is conducted on a small scale, about 1,000 head being sent to the Manila market annually. The average price paid the natives for their cattle is about 25 pesos per head. I am informed that the cattle are paid for frequently with cloth, rope, rice, etc., which are sold

¹ Read before the Manila Medical Society at its June, 1912, meeting.
² A cirujano administrante is a person who has studied medicine in the Santo Tomás University of the city of Manila for a period of not less than two years. He may be registered to practice medicine in remote towns of a province where no civilian doctor of medicine or licenciate of medicine is available. Upon passing a satisfactory examination before the district health officer for the province in which he desires to practice, he pays to its provincial treasurer a fee of 10 pesos for a certificate of registration which shall thereupon be issued to him by the district health officer.
at a shameful percentage above the retail Manila price. The islands, particularly Itbayat, have plenty of pasture land, and cattle raising could be carried on somewhat extensively. Land suitable for garden purposes, however, is scarce and widely distributed over the islands, hence the population must always be a limited one.

The inhabitants have been spoken of as hard working and industrious. My observations do not support this statement. It is true that they must of necessity put in long hours of labor, but this is largely due to the fact that the cultivable land is widely scattered. A great deal of time is, therefore, required to go to and from the fields or rather little garden patches. Having secured something to eat and some clothing, the people seem to be quite content. Day laborers are difficult to secure at any reasonable wage, and the workmen are slow. It is customary for several members of a family to go to the fields in the early morning and return about sunset or later. Some of the families go to the fields on Monday morning returning Saturday afternoon. During the week it is not unusual for them to rest during the day and work at night if there be a moon. Little shacks are put up in the pastures for their protection during these periods.

The homes on Itbayat are made of coral rock foundation, plank floor, and sides and roof of cogon grass. On Batan and Sabtang they are made of coral rock with cogon grass roofs. Rarely a shack is found on these two islands. The better home consists of two parts, a kitchen and a dwelling portion, but in the vast majority of cases there is only one room to serve as kitchen, dwelling-room, and bedroom. Inside of this room a wood fire is made on a few stones and, since the house is unprovided with a chimney, the smoke at times becomes almost unendurable and the dirt is frightful. The great majority of the homes are poorly ventilated, and it is the custom of the natives, as elsewhere in the Philippines, to close their homes as tightly as possible at night. Sudden changes in the temperature are not rare, and there is not sufficient fuel available to keep a continuous fire when it is indicated. Hence the houses are uncomfortably cool and damp at times. The small amount of money at its disposal and the exorbitant prices prohibit a family from having enough clothing for the cooler portions of the year.

Cattle, hogs, goats, and chickens are almost the only possessions of this people and naturally are guarded carefully, especially during the night, when they are kept close to the house. Indeed it is not a rare event to see the family pig or the family
goat inside of the house. There are only twenty-one horses in the province; one on Itbayat and twenty on Batan. Dogs are very numerous. At Sabtang the cattle are kept in corrals at night, because the houses are situated too closely together to permit of their being kept in the dooryard.

A suitable method for the disposal of human excrement is lacking. An effort is now being made to provide water-closets, but as yet they are insufficient for the number of families and furthermore the people have not learned to use them; this applies particularly to Santo Domingo de Basco and Mahatao, Batan Island. On Sabtang and Itbayat Islands, water-closets are very rare.

Each of the islands of the group is poorly supplied with fresh water. Of the six towns only one, namely Mahatao, can boast of a stream. Raile, a barrio of Itbayat, also has a small stream. The other streams of the islands are too far removed from the towns to provide drinking water; these are, however, of importance in furnishing water for cattle. Several springs are to be found on the various islands, but these also are too remote from the towns to furnish drinking water excepting one near the town of Sabtang. Usually rain water is used for drinking purposes. This is collected in earthen jars from the roofs of houses and from trees by means of pieces of split bamboo. The water for miscellaneous purposes is obtained from a limited number of wells and cisterns.

The water jars and cisterns are good places for mosquitoes to breed. On Itbayat there are marshy places which also doubtless serve this purpose. Flies were not very numerous.

Santo Domingo de Basco, Mahatao, and Ibana on Batan Island, and Sabtang on Sabtang Island are each provided with two street cleaners.

From the facts that fresh water is scanty, that the one room serves usually as kitchen, living-room, and bedroom, that domestic animals are kept near the house, and that the natives, excepting the inhabitants of Sabtang, do not care to bathe in sea water, it is clear that these people are not especially noteworthy for their cleanliness.

The diet consists of camotes and fish caught in the Batanes waters and dried; fresh fish is seldom eaten. In addition, a few miscellaneous vegetables are used and meat of one kind or another—beef, pork, goat-meat, or chicken—is eaten on an average of about twice a week. Some eggs are also used. On Itbayat, a rather limited supply of oranges, bananas, pineapples, and coconuts are available in season. Practically the only
pleasure of the people is in drinking *palek*, a native alcoholic beverage made from sugar cane, and in smoking tobacco which is raised for their own consumption.

Basing my opinion upon a rather scanty knowledge of the natives in various parts of the Philippines, I believe the general physical condition of the inhabitants is above the average. This is attributed to their occupation which keeps them in the open a great deal of the time. The women of Ibayat appear to be particularly strong and vigorous. Statistics show that the death rate is about 26 per thousand per annum and that the infant mortality is about 30 per cent. Illegitimate children are very common when one considers that the communities are very small, ranging from about 200 to 2,000. In some of the towns such births make up 30 per cent of the total birth rate. None of these statistics are considered to be reliable. Adult females are more numerous than adult males, the ratio being about 5 to 2 in Santo Domingo de Basco; this is explained by the greater tendency of males to emigrate.

A great deal of the morbidity is connected with the respiratory tract. Due to sudden changes of temperature, dampness in the homes during the rainy season, exposure while fishing or working in the fields by moonlight, and insufficient clothing for the cooler portions of the year, one cold after another is contracted from childhood up so that bronchitis, which is not infrequently associated with spitting of blood but without afternoon temperature, night sweats, or notable loss of weight, is common. This condition, coupled with the fact that homes are habitually closed during the night and that many persons sleep in the same room, creates a favorable soil for tuberculosis. As a matter of fact, "phthisis" is understood by the adult population to be common. A number of cases of this disease were seen. From answers to questions asked, I believe pneumonia claims its victims each year and that pleurisy is not rare.

Careful search for cases of paragonimiasis failed to disclose a single case. Dr. A. G. Sison of the Philippine General Hospital tells me he knows of a case of this disease occurring in a native of the Batanes.

Skin diseases are very common, especially chronic ulcer. Several persons were seen who were incapacitated for work because of the extent to which the ulceration had progressed. Probably not less than 50 persons were seen during my short sojourn in the islands who were suffering from this affliction. The duration of the disease in these cases varied from a few
months to twenty years. Ringworm is common. Two cases of herpes zoster were found. Three patients were suffering from poisoning similar to poison ivy, but in each case the process seemed to be more severe than that caused by poison ivy. The marked frequency and severity of skin disease is attributed largely to the uncleanliness of the inhabitants.

Rheumatism is a rather common disease. Several cases of the chronic form were seen, and the previous history of a number of persons presenting themselves at the clinic suggested the occurrence of this disease.

Bright's disease is also a common affection.

Upon first meeting residents of the Batanes the writer was told of a rapidly fatal fever endemic and at times epidemic on the Island of Itbayat. The disease is said to be more frequent from July to December than in other parts of the year, and it is the consensus of opinion of those best informed relative to its occurrence that it has been less common during recent years than formerly. It is claimed, however, that deaths occur every year from the disease and that the residents of Batan and Sabtang who visit Itbayat are especially apt to contract it. Several persons from these two islands have undoubtedly died of the fever shortly after returning from Itbayat, but the number of such deaths has been greatly exaggerated. Probably not exceeding an average of one person a year from Batan and Sabtang has died from this cause during the past ten years.

Investigation revealed the facts that the disease is characterized not only by fever but also by chills, vomiting, and sweating. Hence malaria was suspected. However, in the examination of a number of persons from Itbayat only one enlarged spleen was found. The blood of this patient, as well as that of a number of others from Itbayat and a few from Batan who claimed to have had the fever within the past two years, was examined for malarial organisms with negative results. The only case in which malarial parasites were found in the blood was in an acute infection contracted by the writer, apparently on Itbayat Island. The parasites were of the aestivo-autumnal type, and the objective symptoms present were recognized by several residents of Santo Domingo de Basco as being identical with those exhibited in typical cases of the Itbayat fever seen previously by them. I became convinced finally that the "fever" so greatly feared by the natives is probably malarial fever of a pernicious form. Further investigation is considered necessary to prove this definitely.
The presence of the malady on the Island of Itbayat has an important bearing on the development of the province. Itbayat is far more fertile than any of the other islands of this group, but the inhabitants of Batan and Sabtang will not move to Itbayat for fear of the fever. Of course, the natives think they have the fever every time they have a temperature from any cause whatsoever. It is thought that benign tertian malaria is present. The term paludismo, malaria, is certainly well understood by the natives, and quinine is a drug with which many families are familiar.

No case was seen suggesting elephantiasis, and the examination of the blood taken at night from 191 adults for the presence of microfilaria resulted negatively.

Despite the fact that dysentery is not infrequently given by the provincial cirujano administrante as the cause of death of both adults and children, a distinct history of dysentery was very rare. No acute case was seen and only 2 chronic ones; each of these was negative for entamoebae. Occasional diarrhoea is common. The stools of 400 inhabitants of Santo Domingo de Basco composed of 100 adults each, and 100 children each, of both sexes, were studied statistically for evidences of intestinal parasitism. Two thin cover-slip preparations were examined of each case. A carthartic was not administered prior to taking the specimens.

The specimens were unfavorable for an examination for the presence of protozoan parasites. Only 5 such infections were found; namely, 1 with monads, 2 with entamoebae, and 2 with Balantidium coli. These findings coupled with the rarity of a history of acute or chronic dysentery argue for the infrequency of entamoebic infections. Two infections with Balantidium coli in such a small number of examinations is unusual and far in excess of the average in other statistical studies in the Philippines. These cases were found in adult males, each of whom had visited Manila for a short period.

Evidence of helminthic infection was readily found in each and every one of the 400 individuals examined. Single infections were present in 46 per cent; double in 42.5 per cent; triple in 11.5 per cent; Ascaris in 92.8 per cent; Trichuris in 46.7 per cent; hookworms in 24.5 per cent; Oxyuris in 1 per cent; and Strongyloides in 0.5 per cent. No cestode infections occurred in the 400 persons examined, but one infection with Taenia saginata was found at Sabtang.

In over 19,000 persons examined in various parts of Luzon by sundry investigators for evidence of intestinal helminthiasis
an average of 85.66 per cent was found to be infected. The highest percentage (95.9) was found by Garrison, Leynes, and Llamas in the study of 1,000 inhabitants of Taytay, Rizal Province. In these studies the average Ascaris, Trichuris, and hookworm infections were 61.36, 40.79, and 30.57 respectively. It is, therefore, clear that the high rate of infection with intestinal worms in the Batanes is due to the great frequency of ascariasis. The results of the present study are given in Tables I and II.

**Table I.**—Frequency of infection with intestinal worms in the 400 persons examined.

<table>
<thead>
<tr>
<th>Sex and age</th>
<th>Examined</th>
<th>Infected</th>
<th>Infections</th>
<th>Single</th>
<th>Double</th>
<th>Triple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children:</td>
<td></td>
<td></td>
<td>100</td>
<td>100</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>Male</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults:</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>400</td>
<td>400</td>
<td>184</td>
<td>170</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td>46.0</td>
<td>42.5</td>
<td>11.5</td>
<td></td>
</tr>
</tbody>
</table>

**Table II.**—Frequency of infection with various parasites in the 400 persons examined.

<table>
<thead>
<tr>
<th>Sex and age</th>
<th>Examined</th>
<th>Ascaris</th>
<th>Trichuris</th>
<th>Hookworm</th>
<th>Oxyuris</th>
<th>Strongyloides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>100</td>
<td>93</td>
<td>50</td>
<td>18</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>100</td>
<td>96</td>
<td>44</td>
<td>14</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Adults:</td>
<td>100</td>
<td>87</td>
<td>42</td>
<td>38</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Male</td>
<td>100</td>
<td>95</td>
<td>51</td>
<td>28</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>400</td>
<td>371</td>
<td>187</td>
<td>98</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Percentage</td>
<td>92.8</td>
<td>46.7</td>
<td>24.5</td>
<td>1.0</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

A part of an epidemic of measles was observed. The infection had been carried to the Batanes by a child who had been in Manila for medical attention in January, 1912. There were numerous cases of measles in Manila at that time. The epidemic was confined to the town of Santo Domingo de Basco.

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3 Willets, *This Journal*, Sec. B (1911), 6, 77.
4 Ibid. (1909), 4, 207.
Thirty-five cases had been reported up to the time I left the islands. Probably many more cases had occurred.

Chicken-pox was present several years ago. No histories were secured suggestive of typhoid fever, diphtheria, or scarlet fever. Cholera was apparently present in 1902. Dengue fever occurs from time to time according to the testimony of the cirujano administrante. Beriberi is apparently absent. No cases suggestive of this disease were seen, and no suspicious histories were obtained. The last case of smallpox seems to have been in 1896. About 2,000 persons were vaccinated while I was in the islands, and sufficient virus to vaccinate 2,000 more was left with the cirujano administrante on May 7. This quantity was sufficient to bring the vaccination up to date. Three cases of insanity were seen, and the history of 4 others secured. No case of yaws was seen.

Since 1906 several lepers have been taken from the Batanes by the Bureau of Health. Some of these were native to the islands, while others were fugitives from northern Luzon. No case of leprosy was found during my investigation.

One apparently typical case of migraine was found. The patient stated that a cousin and an aunt were similarly affected.

One case strongly suggested the occurrence of cerebro spinal meningitis in the islands about four years ago. The patient was a boy 5 years old who had been quite normal until one year of age, when he became acutely ill. His temperature was very high, tremors and convulsions were common; and his grandmother recognized the opisthotonus condition at once and said the child had such a symptom. She also stated that about 10 other children had the same disease about the same time and that they all died. The head, body, and upper extremities of the patient were well developed. The lower extremities were considerably undersized. The patient had never been able to walk, and he was mentally very backward, being able to talk only with great difficulty and his vocabulary was limited.

Venereal disease is rare. Not one case of undoubted clinical syphilis was seen. Some of the old ulcerous cases may have been syphilitic, but a Wassermann reaction would have been necessary to render a positive diagnosis. Four cases of gonorrhea were found, 2 of which were in Filipino cooks who had recently arrived from Manila.

Death claims yearly its victims among the new born and mothers because of the lack of medical attention during par-
turition and the latter part of pregnancy. I have no reason, however, to believe the death rate from this cause to be greater in the Batanes than elsewhere in the Philippines where physicians are unavailable.

Foreign growths were found in several cases. Three cases of cataract, 1 of probable gastric ulcer, and one of probable chronic appendicitis were also seen. From 15 to 20 cases were found which would be benefited by the surgeon's knife.
A STUDY OF THE NORMAL BLOOD OF THE CARABAO

By WILLIAM HUTCHINS BOYNTON

(From the Veterinary Division, Bureau of Agriculture, Manila, P. I.)

The following study was undertaken with the object of ascertaining the normal condition of the circulating blood of the carabao, so that one desiring to make a clinical examination of the blood might have a standard for comparison.

In searching the literature on the subject at my disposal, I have not been able to find any previous work done on the blood of carabao, which circumstance necessitated a systematic study of a large number of healthy animals.

The twenty-five animals used were in apparently normal condition. Their temperatures were taken twice a day, several weeks before the examinations were made. The animals averaged from two and one-half to 6 years of age. Some were work animals used at the laboratory, and had been immunized to rinderpest from six months to two years previous to the time of examination. The majority were susceptible to rinderpest and were kept at the laboratory for experimental purposes.

The blood was obtained in all cases from the ear. The part was first thoroughly cleaned with water, then dried with alcohol, and one of the small veins on the outer side of the lobe was pricked with a sharp-pointed scalpel.

The red corpuscles were counted by means of Thoma's haematocytometer, using Toisson's diluting fluid. The corpuscles in 100 squares in each of 2 slides were counted. If these counts did not agree closely, a third preparation was made, and the results of the three were averaged. The leucocytes were counted in the same preparation as the red cells, the counting chamber used having the Zappert-Ewing ruling. The percentage of haemoglobin was obtained by means of the Tallquist haemoglobin scale, as no other apparatus was available. The specific gravity was obtained by Hammerschlag's method. The time of coagulation was obtained by Wright's method. The relative volume of corpuscles and of plasma was obtained by the haematocrit as modified by Daland. This was placed in a centrifuge, and revolved at a speed of approximately 3,000 revolutions per minute for three minutes. Films were made on glass slides, and fixed

1 Reprinted from Bulletin 21, Bureau of Agriculture of the Government of the Philippine Islands.
2 Archibald R. Ward, chief.
with heat, in pure methyl alcohol or in equal parts of absolute alcohol and ether. The slides were stained with eosin and methylene blue, Ehrlich's triacid mixture, Jenner's stain, and Wright's stain. Jenner's stain was used in making the differential counts, as it is easily handled, and gives very accurate results. The size of the corpuscles was obtained with a Zeiss ocular screw micrometer, using stained films, and taking those parts of the film in which the cells were not crowded.

The technique used in staining the preparation was as follows:

When Jenner's stain was used, the film was dried in air and flooded with the dye, which was left to act for from two to three minutes. It was then washed from ten to fifteen seconds in distilled water, and dried as rapidly as possible in air. When well stained and washed, the red cells had a terra-cotta color.

With Wright's stain, the film was dried in air, and flooded with the dye which was allowed to act for one minute. Distilled water was then added, drop by drop, until a film of metallic luster began to form on the surface. This was allowed to act from two to three minutes longer. The preparation was then washed for ten seconds in distilled water, and dried in air as rapidly as possible. With this stain, the red corpuscles took a pinkish color.

With eosin and methylene blue, the film was previously fixed, either with heat or by submerging in pure methyl alcohol for ten minutes, or in equal parts of absolute alcohol and ether for ten minutes. Then it was flooded with saturated alcoholic solution of Ehrlich's blood eosin for about ten seconds and washed in water. After this it was flooded with a saturated aqueous solution of Grubler's methylene blue for one minute, washed quickly with distilled water, and dried in the air.

With Ehrlich's triacid mixture, the film was previously fixed by placing the slides, film side down, upon a heated copper tray, and held at a temperature just below boiling for fifteen minutes. The film was then covered with the mixture and allowed to act ten minutes, after which it was washed hastily in distilled water and dried rapidly in air.

The red corpuscles in the fresh condition appear as biconcave disks of a homogeneous appearance, yellowish in color, and with a nearly translucent central area and have the general appearance of human blood. With eosin and methylene blue and with Wright's stain, the red corpuscles take a pinkish stain, with Jenner's they are terra-cotta color, and with Ehrlich's triacid mixture they have an orange tint.

Five varieties of leucocytes were noticed in the circulating
blood, which correspond very closely to those found in cattle. Following the widely used classification they are (1) lymphocyte, (2) large mononuclear, (3) polynuclear, (4) eosinophile, and (5) mast cell.

Lymphocytes include cells averaging 7.3 microns in diameter, being a little larger than the red corpuscles, and having a nucleus occupying a greater part of the cell body. The nucleus is usually round, but may show a notch on one side. The cell body shows as a narrow rim around the nucleus. Both nucleus and cell body are coarsely reticular. The cell body has a strong affinity for basic stains, and the nodal points in the reticulum take a deeper stain. With Jenner’s and Wright’s stains, a few small purplish granules are frequently seen in the cell body. With eosin and methylene blue, both nucleus and cell body take a deep blue stain, often the cell body staining deeper than the nucleus. With Ehrlich’s triacid mixture, the nucleus takes a greenish color, the cell body has a purplish tinge, and appears homogeneous. This variety of cell is nongranular, except for the few purplish granules numbering from 1 to 5 frequently seen, as has been previously mentioned.

Large mononuclear leucocytes include cells which are considerably larger than the lymphocytes, averaging 10.8 microns in diameter. The nucleus occupies from one-half to two-thirds of the cell, and is situated at one side of the center. It is either oval or kidney-shaped. Both nucleus and cell body are finely reticular, and stain less deeply than do those of the lymphocytes. Frequently small clear areas or vacuoles are seen in the nucleus, giving it the appearance of undergoing degeneration. With Ehrlich’s triacid mixture, the nucleus takes a greenish tinge, and the cell body has a very faint pinkish tint. With eosin and methylene blue, the nucleus is light blue and the cell body is distinctly blue, but neither nucleus nor cell body takes such a deep stain as the lymphocytes. With Jenner’s stain, both nucleus and cell body are stained blue, but not so strongly as in the case of the lymphocytes. With Wright’s stain, the nucleus is dark violet, and the cell body pale blue.

Transitional forms of the large mononuclears were noticed frequently. In these cells the nucleus is saddlebag-shaped. The staining properties are similar to those already described for the large mononuclears.

In polynuclear leucocytes the nucleus takes on various shapes. It may be S-shaped, W-shaped, Z-shaped, coiled or lobulated, and is coarsely reticular. In appearance they are very similar to those in human blood. The cell body remains practically un-
stained, but contains many fine granules, which are so small that they appear as mere points, and show a rather weak affinity for acid stains. The granules are more numerous than the coarser granules in the eosinophiles. These cells average 9.4 microns in diameter, or nearly twice the diameter of the red corpuscles.

With Jenner's stain, the nucleus is blue and the granules are a bright pink. With Wright's stain, the nucleus has a dark violet color and the granules are distinctly pink. With Ehrlich's triacid mixture, the nucleus takes a pale greenish color, and the granules a pinkish violet. With eosin and methylene blue the appearance is similar to that described for Jenner's stain.

In eosinophile leucocytes the nucleus is very similar to that of the polynuclears, usually being bilobed or trilobed. As a rule the nucleus takes the basic stains readily, the lobes being coarsely reticular. The cell body contains many oval granules, which are much coarser than the granules in the polynuclears, and are strongly acidophile. These cells are a little larger than the polynuclears, averaging 10.9 microns in diameter.

With eosin and methylene blue, the nucleus is stained blue and is coarsely reticular. The granules are a bright pinkish red, taking the eosin stain. With Jenner's and Wright's stains, practically the same appearance is presented as with eosin and methylene blue. With Ehrlich's triacid mixture, the nucleus takes a very faint green appearance, while the granules take a deep copper color.

In mast cells the nuclei are similar in shape to those of the polynuclear leucocytes and eosinophiles, but the nucleus takes the stain so faintly that it is often difficult to determine the shape. The cell body remains practically unstained, but contains many coarse granules, which have a strong affinity for basic stains. The granules are either spherical or slightly oblong in shape, and are practically the same size as the granules in the eosinophile cells.

With eosin and methylene blue, both the nucleus and granules are stained blue. The granules take a much deeper stain than the nucleus. With Jenner's stain the granules are deep violet, and with Wright's stain they are deep purple, the nucleus being very faintly stained. With Ehrlich's triacid mixture, the nucleus is stained a very light green, while the granules are stained a blackish green color.

Table I gives the results of the measurements of 100 cells of each variety except the mast cells in which 26 were measured. These measurements were made from the blood of 10 different animals.
**Table I.—Measurements of blood cells of the carabao.**

<table>
<thead>
<tr>
<th>Kind of cell</th>
<th>Average size</th>
<th>Maximum size</th>
<th>Minimum size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microns</td>
<td>Microns</td>
<td>Microns</td>
</tr>
<tr>
<td>Red cell</td>
<td>5.6</td>
<td>7.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>7.8</td>
<td>11.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Large mononuclear</td>
<td>9.4</td>
<td>9.6</td>
<td>9.1</td>
</tr>
<tr>
<td>Polynuclear leucocyte</td>
<td>10.9</td>
<td>11.5</td>
<td>10.3</td>
</tr>
<tr>
<td>Eosinophile</td>
<td>7.8</td>
<td>7.9</td>
<td>7.6</td>
</tr>
<tr>
<td>Mast cell</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table II contains the results of numerical determinations of the various cellular constituents of the blood, together with other data regarding properties of the blood.

**Table II.—The results of examinations of the blood of 25 supposedly normal carabao.**

<table>
<thead>
<tr>
<th>No. of carabao</th>
<th>Sex</th>
<th>Red corpuscles per cubic millimeters</th>
<th>Leucocytes per cubic millimeters</th>
<th>Hæmoglobin</th>
<th>Specific gravity</th>
<th>Relative volume of corpuscles and of plasma</th>
<th>Time of coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per cent.</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>5,696,000</td>
<td>8,000</td>
<td></td>
<td>90</td>
<td>1.052</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>6,400,000</td>
<td>8,000</td>
<td></td>
<td>95</td>
<td>1.052</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
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<td>6,000</td>
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<tr>
<td>6</td>
<td>M</td>
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<td>9,500</td>
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<td>85</td>
<td>1.052</td>
<td>26</td>
</tr>
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<td>7</td>
<td>M</td>
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<td>M</td>
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<td>18,500</td>
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<td>Average</td>
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<td>92.6</td>
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<td>29.1</td>
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<tr>
<td>Maximum</td>
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<td>100</td>
<td>1.056</td>
<td>43</td>
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<td>85</td>
<td>1.052</td>
<td>25</td>
<td>57</td>
</tr>
</tbody>
</table>

M. a.
It will be noticed from Table II that the highest number of red cells in the counts was 7,000,000 per cubic millimeter. The lowest number was 5,228,000 per cubic millimeter, and the average for the 25 examinations was 6,057,520 cells per cubic millimeter.

The lowest haemoglobin percentage was 85, the highest percentage 100, and the average for the 25 examinations was 92.6 per cent.

The highest number of leucocytes in the counts was 18,500 per cubic millimeter, and the lowest number 6,000 per cubic millimeter. The average for the 25 examinations was 10,389 per cubic millimeter.

The highest specific gravity was 1.056, the lowest 1.052, and the average for the 12 examinations was 1.0532.

In reference to the relative volumes of corpuscles and of plasma, the highest percentage of corpuscles was 43, and the highest percentage of plasma was 75. The lowest percentage of corpuscles was 25, and of plasma 57. The average for 10 examinations gave 29.1 per cent of corpuscles and 70.9 per cent of plasma.

In working out the time of coagulation of the blood, it was found that three minutes and twenty-five seconds was the longest time and three minutes and five seconds the shortest time for coagulation to take place. The average for 8 examinations was three minutes and sixteen seconds plus.

From the table it will be noticed that No. 8 has the highest red-cell count, the highest leucocyte count, 100 per cent haemoglobin, the highest specific gravity, the largest volume of corpuscles to the smallest volume of plasma, and it took the shortest time for the blood to coagulate.

Table III gives a detailed study of the leucocytes. The number per cubic millimeter and the percentage of each are given. These are based on counts of from 800 to 1,200 cells in each case.

The lymphocytes vary from 10,725 to 2,280 per cubic millimeter, and from 71.5 to 36.9 per cent.

The large mononuclears vary from 1,136 to 240 per cubic millimeter, and from 3 to 10.2 per cent.

The polynuclears vary from 1,648 to 6,290 per cubic millimeter, and from 20.6 to 51.9 per cent.

The eosinophiles vary from 0 to 2.522 per cent. Animal 12 showed no eosinophiles in a count of 872 cells. The percentage of these cells varied between 0 to 23.7.
Table III.—Differential counts of leucocytes in carabao blood.

<table>
<thead>
<tr>
<th>No.</th>
<th>Leucocytes</th>
<th>Lymphocytes</th>
<th>Large mononuclears</th>
<th>Polynuclears</th>
<th>Bosinophils</th>
<th>Mast cells</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Number.</td>
<td>PER.</td>
<td>Number.</td>
<td>PER.</td>
<td>Number.</td>
<td>PER.</td>
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<td>3,424</td>
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<td>5,056</td>
<td>516</td>
<td>10.2</td>
<td>1,468</td>
<td>20.6</td>
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<tr>
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<td>6,000</td>
<td>2,880</td>
<td>232</td>
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<td>1,740</td>
<td>23.0</td>
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<tr>
<td>4</td>
<td>8,000</td>
<td>3,588</td>
<td>210</td>
<td>3.0</td>
<td>2,240</td>
<td>28.0</td>
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<tr>
<td>5</td>
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<td>468</td>
<td>3.9</td>
<td>6,228</td>
<td>61.9</td>
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<tr>
<td>6</td>
<td>9,500</td>
<td>4,370</td>
<td>466</td>
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<td>3,029</td>
<td>38.2</td>
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<td>3,429</td>
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<td>5,019</td>
<td>464</td>
<td>4.6</td>
<td>3,599</td>
<td>34.5</td>
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</table>

Average: 10,389, 5,019, 464, 4.6, 3,599, 34.5

Maximum: 15,500, 10,725, 735, 4.9, 3,375, 22.5

Minimum: 6,000, 2,880, 256, 3.2, 3,520, 44.0

The mast cells varied from 0 to 304 per cubic millimeter, and from 0 to 3.8 per cent. In animal 9 no mast cells were found in a count of 920 cells.

In averaging the numbers of each kind of cell when fractions of cells were encountered, every fraction below 0.5 was dropped, and each fraction over 0.5 was counted as 1.

**Summary**

1. In the circulating blood of supposedly normal carabaoos over 2 years old the red corpuscles were found to average 6,057,520 per cubic millimeter.

2. The average percentage of hæmoglobin was 92.6.
3. The average number of leucocytes was 10.389 per cubic millimeter.
4. The average specific gravity found was 1.0532.
5. The relative volume of corpuscles to plasma was found to be 29.1 per cent of corpuscles to 70.9 per cent of plasma.
6. The average time for complete coagulation of the blood was found to be three minutes and sixteen seconds plus.
7. The following five varieties of leucocytes were found in the peripheral blood:
   (a) Lymphocytes. Average size, 7.3 microns in diameter; average number per cubic millimeter, 5.049. They comprise 48.5 per cent of all leucocytes.
   (b) Large mononuclears. Average diameter, 10.8 microns; average number per cubic millimeter, 484. They comprise 4.6 per cent of all leucocytes.
   (c) Polynuclears. Average diameter, 9.4 microns. Average number, 3,598 per cubic millimeter. They comprise 34.5 per cent of all leucocytes.
   (d) Eosinophiles. Average diameter, 10.9 microns. Average number, 1,142 per cubic millimeter. They comprise 11.5 per cent of all leucocytes.
   (e) Mast cells. Average diameter, 7.8 microns. Average number, 115 per cubic millimeter. They comprise 1.2 per cent of all leucocytes.
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By W. E. Musgrave and A. G. Sison

PATHOLOGICAL REPORT
By B. C. Crowell

(From the Departments of Medicine and Pathology, College of Medicine and Surgery, University of the Philippines, Manila, P. I.)

Eleven plates and 5 skiagrams

Since the publication of our preliminary article on the bone lesions of smallpox,² we have had the opportunity to study several other cases of which 12 are briefly reported here. Skiagrams were taken of the affected bones and joints; and in one case post-mortem examination was made by Dr. B. C. Crowell ³ and the findings are included as a part of this report.

DISTRIBUTION OF THE LESIONS

In the preliminary report we stated that “One of the most striking features of the deformities is the constant location of the lesions in the upper extremities.” Further study has shown this statement to be an error. Any of the bones and joints of the body may be affected. However, the long bones and their articulations are more fre-

¹ Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4-7, 1912.
² This Journal, Sec. B (1910), 5, 553.
³ Chief, department of pathology and bacteriology, College of Medicine and Surgery, University of the Philippines.
quently affected than are other bones and joints. In the order of frequency, the following locations of the lesions may be noted:

(1) The bones and joints of the upper extremity.
(2) The bones and joints of the lower extremity.
(3) The bones and joints of the other parts of the body.

The bones of the upper extremity may be involved in the following order of frequency:

(a) Forearm (radius and ulna).
(b) Arm (humerus).
(c) Hand (carpal, metacarpal, and phalanges—proximal and distal).

The bones of the lower extremity may be also involved in the following order:

(a) Leg (tibia and fibula).
(b) Thigh (femur).
(c) Foot (tarsal, metatarsal, and phalanges—proximal and distal).

In regard to age, the bone lesions usually are observed in persons with a history of having had smallpox during early childhood, but the deformity may follow variola contracted at any time before the complete ossification of the bones. The oldest age in which such a complication of variola has been observed occurred in a boy 14 years old.

DISCUSSION

The fact that practically no mention of bone-deforming lesions as a complication of variola is found in current literature should make one very cautious not to interpret coincidence as cause and effect. The fact that the masses of the Filipino people are undernourished and underdeveloped as a result of starvation and metabolism incongruities adds to the difficulty of definite conclusions in interpreting the etiology of chronic bone-deforming lesions contracted during childhood.

Both scurvy and rickets are much less frequently encountered in Manila than would be expected from the habits and faulty feeding customs of the people, and these diseases are less frequent in our large clinics than is reported from similar clinics in other parts of the world.

The pathology of these disturbances, their mode of onset, and the course and results are now pretty well known and do not correspond to the conditions found in our smallpox cases sufficiently close to make the danger of mistake in diagnosis a serious consideration.
The close resemblance in the character of the deformities, their constant association with a history of smallpox during early life, and the absence of similar lesions under other conditions seem to warrant the conclusions that the deformities are a complication of variola.

NATURE OF THE LESIONS

The morbid anatomy and histology of the lesions and deformities have been studied in one case by Crowell. (Case III.)

In our preliminary report we stated that:

The process appears to be due to destructive lesions in the epiphyses of the bones. The shafts of the ulna and radius seem to be normal, except in length. The ends of the bones are enlarged and irregular in shape and similar changes may be encountered in the carpal, metacarpal and phalangeal bones.

The circumferential growth of these bones is not disturbed in the least. There is no sign of underdevelopment in diameter thus proving that the periosteum upon which the circumferential growth depends is not affected.

The bones are markedly shortened and stunted in longitudinal growth, in some cases they are reduced to less than one half the length of the normal bone. The obvious conclusion from this fact is that the seat of the primary lesion is in that part of the bone between the epiphysis and diaphysis which develops actively "ex utero."

Further study of the additional cases reported here with the additional information secured by one autopsy as well as that afforded by Roentgen records apparently confirms the statements made in the preliminary report.

CASE RECORDS

Case I (file and record No. 6992, fiscal year 1912).—V. P., female, Filipina, 27 years old, seamstress, born in Malabon.

She had smallpox during childhood, and as a complication she developed deformity of the left elbow joint, with partial ankylosis and shortening of the bones of the left arm and forearm. (Plate I.)

Case II (file and record No. 5368, fiscal year 1912).—P. B., male, Filipino, born in Mindoro. Had smallpox when a little boy and as a complication he developed deformity of both elbow joints with marked shortening of the bones of the forearms and slightly of the arms, with partial ankylosis of both elbow and wrist joints. Deformity also of both knee joints and some shortening of the bones of both legs. (Plate II and Skiagrams I, II, and III.)

Case III (file and record No. 2464, fiscal year 1912).—A. A., female, single, washerwoman, age 32; born in Silang, Cavite. Had smallpox when a little girl with subsequent deformity of the elbow joints and left wrist, with distinct shortening of the bones of forearms on both sides. Deformity of both knee joints and distinct shortening of the bones of both legs exist. There is a partial ankylosis of the elbow joints. (Plate III, figs. 1 and 2; Plate IV, figs. 1 and 2.)
This patient died March 22, 1912.

Clinical diagnosis: Variola bone lesion; dysentery, acute, bacillary; colitis, chronic, tuberculous; tuberculosis, pulmonary. 

Autopsy was performed by Crowell, who reports his findings as follows:

Anatomic diagnosis: Pneumonia, lobar, left lower lobe; tuberculosis, chronic pulmonary; hydrothorax, left; pleurisy, chronic obliteratorive, double; colitis, acute ulcerative; fatty degeneration of liver; pachymeningitis hæmorrhagica interna acuta; adenoma of thyroid, left lobe; dental caries; deformity of long bones.

Body is that of an emaciated, adult Filipina of short stature, being 132 centimeters in length and weighing 21.80 kilograms. Anterior upper teeth are missing, and the two lateral incisors are loose. Inferior molars are carious. Joints are enlarged and flat. The epiphyses of the long bones are enlarged as are their condyles. Bones of the arms and legs are shortened and bent outward. Apparently there is a partial dislocation of the wrist and knee joints. Posterior aspects of both knees are flat. Costal cartilages are curved backward and inward, pulling the sternum backward, so that the chest is flat and shows a slight depression on its anterior midportion. Scars of smallpox are on the body, being especially numerous over the face. Legs pit on pressure. Rigor mortis is marked. Abdomen is depressed and scaphoid. Panniculus adiposus is scant. Tissues are rather dry.

Peritoneum is smooth. Intestines are contracted. Diaphragm reaches the lower border of the fourth rib on the right and fifth on the left.

Thorax.—Left pleural cavity contains about 60 cubic centimeters of yellowish clear fluid. Left lung is adherent posteriorly. Its lower lobe is consolidated with grayish, firm areas over its surface. In the upper lobe there are 3 old nodules of tuberculosis as large as beans. Section through the lower lobe shows a grayish, uniformly firm surface. On pressure a grayish exudate is expressed. Right lung is also adherent to the chest wall, and shows about 7 tuberculous nodules similar to those of the upper left lobe. Bronchi contain grayish exudate. Bronchial glands are intact.

Pericardium is smooth and shining, and contains a normal amount of fluid. Heart is apparently normal in size and consistence. Its chambers contain post-mortem clot. Valves are thin and delicate. Musculature is firm and brownish in color. Aorta measures at the base 5.4 centimeters, at the isthmus 4.4 centimeters, at the coeliac axis 4 centimeters, at the bifurcation 2.6 centimeters. A few calcareous patches are present on the intima of the coronary arteries. Four or five fibrous areas are present on the base of the aorta. Heart weighs 135 grams.

Spleen is moderately firm, apparently normal; weighs 43 grams.

Kidneys apparently normal; weight, 150 grams.

Liver shows extensive fatty degeneration; weighs 654 grams.

Gall bladder and bile ducts are free.

Stomach contains a whitish, clear, viscid fluid. Its mucosa is intact.

Duodenum shows lymphoid tissue very clearly, otherwise normal.
Pancreas is very firm, and cuts with increased difficulty; section shows slight fibrosis.

Adrenals are small; cortex is yellow; medulla dark.

Intestine.—The colon is ulcerated and slightly hæmorrhagic, especially near the rectum. The ulcers are extensive, superficial, not undermined, not ragged. Close to the cæcum are a few small hæmorrhagic, not undermined ulcers. No parasites are found. Peyer’s patches and solitary follicles are not prominent. Mesenteric nodes and retroperitoneal nodes are not enlarged.

Urinary bladder contains a small amount of turbid urine. Its mucosa is intact.

Generative organs are apparently normal.

Throat organs.—In the left lobe of the thyroid gland is a well encapsulated, thoroughly enucleable, yellowish colored tumor as large as a walnut. Section shows a yellowish, thick substance contained in the small spaces of the parenchyma of the growth.

Brain.—Between the brain and dura mater is a considerable blood clot. The inner surface of the dura is hæmorrhagic, showing threads of fibrin and masses of clot. The convolutions are prominent, opaque, and there is clotted blood in the fissures. This does not extend to the brain substance. There is an increase of intraventricular fluid. Brain is somewhat softer than normal.

Bones.—The elbow joints are the seat of marked deformities, and they are formed by the normal number of bones: the lower extremity of the humerus on the upper part and the upper ends of the radius and ulna on the lower part. The shafts of these bones are more or less bent on their long axes.

Humerus.—The lower end of this bone shows the following anatomical peculiarities: the capitulum or lateral epicondyle is enlarged; radial depression is very shallow or rather absent; coronoid depression is, however, about normal in size; trochlear ridge is more prominent than normal; median epicondyle is smaller than the lateral; on the outer wall of the olecranon depression is seen a flat oval facet which provides articulation for a cartilaginous process that projects from the outer aspect of the olecranon process. This additional structure determines an incomplete adjustment between the olecranon process and its corresponding depression. The olecranon depression measures 2 by 1.7 centimeters in diameter. Trochlea and radial head measure 2.3 centimeters and 1.6 centimeters in width respectively. The distance between the inner and outer condyles is 5.2 centimeters. The articular surface of the bone is rather thickened.

Ulna.—The upper extremity of this bone presents more remarkable anomalies. The top of the olecranon process is very prominent, convex everywhere rather than rectangular in shape; on the outer aspect of the head is a groove 0.5 centimeter in its transverse diameter, extending from the external side of the olecranon process downward and slightly forward along the outer border of the greater sigmoid cavity to be lost in the broadened bicipital hollow. Just behind this groove is a process with a facet on its anterior aspect, semicircular in shape, and directed forward and outward, its greatest diameter being 8 millimeters in length. The lesser sigmoid cavity is entirely absent. In front, the elongated tongue-like coronoid process projects forward, then upward, describing superiorly and inferiorly a regular curved line. Only the projecting portion of this process measures 2.5 by 2 centimeters in its greatest dimensions. The
inner border of the great sigmoid cavity is more prominent than normal. The great sigmoid cavity itself is broad and transversely divided by a narrow, shallow depression, the normal longitudinal ridge being absent. The tubercle below the coronoid process on the anterior surface of the bone is missing. The olecranon process measures 2.6 centimeters in height, while the coronoid process measures 2.7 centimeters in its anteroposterior diameter.

**Radius.**—Not less remarkable changes are present in the upper end of this bone. On its anterior surface is a wide, slightly concave, rather quadrilateral facet which articulates with the capitulum of the humerus. This facet measures 1.7 by 1.5 centimeters. On the inner side of the upper end is another semicircular facet which extends from the top of the extremity downward and is directed inward and backward for the articulation with the equally semilunar articular surface located at the outer side of the head of the ulna. This facet measures 1.1 centimeters in its greatest diameter. Back of the upper end of the bone there are more or less irregular projections, the edges of which form an obtuse angle facing downward and inward. The external side of the head is represented by the prominent border of the quadrilateral facet. The neck is more slender than normal and anteriorly flattened, the tuberosity beyond being more prominent than usual.

**Knee joint.**—In the deformity of this joint the tibia and the fibula are concerned, the femur being apparently well shaped.

The femur bears the normal anatomical characteristics, the only exception is the thickness of the articular surfaces of both condyles which appear to be visibly chondroid. The inner condyle measures 5.8 by 2 centimeters. The outer condyle measures 6 by 2.5 centimeters.

Distance between tuberosities is 7 centimeters.
Distance between inner tuberosity and internal border of outer condyle, 3 centimeters.
Distance between outer tuberosity and external border of outer condyle, 2.5 centimeters.
Intercondylar notch, 2.6 centimeters in width and 2.5 centimeters in depth.

The tibia is the most deformed of the three bones. The condyles of the upper end of the bone are not on the same level, the median condyle being much lower than the lateral. The articular surface of the median condyle is more concave from before backward and from side to side than normally; its edge is rounded anteriorly; the lateral condyle is flat and smaller than the median one; its articular surface is moderately convex from backward and from side to side; at the mid-line the articular surfaces of both condyles fuse together to form a very slight elevation which represents the spine.

Both condyles are markedly inclined backward, overhanging the shaft of the bone so that a prominent projection is formed protruding backward for a distance of 1.5 centimeters. Located inferiorly and laterally is an irregular semicircular facet on the edge of the median condyle to be articulated with a similar facet on the head of the fibula. In front is a transverse, oval tubercle. On both sides, the condyles overhang the shaft. The articular surface measure 6.5 by 4.5 centimeters. Distance between the tuberosities is 7.2 centimeters. The whole articular surface is also apparently cartilaginous.
The head of the fibula is considerably changed in its external architecture. The summit of it is convex externally from before backward and concave exteriorly from side to side. Located anterolaterally is an articular surface which provides articulation for the lateral condyle of the tibia. The inner side of the head is represented by a vertical ridge, terminating below in a sharply pointed process 0.5 centimeter in length. Anteriorly is a convex, slightly elevated surface partially overhanging the shaft of the bone. Posteriorly is a straight ridge which extends from the styloid process obliquely outward. Externally is a tuberosity formed by the union of the posterior ridge and the anterior rounded edge or convex portion of the head. This tuberosity is very prominent, rounded, smooth, and visibly chondroid in consistence.

Summarizing the conditions in the bones above described, it is apparent that the diaphyses of the bones are shorter than normal and that the ends of the bones, representing the original epiphyses, are much altered in configuration. In the shafts of the bones which were removed for examination there is no apparent change from normal in contour or diameter. Mesial longitudinal section through the ends of the bones shows complete ossification of the epiphyseal extremities, and no indication of the line of junction between the epiphyses and diaphyses is present. The deformities of the epiphyses must, therefore, have occurred before the period of full growth, and the interference with the longitudinal growth of the diaphyses must have been due to some disturbance at the line of junction with the epiphyses.

As is to be expected from the above description of the condition of the bones, examined at a period after the cessation of active disease and after healing has taken place, the microscopic examination of the bones shows nothing which will further elucidate the nature of the active process. Complete ossification has taken place, and decalcified sections of the bones show no alterations from the normal condition of bone growth at this period of life. The epiphyseal line of growth is obliterated, and the compact bone and marrow bear normal relations. The only alteration is in the size and conformation of the epiphyses.

Case IV (file and record No. 1367, fiscal year 1912).—J. M., male, Filipino, single, carpenter, 23 years old. Had smallpox when a little child, as a result of which he developed deformity of the left knee, with partial ankylosis and some shortening of the femur. There also is deformity of the toes of the left foot, with shortening of the phalanges, and of both elbow joints with partial ankylosis and shortening of the bones of forearms on both sides. (Plate V, figs. 1 and 2.)

Case V (file and record No. 3611, fiscal year 1912).—C. E., female, Filipina, 29 years old, born in Manila.
Had smallpox when a little child, as a result of which she developed deformity of both knee and elbow joints, with slight ankylosis and some shortening of the bones of legs and forearms. (Plate VI, figs. 1 and 2.)

*Case VI* (file and record No. 2187, fiscal year 1912).—J. F., male, Filipino, about 27 years old, water carrier, born in Manila.

Had smallpox when a little child, as a result of which he developed deformity of both elbow joints and wrist and finger joints, right hand, with partial ankylosis of corresponding joints and shortening of the right humerus and some of the carpal and phalangeal bones of the right hand. (Plate VII, fig. 1.)

*Case VII* (file and record No. 2594, fiscal year 1912).—J. A., male, Filipino, 18 years old, student, born in Iloilo.

Had smallpox at the age of 15 and developed as a complication deformity of both elbow and knee joints of both sides, with very little ankylosis and some shortening of the bones of the arms and forearms. (Plate VII, fig. 2; Plate VIII, figs. 1 and 2; and Skiagram IV.)

*Case VIII* (file and record No. 2967, fiscal year 1912).—J. M., male, Filipino, laborer, 40 years old.

Had smallpox when a child, as a result of which he developed deformity of both elbow joints, with partial ankylosis, and shortening of the bones of arms and forearms. (Plate IX, figs. 1 and 2.)

*Case IX* (file and record No. 4741, fiscal year 1912).—I. G., female, Filipina, housekeeper, 54 years old, born in Gapan, Nueva Ecija.

Had smallpox when a little child complicated by arthrites and subsequent deformity of both wrist joints, with partial ankylosis and shortening of some of the carpal bones and phalanges of the right hand. (Plate X, fig. 1.)

*Case X* (file and record No. 5050, fiscal year 1912).—S. C., male, Filipino, laborer, 26 years old, born in Tondo, Manila.

Had smallpox when a little boy, followed by deformity of both wrist joints with partial ankylosis, but no apparent shortening of the bones, nor diminution in diameter except malformation in the articular and in the elbow joints, especially of the bones of forearms. (Plate X, fig. 2.)

*Case XI* (out-patient service).—A. H., male, Filipino, 35 years old, laborer, born in Taal, Batangas.

Had smallpox at the age of 2, as a result of which he developed ankylosis of temporomaxillary joints with deformity of the left ramus of the lower mandible, consisting of diminution in length and certain irregularities along its anterior surface. There is complete ankylosis of the left elbow joint and deformity of the lower end of the humerus, with shortening of this bone.

There also is shortening of the bones of the forearms. (Plate XI, figs. 1 and 2.)

The biceps, triceps, brachialis anticus, and deltoid muscles are atrophied. An interesting feature of the case is the presence of a slit-like opening between the left incisors through which the patient takes food, it being impossible for him to open his mouth because of the ankylosis of the jaw.

*Case XII* (out-patient service).—V. P., male, Filipino. Had smallpox when a child, and as a complication he developed deformity of the wrist and elbow joints in the right arm, with shortening of the bones of the forearms. (Skiagram V.)
ILLUSTRATIONS

Plate I
Case I, showing deformity of the left elbow joint, with partial ankylosis and shortening of the bones of left arm and forearm.

Plate II
Case II, showing deformity of both elbow joints with marked shortening of the bones of the forearms and slightly of the arms, with partial ankylosis of both elbow and wrist joints. Deformity also of both knee joints and some shortening of the bones of both legs.

Plate III
Case III, showing deformity of the elbow joints and left wrist, with distinct shortening of the bones of forearms. There is a partial ankylosis of the elbow joints.

Plate IV
Case III, showing deformity of both knee joints and distinct shortening of the bones of both legs.

Plate V
Case IV, showing deformity of the left knee, with partial ankylosis and some shortening of the femur. There also is deformity of the toes of the left foot, with shortening of the phalanges, and of both elbow joints with partial ankylosis and shortening of the bones of the forearms.

Plate VI
Case V, showing deformity of both knee and elbow joints, with slight ankylosis and some shortening of the bones of legs and forearms.

Plate VII
Fig. 1. Case VI, showing deformity of both elbow joints and wrist and finger joints, right hand, with partial ankylosis of corresponding joints and shortening of the right humerus and some of the carpal and phalangeal bones of the right hand.
2. Case VII, showing deformity of both elbow and knee joints of both sides, with very little ankylosis and some shortening of the bones of the arms and forearms.

Plate VIII
Same case as Plate VII, fig. 2.
Case VIII, showing deformity of both elbow joints, with partial ankylosis and shortening of the bones of arms and forearms.

Plate X

Fig. 1. Case IX, showing complication by arthritis and subsequent deformity of both wrist joints, with partial ankylosis and shortening of some of the carpal bones and phalanges of the right hand.

2. Case X, showing deformity of both wrist joints with partial ankylosis, but no apparent shortening of the bones, nor diminution in diameter except malformation in the articular and in the elbow joints, especially of the bones of forearms.

Plate XI

Case XI, showing ankylosis of temporomaxillary joints with deformity of the left ramus of the lower mandible, consisting of diminution in length and certain irregularities along its anterior surface. There is complete ankylosis of the left elbow joints and deformity of the lower end of the humerus, with shortening of this bone.

Skiagrams I-III

(See Plate II.)

Skiagram IV

(See Plate VIII.)

Skiagram V

Case XII, showing deformity of the wrist and elbow joints in the right arm, with shortening of the bones of the forearms.
PLATE I. CASE I. DEFORMITY OF LEFT ARM AND FOREARM.
PLATE III. CASE III. DEFORMITY OF UPPER EXTREMITIES.
Fig. 1. Case IV. Deformities of upper extremities.

Fig. 2. Case IV. Deformities of left leg and foot.

PLATE V.
Fig. 1. Case V. Deformity of upper extremities.

Fig. 2. Case V. Deformity of lower extremities.

PLATE VI.
Fig. 1. Case VI. Deformity of upper extremities.

Fig. 2. Case VII. Deformity of upper extremities.

PLATE VII.
Fig. 1.  PLATE IX. CASE VIII. DEFORMITY OF UPPER EXTREMITIES.
Fig. 1. Case IX. Deformity of both wrist joints, and bones of right hand.

Fig. 2. Case X. Deformity of upper extremities.

PLATE X.
Fig. 1. Case XI. Deformity of facial bones and bones and joints of left arm.

Fig. 2. Case XI. Deformity of left arm.

PLATE XI.
WRIST JOINTS.
SKIAGRAM I. ANKYLOSIS OF WRIST JOINTS.
MUSSGRAVE ET AL.: BONE LESIONS OF SMALLPOX.

SKIAGRAM II. ANKYLOSIS OF ELBOW AND WRIST.
MARKED SHORTENING OF BONES OF THE FOREARM.
Skiagram II. Ankylosis of elbow and wrist joints with marked shortening of bones of the forearm.
IS OF WRIST JOINTS.
SKIAMGRAM III. ANKYLOSIS OF WRIST JOINTS.
BROW AND KNEE JOINTS.
Musgrave et al.: Bone Lesions of Smallpox.


Skiagram IV. Deformity of Elbow and Knee Joints.
SKIAGRAME V. DEFORMITY OF THE WRIST AND ELBOW JOINTS IN SMALLPOX.
SHORT ARM, WITH SHORTENING OF THE BONES OF THE FOREARM.
SKIAGRAM V. DEFORMITY OF THE WRIST AND ELBOW JOINTS IN THE RIGHT ARM, WITH SHORTENING OF THE BONES OF THE FOREARM.
STATUS THYMICO-LYMPHATICUS AMONG FILIPINOS

By B. C. Crowell

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Two plates

The term “lymphatism” is about as old as that of “scrofula,” and for many years it conveyed an idea about as indefinite. As the advances in our knowledge of human diseases and their causes have practically abrogated the use of the latter term, its place being taken by a more definite nomenclature, so have the conditions suggested by the older term lymphatism been more accurately defined, the conditions associated with it been more closely circumscribed, and the interpretations of the anatomical changes become more elaborate.

To the older school the term lymphatism conveyed an indefinite idea of an individual with a pasty, sallow skin, flabby subcutaneous tissues, poorly developed musculature, a tendency to hyperplasia of the lymphatic organs, and a vulnerable glandular system.

The object of this paper is to trace briefly the steps by which our knowledge of this condition has become more accurate, to show that the entity, which has evolved from this indefinite conception, namely, status thymico-lymphaticus, occurs with considerable frequency in the Philippine Islands, and that here it has one unusual feature in that it has given rise to confusion with at least one condition frequently encountered here.

Whitmore(1) in 1911 reported 16 cases showing various grades of status lymphaticus out of a series of 565 autopsies performed in India. As far as ascertained, this is the only record of such cases in the Orient. He remarked that the condition is in no way dependent on race or environment.

It has long been recognized that sudden death is not an infrequent occurrence in persons of this type, and the almost

1 Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4-7, 1912.

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constant presence of an enlarged thymus gland, coupled with
the clinical evidence of respiratory embarrassment in many cases,
has led many authors to claim that direct mechanical pressure of
the thymus is accountable for the sudden death. Thus has
arisen the much discussed question of the so-called “mors thy-
mica.” The mechanical pressure of the thymus has been claimed
by some to be exerted on the trachea, by some on the heart, by
others on the vessels, and by still others on the nerves. Pressure
on the trachea, however, has attracted most attention, and at
the present day there are still many who claim evidence of such
a manner of death.

It may here be said that the standard for the size of the
thymus has been very unsatisfactory, and comparatively recent
work has shown that many glands which were accounted en-
larged were but normal. The old idea that the thymus dimin-
ished in weight from the second year of extra-uterine life is
now replaced by the certain knowledge that in normal indi-
viduals it increases in weight up to about the fifteenth year,
after which it normally undergoes gradual involution. The
thymus, however, suffers so severely as the result of nutritional
disturbances that it has been said that the condition of the
thymus is the best single indicator of the state of nutrition of
the body. This alteration in the thymus as the result of general
acute or chronic disease was termed “accidental involution” by
Hammar,(2) and the recognition of this has played a very im-
portant part in the interpretation of statistics.

The evidence adduced to show that the thymus exerts suf-
ficient pressure to produce suffocation is not all convincing and
Wiesel’s(3) conclusion that there may be a few cases dying from
this cause, while the majority are not thus accounted for, seems
justifiable. The fact that many infants who had large thymus
glands died with symptoms of stridor or laryngismus and pre-
sented on superficial anatomical examination little else to explain
the death is accountable for the importance attached to the
large thymus. As long ago as 1858, Friedleben(4) investigated
many of these cases and issued the dictum, “Es gibt kein Asthma
thymicum,” but it remained for Paltauf in 1889(5) to promul-
gate the theory that the enlarged thymus was only one evidence
of a general vulnerable bodily constitution which he designated
“status lymphaticus.” He described patients with this condi-
tion as exhibiting “great pallor of the skin, usually a well de-
veloped panniculus adiposus, more or less congested organs
without special changes in consistence; the spleen is mostly
enlarged and shows prominent follicles; in the epiphyseal cartilages are found evidences of active rachitic proliferation; the follicles of the lymphatic glands are much enlarged and the thymus well developed.” He recognized the general lymphatic changes which are present, and the cardiovascular hypoplasia, and summarized the whole as evidence of a “lymphatic-chlorotic constitution.” The cause of death he considered to be due to this anomalous constitution of the body, and the enlarged thymus was for him but a symptom of that general nutritional disturbance which was further evidenced by the enlarged lymphatic glands, tonsils, etc.

Bartel,(6) in Weichselbaum's laboratory, added another idea in his conception of the lymphatic changes and those which he found frequently associated with them as evidence of a “status hypoplasticus”; the associated changes found were anomalies of development in other organs, as hypoplasia of the arterial system and the genitalia, syringomyelia, hydrocephalus, glioma, and frequently horse-shoe kidney, and embryonic lobulation of the kidneys and lungs.

Very few ideas have been added to those of Paltauf and Bartel in the interpretation of the findings in such cases. The conditions under which they are found, and the diseases with which they are associated, however, have been much elaborated, and some authors, especially Von Neusser,(7) have indicated certain external appearances of the body which assist in making the diagnosis clinically.

This condition occurs in both infants and adults, although it is more easily recognized in the latter class. It is now well known that persons who are subjects of this condition have a peculiarly vulnerable constitution and are liable to death from seemingly insufficient causes. The cause of death in these cases is still a matter of much discussion, but Paltauf's contention that death is due to a paralysis of the heart is fairly generally accepted. Shridde's(8) hypothesis that death is due to a kind of endogenous poisoning through excessive or disordered function of the thymus Wiesel considers to be based on insufficient evidence. Wiesel(9) himself has noted an hypoplasia of the chromaffin system in cases of status lymphaticus, and this undoubtedly is an important element acting through the production of diminished tone of vessels and nerves.

All grades of the condition are found and are termed variously status thymicus, status lymphaticus, status thymico-lymphaticus, and partial status lymphaticus. It is possible and not improb-
able that there are two classes of cases; one in which the condition is a pure status lymphaticus in the sense of Paltauf, and another in which the various changes are more or less caused by the disease with which they are associated.

Kolisko(10) has given the best description of the anatomical conditions found in cases of pure status lymphaticus, while Von Neusser(7) has probably furnished the best data on which to base a clinical diagnosis.

For the diagnosis of status lymphaticus in the cadaver, Kolisko has given the following signs:

1. The failure of the involution of the lymphatic organs which otherwise occurs regularly at puberty. The enlarged tonsils consist of lymphoid tissue while their epithelial crypts are small; the follicles at the base of the tongue cause by their enlargement a rough granular condition of that part, the upper part of the pharynx is beset with lymphoid nodules as large as peas, mostly in the pharyngeal vault, but also on the posterior pharyngeal wall, in the pyriform sinuses, and even in the larynx on the inner side of the epiglottis.

2. The cervical lymphatic glands are bean-size to, at the most, cherry-size, tolerably firm and mostly pale, sharply circumscribed one from the other.

3. The thymus persists beyond puberty, and frequently undergoes hyperplasia.

4. The endocardium of the left ventricle shows a diffuse cloudiness on the valves, the mural endocardium, and in the heart muscle, without inflammatory manifestation. The alterations in the lining of the heart lead Kolisko to conclude that abnormal pressure with a tendency to dilatation has been acting on the ventricular endocardium.

5. The enlarged spleen shows hyperplastic follicles on the cut surface, and its capsule is tense and often very friable.

6. The mucosa of the intestine shows distinct hyperplasia of the follicles, and the mesenteric lymphatic glands are proportionately enlarged. So are all other lymphatic glands of the body enlarged in various degrees, the smaller being mostly rich in blood, the larger bloodless and not juicy.

7. The bone-marrow frequently exhibits the appearance of medulla rubra.

8. As the size and weight of the thymus normally are subject to remarkable alterations and as the other lymphoid tissue of the child's organism is so variable in its growth, and hyperplasia of it is so frequent as the result of inflammatory irritation or disturbances of nutrition as in rickets, it is clear that the interpretation of the pathologico-anatomical findings in relation to the diagnosis of status lymphaticus in the cadavers of children up to the sixth year is beset with extraordinary difficulties. Therefore, for diagnostic purposes one must emphasize the occurrence of the size of the thymus, disproportionate to the age of the child, as well as the frequency and similarity of the hyperplasia in the lymphatic system, and also the condition of nutrition must be borne in mind.

9. A frequent, but not constant, finding is the narrowness and delicacy of the aorta and the rest of the arterial system, yet hypoplasia of the arteries can exist even without a macroscopically recognizable hyperplastic lymphatic system. Also, the hypoplasia of the genitals often accom-
panying the hypoplasia of the arteries is no constant phenomenon in the lymphatic constitution. The aorta as well as the peripheral arteries show an unusual narrowness, with thin walls and abnormal elasticity to such a degree that a section of vessel after removal retracts to almost half its original length.

10. In the heart sometimes a surprising smallness (hypoplasia) is encountered, but the heart can also be of normal size or even hypertrophic. The hypoplasia of the arterial vascular system is possibly commensurate with the abnormal blood pressure in such individuals, and stands apparently in relation to the hypoplasia of the adrenals and chromaffin system found by Wiesel in many cases.

Von Neusser's work has been consulted in preparing the following description of the clinical appearance of such cases, but his work was practically confined to adults, while Escherich (11) and Howland (12) have furnished data for the diagnosis in children. It goes without saying that one who is on the lookout for such cases and who is familiar with their appearance and with the diseases with which they are associated will recognize them more frequently than one not so prepared. On account of the relative obscurity of the findings this prepared mind is of more importance in the detection of this disease than of many others.

In males the individuals are usually well nourished and gracefully molded, with smooth velvety skin. The features are not infrequently of the classic type, and the neck rather columnar in shape, being set rather abruptly upon square shoulders without the usual sloping. The extremities are gracefully constructed, and the thighs are rounded and usually arched or rounded both anteriorly and laterally. The hair is scant, including the beard and mustache, thoracic and axillary, abdominal, pubic, and that over the extremities. The pubic hair is usually cut straight across as in the female. In fact, the whole is practically a feminine type of body.

The female, on the other hand, while preserving in part the graceful feminine outlines of the body, and having for the most part the same scantiness of hair, frequently presents heterosexual characteristics, with deep base voice and some beard and mustache.

The diseases with which it is most frequently associated are those of the ductless glands, as in Addison's and Basedow's diseases, acromegaly, osteomalacia, and in brain tumors, and in anomalies of the sexual organs such as hermaphroditism. In Basedow's disease it is very frequent, and it is considered by Shridde (8) and many others that the lymphocytosis which is so frequent in Basedow's disease is dependent upon the lymphatic
constitution and that a lymphocytosis of more than 40 per cent is a contraindication for operation. Many investigators, especially those of the French school, have gone so far as to attribute the symptoms of Basedow's disease to the disordered function of the thymus and to treat by thymectomy rather than thyroidectomy. Thymus feeding in such cases has not been uniformly successful. However, in view of the frequent fatalities following thyroidectomy in exophthalmic goitre, advanced or extensive lymphatic and thymus change should be excluded as far as possible by differential blood count, roentgenization, and percussion of the thymus region. Capelle\(^{(13)}\) collected statistics from the literature in regard to the association of status thymico-lymphaticus and Basedow's disease, as follows: Of patients with Basedow's disease dying of intercurrent infections, 44 per cent showed hypertrophic thymus; of those dying purely from Basedow's without associated disease this became 82 per cent, and of those dying at or after operation 92 per cent, while if those were excluded who died from haemorrhage it became 100 per cent. He, therefore, concluded that the thymus enlargement was practically an indicator of the severity of the disease.

But certainly not all cases of status thymico-lymphaticus are associated with such extensive alterations in other ductless glands, and the anatomical records of homicides and suicides are particularly interesting. These have been studied by many workers, from two of whom I shall quote.

Bartel\(^{(14)}\) in 1910 studied 122 cases of suicides, and out of 52 cases found—

| Status thymico-lymphaticus in | 36 |
| Status lymphaticus in | 26 |
| Partial status lymphaticus in | 20 |
| and had incomplete protocols in | 18 |

Miloslavich\(^{(15)}\) studied, in 1912, 110 cases of suicides among soldiers, and found 80 per cent with a lymphatic constitution as follows:

| Status thymico-lymphaticus | 47 |
| Status lymphaticus | 21 |
| Status thymicus | 8.5 |
| Partial status lymphaticus | 3.5 |
| Negative as to status | 20 |

Bartel considered lymphatism as a certain and very pregnant sign of a constitutional anomaly, and Miloslavich considered its presence in suicides evidence of mental instability.
REPORT OF CASES

In a series of 300 autopsies, there have occurred 20 cases of well-marked grades of this condition as follows:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status thymico-lymphaticus</td>
<td>13</td>
</tr>
<tr>
<td>Status lymphaticus</td>
<td>7</td>
</tr>
</tbody>
</table>

In addition there have been very numerous cases of lesser grades of lymphoid hyperplasia of the various viscera. It may here be stated that the autopsy service which includes these cases is derived from a large general hospital, a hospital for contagious diseases, and the medico-legal cases occurring in the city of Manila, so that all classes of cases are encountered.

A more detailed examination of these cases follows. 3

I. CASES OF STATUS THYMICO-LYMPHATICUS

1. (1948.) One case was in a male, 8 years old, whose duration of illness is given as one day, and the clinical diagnosis was undetermined. He had a thymus weighing 27 grams, and hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, intestine (duodenum, ileum, and colon), and lymphatic glands (mesenteric, femoral, inguinal, and axillary). There were also ecchymoses on the pleura and epicardium, and small external genitalia.

2. (2000.) One case was in a 30-year-old male who died soon after partial thyroidectomy performed under local anaesthesia for the relief of symptoms of Basedow's disease. He had a 40-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring and intestine (ileum and colon).

3. (2011.) A 10-year-old boy died as the result of an automobile accident which caused extensive fractures of the skull. He had a 26-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring, oesophagus, stomach, intestine (duodenum, ileum, and colon), and the cervical and mesenteric lymphatic glands. The adrenals were hypoplastic, and there were ecchymoses on the pleura and epicardium.

4. (1979.) A 12-year-old boy died from drowning. He had a 37-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, intestine (duodenum, ileum, and colon), mesenteric, inguinal, and axillary lymphatic glands.

3 Cultures from the viscera of the cases were sterile unless otherwise stated.

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The adrenals were hypoplastic, and there were ecchymoses on the leptomeninges.

5. (1980.) A 7-year-old boy died of streptococcus septicæmia, the duration of his illness being given as three days. The fever in this case along with the enlargement of the superficial lymphatic glands led to the clinical diagnosis of probable bubonic plague. He had a 30-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring, intestine (duodenum and ileum), mesenteric, inguinal, axillary, and cervical lymphatic glands. Epicardial ecchymoses were also present.

6. (1982.) A 9-year-old boy died with slight broncho-pneumonia, the duration of illness being given as twelve days. He was also suspected of having plague for the same reasons mentioned in the previous case. He had a 40-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, and inguinal and axillary lymphatic glands.

7. (1971.) A 25-year-old male, whose duration of illness could not be ascertained and on whom no clinical diagnosis could be made, had chronic pulmonary tuberculosis with hemorrhage. He also had a 22-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring, intestine (ileum and colon), and mesenteric, retroperitoneal, and femoral lymphatic glands.

8. (1920.) A 3-year-old boy, who was ill six days with undetermined diagnosis, had slight pleural and intestinal tuberculosis. In addition, he had a 50-gram thymus and marked hyperplasia of the lymphoid tissue of the pharyngeal ring, intestine (duodenum, ileum, and colon), spleen, and inguinal and femoral lymphatic glands. Epicardial, pleural, and gastric ecchymoses were also present.

9. (1845.) Another 3-year-old boy, who was said to have been ill fourteen hours, had general glandular and intestinal tuberculosis. In addition, he had a 25-gram thymus and hyperplasia of the lymphoid tissue of the tonsils, spleen, and intestine.

10. (1761.) A 1-year-old male, ill two hours, supposed to have had nephritis and rachitis, had an “abnormally enlarged” thymus, hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, intestine, and mesenteric lymphatic glands. He had also a dilated heart and rachitis.

11. (1901.) A 7-year-old boy, who was ill three days and died suddenly, had a “large” thymus, hyperplasia of the lymphoid tissue of the pharyngeal ring and spleen, and an acute gastro-enteritis.
12. (1894.) A 19-year-old male, who died after three days' illness with bubonic plague, had in addition to the lesions attributable to plague a "large" thymus and an hypoplastic aorta and lymphoid hyperplasia in the intestines. Bacillus pestis was isolated from this case.

13. (2114.) A 6-year-old male, whose duration of illness was given as four days and on whom a diagnosis of probable grippe was made, had a 37-gram thymus, hyperplasia of the lymphoid tissue of the pharyngeal ring, intestine, mesenteric and superficial lymphatic glands, acute suppurative otitis media, and chronic fibrous pleurisy. A Gram-positive diplococcus resembling a pneumococcus was found in films from the left middle ear. Cultures from the spleen were negative.

II. CASES OF STATUS LYMPHATICUS

14. (1933.) A 2 1/2-year-old girl, who was ill one hour with undetermined diagnosis, had a slight enteritis, hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, intestine, and mesenteric lymphatic glands, dilatation of the right ventricle, and congestion and oedema of the lungs. The thymus was of normal size.

15. (1929.) A 7-year-old boy, who was said to have been ill thirty minutes with undetermined diagnosis, had a 13-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, mesenteric lymphatic glands, and intestine (ileum and colon), slight acute enteritis, dilatation of the right ventricle, and an "unusually small" thyroid.

16. (1799.) A 15-month-old female, whose duration of illness and clinical diagnosis were unknown, had an 18-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, intestine, and mesenteric lymphatic glands. In addition, there were ecchymoses in the pleura and duodenum, and no other gross pathological phenomena aside from congestion of the kidneys.

17. (1764.) A 5 1/2-year-old girl, who had numerous fractures from collision with a trolley car, had a 12-gram thymus and hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, and intestine.

18. (1767.) A 17-year-old female, who was ill ten days after parturition and was supposed to have had acute beriberi, had a cyst of the pituitary gland, a small thymus, and hypoplastic
aorta, hyperplasia of the lymphoid tissue of the pharyngeal ring and spleen, and hypoplasia of one adrenal.

19. (1522.) A 4½-month-old female, who was ill a month with what was diagnosed infantile beriberi, had slight lobular pneumonia, congestion of the kidneys, a 5-gram thymus, anomalous lobulation of one lung, and hyperplasia of the lymphoid tissue of the spleen, intestine, and mesenteric lymphatic glands.

20. (1275.) An 18-year-old female, who was said to have been ill two weeks and whose record does not show the manner of death, had a "small" thymus, hypoplasia of the heart, aorta, and adrenals, hyperplasia of the lymphoid tissue of the pharyngeal ring, spleen, and mesenteric lymphatic glands, a calcific nodule on the pleura, localized atheroma of the aorta, pulmonary congestion, and oedema and parenchymatous degeneration of the kidneys.

Analyses of these cases shows the following features of interest:

1. In 3 cases (1, 16, 20) no essential lesions were found save those of status thymico-lymphaticus.
2. The condition was encountered in 3 cases (3, 4, 17) of accidental death.
3. In 7 cases (6, 8, 11, 13, 14, 15, 19) the other anatomical lesions which should be considered as contributing factors to the cause of death were slight.
4. In 4 cases (5, 7, 9, 12) there were definite anatomical lesions sufficient to cause death, which were evidently unrelated to status lymphaticus and on which the lymphoid hyperplasia could scarcely be considered dependent.
5. In 2 cases (2, 18) there were definite anatomical lesions in other ductless glands, and in 1 case (10) there was a definite disease due to metabolic disturbance.
6. The cases occurred between the ages of 1 and 30 years, 14 being below the age of puberty.
7. In 6 cases (1, 3, 4, 5, 8, 16) there were encountered ecchymoses on serous surfaces, and in 2 cases (8, 16) on mucous surfaces such as are found in beriberi; and doubtless had the records been more complete, the number of these would be greater.
8. The distribution of the lymphoid hyperplasia is not constant, different organs being affected in various grades in the different cases.
9. There occurred definitely recognizable hypoplasia of the adrenals 4 times (3, 4, 18, 19), of the aorta twice (18, 19), of the
heart once (19), of the thyroid once (15), and of the external genitals once. Careful measurements of the heart and aorta were not made in all cases, and these figures probably do not represent the true state of affairs. This question will be more carefully investigated at another time, but my opinion is that the size of the aorta was below normal in the majority of these cases.

10. The duration of illness was brief in all but 4 cases (6, 18, 19, 20).

It is not proved nor believed that the lymphatic constitution is of greater frequency among Filipinos than among other races, but it is desired to draw attention to its occurrence and to the importance of its recognition. In children its manifestations are the same as in other races; on the other hand, in a large number of adults of the Oriental races the growth and distribution of the hair differs so essentially from that in Caucasian races that the same importance cannot be attached to this point in diagnosis as is given it by those whose investigations do not include Orientals. I refer to the almost complete absence of beard, mustache, axillary and thoracic hair, and that on the extremities which is common among Orientals. Also, in tropical climates where acute and chronic infections of the skin of the lower extremities are so frequent on account of the custom of the natives of going barefooted, enlargement of the femoral and inguinal glands of inflammatory origin is very frequent, and enlargements of this type should not lead to the diagnosis of the lymphatic constitution. However, during the recent period of activity of the Bureau of Health of Manila in causing investigation of all cases of death with enlarged superficial lymphatic glands as one means of detecting cases of bubonic plague, several of the cases of the present series were encountered. I am also informed by workers in the Bureau of Health that several cases were encountered clinically with enlarged femoral or inguinal glands in which there was a brief period of hyperpyrexia of unexplained origin, the enlargement of the glands persisting after the subsidence of the fever. Such cases were sometimes referred to as cases of "glandular fever," and it seems not impossible that some of these cases may come within the category under consideration. This seems more probable in view of the recognized susceptibility of individuals of the lymphatic constitution to slight infections.

From another standpoint, the recognition of the lymphatic constitution is of importance among Filipinos; that is, on account
of the similarity of some of the clinical and pathological manifestations of status lymphaticus and those of beriberi. In a well-developed case of beriberi with neuritis, œdema, and gastric and cardiac disturbances, there is no danger of confusion of the two diseases. But in cases of sudden death where clinical observations have been meagre or not made at all, both conditions should be borne in mind. Their occasional similarity, coupled with the extreme frequency of the diagnosis of beriberi and the complete absence of that of status lymphaticus in the records of the statistical division of the Bureau of Health, would seem to make it very probable that some cases of status lymphaticus have been classified as cases of beriberi. The clinical symptoms of infantile beriberi recently described by Andrews(16) of this department are frequently those which have been recognized as characteristic of status lymphaticus, and the appearance of the child is practically the same. This is especially true of those cases referred to by Andrews as those of the acute pernicious type of infantile beriberi. The anatomical findings in the two diseases are frequently very similar, the degeneration of the nerves which can only be recognized by appropriate microscopic technique being the only characteristic of infantile beriberi which has not been found in cases of status lymphaticus. The possibility of the association of the two conditions should be recognized.

LITERATURE

(1) Whitmore, A. Lancet (1911), 181, 752.
(4) Friedlæben, A. Die Physiologie der Thymusdrüse in Gesundheit und Krankheit. Frankfurt (1858).
(6) Bartel. Ibid. (1908), 21, 783.
(15) Miloslavich. Virchow's Arch. (1912), 208, 44.
ILLUSTRATIONS

PLATE I
Lower end of ileum showing hyperplasia of solitary follicles and Peyer's patches in status lymphaticus. (Drawing by Castro.)

PLATE II
Organs of the neck showing hyperplasia of faucial and lingual tonsils and lymphoid tissue in the pyriform fossae in case 2. (Photograph by Cortes.)
PLATE I. HYPERPLASIA OF LYMPHOID TISSUE OF ILEUM IN STATUS LYMPHATICUS.
PLATE II. HYPERPLASIA OF LYMPHOID TISSUE OF PHARYNGEAL RING IN STATUS LYMPHATICUS.
PRIMARY SARCOMA OF THE SMALL INTESTINE

By R. W. Hammack

(From the Department of Pathology and Bacteriology, University of the Philippines, Manila, P. I.)

Within the last year three cases of primary sarcoma of the small intestine have been encountered in the service of this department. In view of the infrequency of this condition, these cases have been summarized and are here presented, together with a brief consideration of the pathological anatomy of the disease.

CASE 1. (S. P. 654.)—The patient was a male Filipino, aged 33, a cigar maker. For four years he had had abdominal pain after eating, which could be relieved by induced vomiting, and the bowel movements had been irregular. In spite of symptoms of obstructions, he was fairly well nourished. A movable tumor was felt in the abdomen. The tumor was removed at operation, and the patient was in good condition three months later, after which he was lost sight of.

Description of tumor.—The available records do not state exactly which part of the intestine was removed.

A segment of the small intestine, 10 centimeters in length, was completely encircled, and the wall diffusely infiltrated by the tumor. In the distal portion of the segment, where the wall measured 1 centimeter in thickness, the lumen was markedly narrowed, while above it was dilated and the wall thinner. There was no ulceration, the mucous surface being smooth and without folds. Enlarged lymphatic glands were found in the mesentery.

Microscopic examination.—A section through the infiltrated intestinal wall showed partial absence of the epithelium, but the denuded portions showed no necrosis or evidence of ulceration. The various coats of the intestine could not be differentiated since the entire wall was infiltrated by tumor cells. The cells were round, slightly larger than lymphocytes, and had pale-

1 Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4-7, 1912.
2 Instructor in pathology and bacteriology, College of Medicine and Surgery, University of the Philippines.
staining nuclei and a small amount of cytoplasm. They were for the most part fairly uniform in size and shape, although occasionally large forms were seen. Connective-tissue fibers and cells and blood vessels were numerous. Here and there were bundles of muscle fibers, many of which were swollen and evidently degenerated. Where the epithelium was present, the tumor cells extended up to it, and the glands were few and short, and the villi absent. Diagnosis: Lymphosarcoma.

Case 2. (Necropsy, 1777.)—The tumor occurred in a male Filipino, aged 31, a sailor. The first symptoms occurred about three months before death when he began to have paroxysmal attacks of pain in the right hypochondrium, followed about a week later by jaundice which soon disappeared. When admitted to the hospital two months after the beginning of the symptoms, the patient was emaciated, and a soft tumor, the size of an orange, could be palpated below the right costal margin. Laparotomy was performed three weeks before death, but the condition was found inoperable.

Necropsy findings.—In the right hypochondriac region were numerous fibrous adhesions binding the liver to the diaphragm and the pyloric portion of the stomach to the liver. Behind the transverse colon lay the greatly enlarged duodenum. The stomach and upper part of the small intestine contained a large amount of blood. The pylorus was large, and opened into a greatly dilated duodenum. This dilated portion measured 16 centimeters in length, ending at the beginning of the transverse portion of the duodenum, and its lumen measured 20 centimeters in circumference at the widest part. The mucosa was destroyed in practically all of this dilated portion, the surface roughened and fissured, and from the posterior wall small irregular yellow and reddish black necrotic masses projected. The wall of the cavity was thickest posteriorly, and was everywhere thicker than the normal intestinal wall. The papilla of the common bile duct, with the pancreatic duct opening into it, was found 11 centimeters from the beginning of the dilation. The mucosa of the lower 2 or 3 centimeters of the common duct was thickened and slightly roughened, but proximal to this was normal. The growth had invaded the upper portion of the head of the pancreas, giving to it a moist homogeneous appearance. The lymphatic glands behind the duodenum were much enlarged, and some were almost entirely necrotic. Ten centimeters below the dilation was a small plaque projecting into the intestine over which the mucosa was intact. In the upper part of the ileum were found 3
small infiltrative growths, the largest completely encircling the intestine and measuring 5 centimeters in length and 5 to 7 millimeters in thickness. This appeared as a grayish patch from both the mucous and the serous surfaces, and projected slightly into the lumen, but did not diminish it appreciably. The folds of the mucosa were partly obliterated, but there was no ulceration. The other two growths nearby had the same general appearance, but were not completely circular. The mesenteric glands opposite all of these were enlarged and firm. There were no metastases to other organs.

Microscopic examination.—The tumor was very cellular, the cells showing great variations in size although the majority were slightly larger than small lymphocytes, round, with pale-staining nuclei and scanty cytoplasm. Multinuclear cells, which seemed to occur in groups, and mitotic figures were not infrequent. In the duodenum the intestinal wall was made up practically of tumor tissue and the inner surface was largely necrotic. Examination of one of the tumors in the ileum showed that growth had occurred in the submucosa and mucosa, while the muscular coats were not invaded. The infiltration extended between the crypts, which were rather few, to the surface, which was covered only partially by epithelium and showed occasional small necrotic areas. The tumor cells of this section were more typically lymphoid, and the multinuclear cells were infrequent. Mallory’s aniline blue stain revealed a delicate connective-tissue reticulum throughout the tumor tissue. The enlarged mesenteric and retroperitoneal lymphatic glands showed the same general structure with frequent necrosis. Diagnosis: Lymphosarcoma.

Case 3.5 (Necropsy, 1963.)—The patient was a male Filipino, aged 27, a soldier. The first symptom was an attack of abdominal pain on June 7, 1912, which kept him from duty for three days. On June 23, an abdominal mass was noticed. At this time, on account of rapid loss of weight, he was sent to Manila for hospital treatment. On July 10, a laparotomy was performed and an inoperable tumor was found. The patient died August 10, 1912.

Necropsy findings.—The body was greatly emaciated. The tumor occurred in the ileum, 1.5 meters from the ileocæcal valve. It almost completely encircled the intestine lying within its walls and extending for 2 or 3 centimeters between the layers of the mesentery. Its longitudinal diameter was about 8 centimeters.

*I am indebted to Maj. S. C. Gurney, medical division, Philippine Constabulary, for the clinical notes on this case.
At the point of greatest infiltration, the intestinal wall was about 1 centimeter in thickness. The lumen of the intestine was not encroached upon but rather enlarged. There were one or two small erosions of the mucosa, and at the thickest portion of the tumor was found a deep ulcer 3 centimeters in diameter with smooth, sloping walls.

The mesenteric glands were large, firm, and pale, and about them was often found a diffuse infiltration of the mesentery and of the retroperitoneal tissues. The retroperitoneal glands were also enlarged and some were softened. On the anterior surface of the right ventricle of the heart was a small, pale, circular nodule, slightly elevated, measuring about 0.5 centimeter in diameter. There were no metastases in other organs.

Microscopic examination.—Sections from the thickest part of the intestine showed the tumor to be made up of small groups of cells of lymphoid type, between which were coarse bands of connective tissue, often hyaline, and numerous cells which were evidently of inflammatory origin. In the younger metastatic tumors of the lymphatic glands, the connective tissue and adventitious cells were much less prominent and only a fine reticulum was present. Eosinophilic cells were rather numerous, and multinuclear cells were present, but mitotic figures were rare. Blood vessels were numerous, especially in the metastatic growths.

The nodule in the heart consisted of a small area not definitely circumscribed, showing diffuse infiltration of the muscle by tumor cells with little change in the muscle fibers. Diagnosis: Lymphosarcoma.

That primary sarcoma of the intestine is infrequent is evidenced by the statistics of various pathological institutes. Thus Smoler(1) reports that among 13,036 necropsies performed at Prague, in a period of fifteen years, there were but 13 cases, of which 10 were of the small intestine. Corner and Fairbank(2) in 1904 were able to collect from the literature but 65 cases, although, according to Kaufmann,(3) Rademacher(6) reviewed 140 cases in 1908, adding one new one. In our series of 2,200 necropsies, the two given above are the only ones of undoubted primary sarcoma of the intestine. The disease may occur at any age, the youngest case reported being a congenital one and the oldest 70 years of age, although it appears that the greatest number occurs in the fourth decade. In the cases reported, the proportion of males to females has been about two to one.
The small intestine is evidently more commonly the site of origin of sarcoma than is the large intestine, since Jopson and White,(4) in 1901, could collect but 22 cases of sarcoma of the large intestine, while Libman(5) the year before had brought together 64 of the small intestine. The ileum is the portion most commonly affected.

Of the types of sarcoma found, lymphosarcoma is the most frequent, but spindle-celled, round-celled, and melano-sarcomata as well as endotheliomata occur.

The tumor most frequently occurs as a spreading, infiltrating growth, completely or nearly encircling the intestine, but may occur as a polypoid mass projecting into the lumen. The growth is usually confined to the intestinal wall, leaving the serosa intact. Ulceration, on the other hand, is frequent and may cause perforation. It is remarkable, however, that very extensive infiltration of the intestinal wall, including the mucosa, can take place without ulceration, as is exemplified by case 1 of this series. Dilatation is more frequent than stenosis. This is apparently due to the widespread infiltration and destruction of the muscular coats with possibly the additional factor of accumulation of intestinal contents on account of the absence of peristalsis. The annular form of the tumor, however, can cause constriction as is shown also by case 1. When complete obstruction occurs, it is most often due to intussusception.

Metastases from intestinal sarcomata occur most frequently in the abdominal lymph nodes, particularly with lymphosarcomata, but metastases in the intestine, liver, spleen, kidneys, lungs, and brain have been recorded. Involvement of other abdominal organs seems to occur most often by direct extension from the metastatic growths in the lymphatic glands.

All of the cases here presented have been lymphosarcomata, and all have exemplified the annular form, while one shows the infrequent condition of stenosis. In all there have been metastatic growths in the abdominal lymphatic glands with more or less widespread extension from these. In but one case, in which there was a small nodule in the heart, was there metastasis beyond the abdomen.

LITERATURE

(1) Smoler. Prager med. Wochenschr. (1898), 23, 145.
(3) Kaufmann. Lehrbuch der Speziellen Pathologischen Anatomie (1912), 528.
(5) Libman. Ibid. (1900), 120, 309.
TUMORS OF THE PITUITARY GLAND

REPORT OF A CASE OF PITUITARY GLIOMA

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Two plates

The absence in the literature of reports of similar pituitary tumors justifies the publication of the present case. Weichselbaum(21) was one of the first investigators to whom we owe the description of pituitary cysts, lined with ciliated epithelium, and containing a homogeneous or granular material. Beck(3) and Weigert(22) have described several dermoid cysts of the pituitary body. The first case of neuromyoma made up of striated muscle and medullated nerve fibers was reported by Hale White.(1) Rayer,(13) Rokitansky,(16) and Langer(1) have observed several cystic tumors which it is presumed originated from the infundibular canal. The first case of lipoma is that of Weichselbaum. (21)

The report of two cases of angioma and chondroma, respectively, is attributed to Lancereaux.(1) Several cases of adenoma have been encountered (Breitner,(6) Einsenlohr,(8) and Von Hippel(9)). Adami and Nicholls(1) have described an endothelioma and a perithelial angiosarcoma. Colloid carcinomata and melanomas have also been reported. Soemmering and Lancereaux(1) found several cases of echinococcus cysts. Battiscombe(7) has reported an abscess. Beadless,(2) Boyce and Beadless,(5) and Weigert(22) described several caseating tumors; and the description of syphilomata belongs to Lancereaux.(1)

The above facts prove the absence of reports of pituitary gliomata in the literature, none of the authors cited having reported such a pituitary neoplasm.

Of the 2,000 autopsies performed in the city, San Lazaro, and Bilibid morgues during five consecutive years, there is no analogous case.

1 Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4-7, 1912.

2 Instructor in pathology and bacteriology, College of Medicine and Surgery, University of the Philippines.
As an introduction, some consideration of the histology and physio-pathology of the pituitary body may be recorded.

The anatomical formation peculiar to the gland is that of a pea-shaped mass, connected to the base of the encephalon by means of a delicate peduncle. It weighs generally from 3 to 6 decigrams, and occupies a cavity in the cranial floor called the "sella turcica." It consists of an anterior and a posterior portion, together with a ramus or infundibulum. The anterior or glandular portion consists of a stroma of connective tissue, rich in capillaries, in which are closely packed epithelial cells grouped in the form of acini. The cells which constitute these acini are polyhedral, round, or oval, and are of two principal varieties, chromophilic and chromophobic. In the chromophilic cells the cytoplasm is abundant and contains granules which are stained red or yellow with eosin or take a purple coloration with hæmatoxylin. Klotz(1) has remarked the constant presence of acidophilic, mononuclear cells. The chromophobic or principal cells are smaller, more numerous, and have a transparent protoplasm. According to Berkeley,(4) the nerve elements are derived from the sympathetic plexus of the carotid. At the periphery of the lobe and particularly at the boundary line between the two lobes, the so-called pars intermedia, the acini, surrounded by cubical cells, are often distended by a colloid substance, which is also present in the lymphatic and vascular spaces.

The posterior or infundibular portion of the gland is composed of vascular connective tissue, in which exist numerous fusiform or stellate cells which are often pigmented. The structure is practically that of the neuroglia. Berkeley(4) has reported the presence of ganglion cells and of varicose nerve fibrils, but Kölliker(1) denies the existence of nerve elements in this portion of the gland.

At the line of junction between the two lobes, the vessels are particularly numerous, and in man there is a bilobular cavity bounded by cylindrical epithelium.

Physiology.—The nature of the function of the gland, although obscure as yet, is becoming more and more clear. Evidence is accumulating which tends to prove that, like the thyroid and the suprarenals, it belongs to the group of ductless glands, and that it elaborates an internal secretion essential to metabolism. As regards its vascularity, presence of acini, and lymphatic vessels containing colloid, a close analogy exists with the thyroid, which
according to general opinion elaborates an internal secretion. Schnitzler and Ewald(18) have shown experimentally the presence of small quantities of iodine in the pituitary gland. In addition, there is an hypertrophy of the organ after thyroidectomy in animals (Rogowitch,(15) Hofmeister(1)), and in cases of thyroid atrophy in man. The abnormalities of growth and development known as acromegaly and gigantism are in the majority of cases associated with lesions of the pituitary body, such as cysts, hypertrophies, or tumors. In acromegaly it has also been found that the thyroid is sometimes atrophic or cystic, and that the disease can be complicated by myxedema or exophthalmic goitre. Some relation seems to exist also between the pituitary gland and the genital organs. Nicholls performed an autopsy on a woman about 30 years old with a pituitary tumor, in whom the genitalia were in a marked state of infantilism; a similar phenomenon has been observed in man by Pechkranz.(12) Genital deficiency, genital hypoplasia, and malformations have been noted with frequency in acromegaly by Garnier et Santenoise,(1) Thoma,(20) and Babinsky.(1)

Through the observations of Marie(10) it has been recognized that there exists a close relationship between the abnormalities of the pituitary body and the hypertrophy of the bones of the face and extremities, a pathologic condition known as acromegaly. This condition takes place during the age of virility, and its development is slow, up to twenty or more years. There is an enlargement of the face, the superior and inferior maxillary bones being particularly affected; the ears reach a very large size; the nasal fossae are widened; and the eyelids thickened. The hands and feet are so characteristically enlarged as to be disproportionately enormous.

In generalized gigantism one frequently observes enlargement of the pituitary body. Marie inclined to the opinion that absence of active pituitary secretion induced acromegaly with replacement of the pituitary tissue by neoplasm. Schäfer(17) to whom together with Oliver and Herring we owe our recent knowledge of the subject, believes with Tamburini and Woods Hutchinson (1) that the neoplasm is due to overdevelopment of the anterior lobe, as in some cases a simple hyperplasia of this region has been observed; Von Hansemann(1) affirms that those tumors of the anterior lobe are simple adenomata and not sarcomata, or rather colloid accumulations which are associated with the disease. Schäfer suggests that the observations of Marie, in the
cases cited by the latter, can happen on the supposition that the condition commences by simple hyperplasia, the neoplasm being the terminal phenomenon.

But if the facts indicate that this anterior glandular portion furnishes hormones or other products which stimulate the development of bone or connective tissue, the observations of Herring show that the intermediate portion, also glandular, develops colloid material which finds its way through the nervous posterior portion of the gland, which is directed toward the infundibulum, and thence to the cerebral ventricles. This internal secretion elaborated in these two portions has different but equally useful properties, which, as Howell (1) has shown, is not present in the extract obtained from the anterior portion. According to experiments carried out by Oliver and Schäfer, (1) an extract of these two portions produces a rise of blood pressure very similar to, but more prolonged than, that induced by suprarenal extract, plus an increase of urinary secretion or polyuria absolutely independent of blood pressure, but directly due to the action of the extract on the renal tissues. Similarly to adrenalin, this extract is indifferent to warmth or heat. The indications are that the gland produces not one but several hormones. Like the suprarenals this diminutive gland, which in man weighs only 0.5 gram, is essential to life, and according to the experiments of Paulesco (11) and Reford and Harvey Cushing (14) its extirpation in animals in the laboratory is followed by death within forty-eight hours or more, with marked symptoms of inanition. The essential cause of this death is yet unknown. It is, nevertheless, a significant fact that this diminutive organ, essentially glandular, should exercise such an extraordinary influence in the life of animals.

**REPORT OF CASE**

The condition of the patient and the impossibility of obtaining a history made it impossible to get many clinical facts about the case. The post-mortem examination showed a case of simple pituitary lesion, that is, one without the accompaniment of acromegaly.

**Clinical history.**—It was an emergency case. The patient was found in the street in an unconscious state.

**Personal data.**—The patient was a male, name, age, and occupation unknown. However, his age must have been between 30 and 35 years. Family antecedents and social position were unobtainable.

**Actual condition of the subject.**—When first seen, the patient was in bed with dyspnoea and general convulsions. The pulse was small, slow, and thready; no fever; hypothermia was present; there was no paralysis. He was of strong frame and well nourished; his development was normal. It was impossible to obtain further symptomatological facts than those
noted, owing to the unconscious state of the patient and the absence of relatives or friends capable of supplying them.

Observations.—Five hours after admission. The patient has remained comatose since admission, in spite of the treatment instituted. A few moments afterwards the convulsions lessened gradually, and a general progressive adynamia declared itself.

The cadaver was brought to the city morgue with diagnosis undetermined. The following is the report of the autopsy performed seventeen hours after death, with histological examination of the affected viscera.

The cadaver is that of an individual of good musculature, corpulent, of medium height (163 centimeters), with a weight of 53.6 kilograms. There is cadaveric rigidity present in both superior and inferior extremities and lividity in the dependent parts. Some eschars are present on the body. There is a tattoo with initials L. R. on the anterior surface of the left forearm. No enlargement of the superficial lymphatic glands can be detected. Pupils are slightly dilated; face normal; thorax well shaped; abdomen plane. Genitalia are well developed without apparent solution of continuity, recent or remote. No evidences of hypertrophy of the osseous system are present.

The subcutaneous adipose tissue is in normal quantity, and has a dull yellow color. The muscular tissue is perfectly developed. The tissues have a normal appearance.

The peritoneum is smooth and glistening. There is a normal quantity of peritoneal fluid. The appendix and intestines are normal. The omentum covers the guts like an apron, and is 3 centimeters below the umbilicus. The liver is not prolapsed. There are adhesions between the anterior surface of the liver and the abdominal surface of the diaphragm. This latter rises to the level of the fourth interspace on the right side and to the level of the fourth rib on the left.

The pericardium is smooth, whitish blue, and contains approximately 5 cubic centimeters of an orange-yellow liquid. The heart is soft, and normal in size; it contains post-mortem coagula; the musculature is firm, normal in aspect; the valves are fine and elastic. The aorta is normal except at the base where there is a slightly raised area, which is translucent, fibrous, and 6 millimeters at its widest diameter.

Lungs.—The lungs are adherent to the thoracic and diaphragmatic walls. They are crepitant except in certain spots. The surface is somewhat rough, due to adhesions at the apex of the superior and base of the inferior lobes, and is dark gray anteriorly and reddish posteriorly. Several nodular indurated areas are felt on palpation. Section across these indurated areas shows congested collections of tissue from 5 to 15 millimeters in diameter situated, apparently, about the bronchial terminations, and slightly raised from the pulmonary parenchyma. Scraping the surface loosens some fibrin filaments from the bronchial infundibula, together with a serosanguineous exudate from the air vesicles.

The bronchi are slightly congested, and contain frothy exudate. Microscopically, there is a dense infiltration of red and white cells around the bronchioles. The epithelial lining of the bronchi is detached in part from its basement membrane, and the cells occupy the lumen of the bronchi, together with a fibrinous exudate in the form of a network in whose meshes are red blood-cells and leucocytes. The air vesicles, vessels, and lymphatic spaces are loaded with red globules.
Spleen.—It is adherent to the diaphragm and to the abdominal wall. The splenic substance is firm, and on section shows a reddish pulp with a few grayish spots. The Malpighian corpuscles are well defined, as also are the trabeculae.

Kidneys.—These are relatively small. The capsule strips with some difficulty, tearing in places and leaving a slightly rough surface, with a few retention cysts wedged in the renal substance. Section shows a cortex 5 millimeters thick, with a pale gray color in which one can distinguish the striae with difficulty, the glomeruli remaining completely invisible. Fœtal lobulations still persist. Microscopically there is a moderate hyperplasia of the intertubular connective tissue. The epithelium in many uriniferous tubules is loose and lying in the lumen. There is proliferation of round cells around the glomeruli and in the intertubular spaces.

Suprarenal capsules.—These are about normal in size. The cortex is thin and has a pale yellow tint with yellow areas scattered in the surface, and encloses a small quantity of milky white medulla.

Liver.—This organ is adherent to the diaphragm and high-toned in color, of firm consistence, and has a sharp border. Section shows a congested surface with clearly defined lobulations. Microscopically there is a rather extensive extravasation of blood between and over the hepatic cells. The central and interlobular veins are distended by the presence of red globules.

Gall bladder.—It is adherent to the under surface of the right lobe of the liver. It contains sirupy, greenish yellow bile. The walls of the viscus are slightly hypertrophied. The biliary tract is free.

Stomach.—It contains a dark red, viscid fluid mixed with the rugosities. There is slight hyperplasia of the lymphoid tissue.

Duodenum.—This organ is normal.

Pancreas.—It is of firm consistence. Section shows grayish lobulations, spotted with a bloody tint. The duct of Wirsung is free.

Intestines.—These are normal with the exception of the presence of a few ascarides. The mesenteric nodules are palpable, but not swollen.

Organs of the throat.—These are normal excepting a slight hyperæmia of the trachea. The thyroid gland is not markedly hypertrophied, but is slightly more bulky than normal. The thymus is atrophied and fatty.

Cerebrum.—This shows at its base an encapsulated bilobar tumor involving the pituitary body and its infundibulum. The capsule isolates it completely from the cerebral substance, and internally it is slightly adherent to the neoplastic contents. The two lobes of the tumor are joined one to the other, the anterior lobe being larger than the posterior. The former has a more or less spherical form with a diameter of 8 centimeters, lies between the pons varolii and the olfactory bulbs in the anteroposterior axis, and extends over the internal surface of the temporal lobes in the transverse axis. The second or smaller lobe is 3 centimeters in diameter, and is the portion of the tumor which, circumscribed by the circle of Willis, is lodged in the sella turcica. It is somewhat firm and of a clearer color than the larger lobe. Over the surface of both lobes there are various nodular growths more or less of the size of a pea. The tumor on the whole is soft, fluctuating, and can be entirely enucleated from the cranial floor. The surrounding cerebral substance is softer and more friable than normal. The contents of the tumor is soft, semiliquid, dark, and seems to be a voluminous hæmatoma with
grayish bands which cross through the mass in different directions. By
the side of the tumor passes the optic nerve which is more or less com-
pressed as well as the left olfactory bulb, and probably also the optic
chiasm. The third or oculomotor nerve appears to be intact. The base
of the encephalon is considerably compressed by the larger lobe of the
tumor, above which there is a well pronounced depression in the cerebral
substance. Also the base of the left frontal lobe appears so flattened
from behind forward that its anteroposterior diameter has been reduced
to half its normal size. The same compression is exercised over the
temporal lobes, increasing the distance between the two.

The cranium with the exception of a few small cavities, irregularly
margined, in the enlarged sella turcica, does not show any other
anomaly of importance.

Microscopical examination of the tumor.—The tissue was fixed and
stained by the method of Mallory for the neuroglia (phosphotungstic acid
and hematoxylin stain) and by the ordinary procedures of staining with
eosin and hematoxylin.

On the surface of the section there is a band of fibrous connective
tissue which represents the capsule. Immediately within this there is
a zone of cells, more or less closely packed, with round bluish nuclei
and a moderate quantity of protoplasm which possesses a certain affinity
for eosin, with outline more polygonal than circular, although there are
some few of the latter type. Interposed between these cells are some
rather pale-red globules. Sometimes these cells group themselves around
spaces more or less oval, simulating blood vessels loaded with red globules.
Some fusiform, long, delicate cells are insinuated between the groups
of cells.

The subjacent zone is composed of cells of almost the same size as
the preceding, with round nuclei, less dark, and with a small quantity
of transparent protoplasm, in whose periphery are projections of radiat-
ing apophyses which give to the cell a stellate shape. These appendices
appear to be connected with one another, but there are some free and
isolated. One can find, irregularly distributed, some spaces more or
less irregular, without a definite wall, and surrounded by one or two
rows, often interrupted, of cubical cells so similar to the stellate cells in
size that it is difficult to establish an exact distinction owing to the
close packing of the former. Within these spaces there seems to be
some homogeneous rosy substance. Inserted between these are found
spaces without evident walls, as a mechanical effect of mere separation
of the cells to give access to a group of red globules which, densely
packed in the cavity, spread themselves among the stellate cells of the
periphery.

The innermost or deepest zone offers a more remarkable aspect. Islets
are seen, generally connected with one another, isolated occasionally,
made up entirely of stellate cells identical with those of the anterior
zone; between these islets there are spaces which, insinuating themselves
between the cell masses, distribute the blood to all parts of the zone.
These extensive spaces lack walls, and their borders are simply limited
by the peripheral cells of the islets. Within the latter there are leuko-
cytes, probably of a migratory character as they are few in number.

Scattered between the last two zones are found stellate cells disposed
around a more or less oval space, apparently without lining, but examin-
The Diagnosis

From the considerations just adduced, it is evident that the occurrence of widespread haemorrhage owing to its vascularity with possible formation of new blood vessels, and largely the lack of evidence of metastasis anywhere in the body are facts that point undoubtedly to a diagnosis of telangiectatic glioma of the pituitary body.

Effect of the Tumor on the Circulation

The tumor was treated in the following manner: Pieces cut from the two lobes of the tumor were inserted in a flask and placed in running water to displace the formalin; after sixteen hours they were removed and pulverized in a mortar. To the powder thus obtained, its estimated weight being 5 grams, were added 30 cubic centimeters of normal saline solution. The mixture was then filtered, and the filtrate used for the injection. The carotid artery was used for taking the blood pressure, and intratracheal respiration was used for the respiratory curve.

Two cubic centimeters of the filtrate were injected into the circulation of a dog, and the effect was a rapid fall of the blood pressure. Schäfer and Herring (1) found that watery extracts of the intermediate and nervous portions of the normal pituitary body produced antagonistic effects on the blood vessels. They assert that in the first injections a vasoconstrictor action predominates while in the subsequent ones a depressor action prevails. From these facts, it is justifiable to suppose that the vasoconstrictor action of the pituitary body in the present case was totally destroyed by the tumor, only the active depressor principle remaining, or that both active principles were eliminated during the process of replacement of the pituitary tissue by the neoplasm, and that the depressor effect of the emulsion was due to decomposition, or that, at least, the vasoconstrictor principle had been dissolved in the formalin in which the specimen was preserved. That this latter is possible is demonstrated by the fact pointed out by Suzuki (19) that formalin dissolves or draws out the vasoconstrictor bodies in the suprarenal glands. In default, therefore, of a true biological explanation of the phenomenon, it is as well to point out here those three possibilities.
BIBLIOGRAPHY

(5) BOYCE and BEADLESS, Journ. Path. & Bact., Edinburgh (1893), 1, 359. Enlargement of the hypophysis in myxedema, with the following cases:
   Case 1. Tubercular-like Granuloma of the anterior lobe.
   Case 3. Enlargement of Hypophysis Cerebri.
   Case 5. Cystic tumor of Hypophysis Cerebri.
   Case 6. Case of Acromegaly with Cystic Hypophysis Cerebri.
   Case 7. Tumor of Hypophysis Cerebri (probably secondary).
   Case 8. Cystic tumor of Hypophysis (probably dermoid).
(7) BATTISCOMBE. Lancet (1888), 1, 970, 971. Abscess of pituitary body and sella turcica.
(9) HIPPEL. Ibid. (1891), 126, 124. A glandular tumor.
(10) MARIE. Brain, London (1889), 12, 59.
(11) PAULESCU. Journ. Physiol. (1907), 9, 523.
(13) RAYER. Arch. gén. méd. (1823), 3, 365. Infundibulum 8-9 lines in diameter.
(15) ROGOWITCH. Ziegler's Beiträge (1889), 4, 453.
(18) SCHNITZLER und EWALD. Wein. klin. Wochenschr. (1896), 9, 657.
(22) WEIGERT. Ibid. (1875), 65, 212, 219, 223. Teratoma, struma pituitaria, and gummata.
ILLUSTRATIONS

(Colored drawing by Castro; photographs by Cortes)

PLATE I

Fig. 1. Photograph from colored drawings of microscopical section of the hypophysial tumor.
2. Same as above.

PLATE II

Pituitary telangiectatic glioma.

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Figs. 1 and 2. Microscopical section of the hypophysial tumor.

PLATE I.
HiLARIG: TUMORS OF THE PITUITARY GLAND.

PLATE II. PITUITARY TELANGIECTATIC GLIOMA.
THE OUTBREAK OF PLAGUE IN MANILA DURING 1912

THE INSIDIOUS BEGINNING, WITH A DISCUSSION OF PROBABLE FACTORS CONCERNED IN ITS INTRODUCTION

By Victor G. Heiser

(From the Bureau of Health, Manila, P. I.)

One map

After an absence of six years in human beings, and five years among rats, plague was again found in the Philippine Islands on June 19, 1912. On account of the almost daily communication which Manila has with badly plague-infected foreign ports which are within a few days' steaming distance for the average vessel, and since, therefore, passengers, crews, rodents, and vermin may arrive well within the incubation period of the disease, it seems remarkable that the Philippines should have remained free from plague for so many years. During this period, plague has been detected from time to time, among human beings upon incoming vessels, but such infections were invariably intercepted at quarantine. All vessels plying between the Philippines and Oriental ports have been fumigated with sulphur, at not greater than six-month intervals, to destroy rodents and vermin.

In view of the fact that we are still in the midst of the outbreak, this paper will be confined strictly to a statement of fact, as it is not believed to be advisable, at this time, to attempt to draw any conclusions.

CHARACTER OF PLAGUE AT QUARANTINE

A most insidious form of plague was encountered at Manila last spring. On April 6, a death was reported on the steamer Zafiro which had arrived the day previous from Hongkong, and

1 Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4-7, 1912.
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had, therefore, been in the harbor, at the time of the death, for a period of twenty-four hours. At the medical inspection of the vessel made at the time of its arrival, no illness was detected. The investigation showed that the victim had been on deck on the night of April 5, apparently in good health. The next morning, at 6 o'clock, he was found dead in his bunk. The necropsy and subsequent biological findings showed that death had been due to pneumonic plague. On April 7, the steamer Loongsang arrived in Manila from Hongkong, and the captain reported that a death had occurred the day previous in a Chinese member of the crew. Upon an investigation of this case, the captain stated that the man was, apparently, in good health, but that while hauling on a rope he fell over in an apparent faint, was placed in a chair, and in the course of a few hours was dead. The necropsy and animal inoculation showed that he had died of pneumonic plague. From this time forward, the temperatures of all the crews and passengers on incoming vessels were taken as a further safeguard.

On the arrival of the steamer Taisang at the Mariveles Quarantine Station, about 6.30 a.m., April 30, 1912, the entire personnel was carefully examined and found free from sickness of a suspicious nature, nor were there any elevations of temperature. Seventy-three persons were detained. At the afternoon inspection, a passenger, Co Sing, aged 51 years, Chinese, was found to have a temperature of 39° C. and pulse of 100. He was immediately placed in the hospital, but protested vehemently that he was not sick. The man was carefully watched from the first. There was a slight cough; physical examination of the chest revealed only a few râles. Smears made of the sputum and stained for plague were negative. On the fifth day the fever still continued, but the patient still insisted that he was not ill, and demanded to be released from the hospital. On this day the expectoration was blood-stained, but no suspicious organisms were noted in the smears, nor could any physical signs of pneumonia be detected. No enlarged glands could be palpated anywhere. On the morning of the seventh day the temperature and pulse dropped, and the general condition was distinctly worse. The patient now admitted that he felt ill. Several hours later the patient flinched when pressure was made in the right axilla, and slight lymphatic enlargement was made out. By the evening of this day the bubo in the axilla had increased markedly in size, the outlines of the swelling being, approximately, 3 by 7 centimeters. Glands now became palpable in other portions of the body, particularly those in the cervical
region. There were both inguinal and femoral buboes. The condition of the patient became rapidly worse, and he died at 7 o' clock on the morning of the eighth day of his illness.

At necropsy, the glands of the right axilla and those of the right side of the neck were found considerably enlarged, and the lymphatic system generally showed enlargements. There was consolidation of the lower lobe of the right lung; the spleen was about twice the normal size. In general, the necropsy findings of a typical case of septicæmic plague were present. Smears made from the spleen and right axillary glands showed enormous numbers of typical bipolar-staining organisms. Cultures and fresh pieces of tissue, after animal inoculation had been made therefrom, were reported upon by the Bureau of Science as positive for plague.

HUMAN PLAGUE IN MANILA

On June 19, a Filipino employed as a watchman at 236 Calle San Jacinto, which is in the Chinese district, and who resided at 920 Calle Antonio Rivera, was found dead at his home, after an illness of about three days. On post-mortem examination, typical plague buboes were found in the right groin and axilla. Smears made from the spleen showed Gram-negative, bipolar-staining organisms, and inoculations made into guinea pigs resulted in typical attacks of plague. Rat catchings have been made regularly, at weekly intervals, in Manila, since the last cases of plague among rats, in 1906, and dead rats in which the cause of death was not obvious were sent to the laboratory, but no plague rats had been found since that time. The victim was a permanent resident of Manila, and had not been away from the city in many months. He lived far from the water front, did not associate with persons who had been out of the city, and since, so far as known, the nearest focus of the disease was Hongkong, the source of this infection is most difficult to explain.

The next case occurred on June 26 in a Filipina woman who lived at 1615 Calle Azcarraga, near the Arranque market. She was found alive, having been ill for three days, and, at the time she was transferred to the San Lazaro Plague Hospital, had a temperature of 41° C., and was in a moribund condition. The necropsy showed slightly enlarged glands of the left groin, but the other usual necropsy findings of plague were conspicuous by their absence. Smear preparations made from the glands of the groin and from sections of the spleen showed Gram-negative bipolar-staining organisms, and the subsequent biological exami-
nation proved positive for plague. The next case of plague did not occur until August 4—thirty-nine days later. This victim of the disease resided at 139 Calle Villalobos, in Quiapo. The case was followed by another, on August 8, at 129 Calle Villalobos (five houses removed from the former case), and by a third case, on August 21, at 352 Calle Echague. This address is just around the corner from where the Calle Villalobos cases resided. A noteworthy fact in connection with these three cases is that all were schoolboys, the oldest being 16 years of age. The disease then occurred at irregular intervals, there being but 3 cases in September, and, during October, to the 20th, there were 4 cases, all of which occurred on different streets, as may be seen from the accompanying map, the total of all cases to that date being 13. Then, between the dates of October 20 and October 22, 13 new cases occurred, so that, in a period of two days, there were as many cases as there had been during the four previous months.

EXPLOSIVE HUMAN OUTBREAK

An investigation of the large number of cases that occurred within the two days mentioned soon showed that they were all confined to laborers who worked at the Manila and Dagupan Railroad freight station. Large numbers of rats had been seen dying in, first, the north warehouse, and, a few weeks later, in the south warehouse. About three weeks after the heavy rat mortality was noticed in the north warehouse, plague appeared among the laborers in the south warehouse. It is believed reasonable to infer that the large number of cases of plague among these laborers is due to the fact that as the rat mortality was rapidly eliminating the normal supply of nourishment for the fleas, the latter began to attack human beings. This outbreak was brought to a speedy close by eradicating the rats and sprinkling the premises with kerosene to kill the fleas. The total number of cases in Manila, to November 5, was 33, and of these 30 died.

PLAGUE AMONG RATS

As soon as the first case of plague was discovered among human beings, an active rat-catching campaign was begun, efforts being concentrated to the vicinities in which human cases had occurred. Rats were caught at the rate of approximately 3,000 per month, but it was not until August 31 that a plague rat was found. This was caught in a spring trap at 351
Calle San Sebastian, which is in the same block in which the human cases on Calle Villalobos occurred. On September 7, a plague rat was found at 104 Calle Santa Rosa, and another at 215 Calle Echague, both of which addresses are within a block of the cases that had occurred on Calle Villalobos. On October 4, a plague rat was found at 644 Calle Ilaya, and another at 637 Avenida Rizal. Up to October 1 the percentage of infected rats found has been 0.005, which is unusually low, 2 per cent being considered a low average where human cases are occurring. At Hongkong, for instance, 7 per cent of the rats examined have proved to be plague-infected.

During the early part of October the rat-catching efforts were increased, and rats were being caught at the rate of approximately 9,000 per month. Other plague-infected rats were found, on October 16, at 520 Calle Jaboneros, 417 Calle Príncipe, and 614 Calle Salcedo; and on October 17, at 323 Calle Barcelona and 1057 Calle Padre Chavez. It is interesting to note that human cases were also found near all of these addresses.

**SANITARY MEASURES EMPLOYED**

Wherever a case of human or rat plague was encountered, the premises were immediately and thoroughly sprinkled with kerosene, and then disinfected in the usual way with some form of emulsion of a coal-tar product. The blocks surrounding the infected block were regarded as an infected center, and cleaning and rat-proofing measures were instituted from the periphery of such a zone and continued toward the center. This was done with the idea of driving the rats to the infected block and gradually eliminating them, and thus guarding against the spread of the infection throughout the city. For an infected center of this kind, approximately 75 laborers were employed whose duty it was to remove all accumulations of garbage and rubbish, and particularly to move wood piles, goods in warehouses, boxes, and other things among which rats might hide, and then to spray with kerosene. For the purpose of catching rats that attempted to escape during such operations, fox terriers were used with considerable success. In this outbreak, the experience had with former outbreaks, that is, that wood piles are great harboring places for rats, was repeated.

All householders in an infected zone were required to provide themselves with metal garbage cans at least 30 centimeters in height, with tight-fitting covers. The height was insisted upon particularly, in order to prevent dogs and cats from upsetting
the cans and throwing the contents into the street, thus furnishing rats with food.

In any house in which a case of rat or human plague occurred, in addition to the measures mentioned above, all hollow walls and ceilings were ordered removed and other rat proofing done.

In infected districts, wherever necessary, ground surfaces were ordered cemented, rat runs destroyed, and other rat proofing was carried out.

Wherever possible, the sewers were fumigated with sulphur dioxide, but, on account of the many openings in the sewers at unknown places, it is impossible to state whether rats were killed in them or whether they escaped upon the gas being applied. It was noted, however, that whenever the fumigation of the sewers was going on, the number of rats caught in traps or by poison always increased.

On account of the experience had in Java, special attention was directed toward bamboos which were large enough to harbor a rat, but, on account of the fact that human and rat cases nearly all occurred in houses which were built of strong materials, there was not much opportunity to make observations on this point.

At first, reliance was placed largely upon wire-cage and spring traps, but the results were not very encouraging. The usual formulas for rat poisons: Mixtures of bacon, various grains with powdered glass and strychnine sulphate or arsenous acid, and phosphorus pastes and preparations were tried, but none of these proved very successful. The poison then adopted consisted of arsenous acid and rice boiled together in the proportions of 1 to 5. This bait has been more successful than all of the others combined. It has proved especially satisfactory because it can be used day after day without the rats becoming suspicious of it.

A persistent campaign of education was carried on by means of the newspapers and by printed circulars which explained the method of transmission of the disease. The contents of these circulars were later taught in the public schools.

PROBABLE FACTORS CONCERNED IN THE INTRODUCTION OF PLAGUE INTO THE PHILIPPINES

Immediately after plague made its appearance in Manila, large numbers of rats were caught along the water front and around the wharves, but none of these were found to be plague-infected. On account of the fact that the first cases occurred among permanent residents, among persons who had not been out of Manila in many months, among persons who did not associate with
people who work along the water front or with persons who had recently been in a plague-infected country, it seems reasonable to infer that the disease was not introduced by human beings. On account of the facts that no infection has as yet been found among the rats of the water front, and especially since the wharves have remained free from rats and are practically rat proof, it does not seem probable that infected rats could have come from a ship by means of gangways, cargo chutes, or lines of a vessel. This is rendered still more unlikely because vessels from plague-infected ports are fumigated with sulphur at least twice annually. Rice vessels and other ships that are especially liable to have rats on board are fumigated upon every trip. After the detection of human cases, in April, on arriving vessels, all ships coming from Hongkong and Amoy were fumigated on every trip. All dead rats found aboard ships were sent to the laboratory, but all proved negative for plague. From the foregoing it is evident that, reasoning by exclusion, infected rats were probably introduced in cargo. This seems very probable, in view of the enormous quantities of food supplies and other cargo which come almost daily from plague-infected centers in China and Japan. For instance, there are literally thousands of baskets of eggs, garlic, onions, and similar foodstuffs among which rats could easily take refuge, that come from places like Canton or Amoy, within the period of five days. It is well known that plague has existed in Canton almost continuously during the past ten years, and it is not improbable that plague might have been introduced in this way. There are also large quantities of cargo arriving from Japan, especially glass and chinaware, and other things which are packed in hay and straw, and which afford favorite harboring places for rats.

The disease might have been introduced through the means of infected bedbugs. In a case of human plague which was taken from 508 Calle Magdalena, bedbugs were caught from the petate (straw mat) upon which the man died, and smears made from the intestinal contents showed plague-like bacilli; the pathological findings, however, were not confirmatory. It is not impossible that bedbugs may have been concerned in the introduction of the disease. On account of the fact, however, that all second- and third-class passengers' clothing and effects are steamed at Mariveles, it does not seem likely that bedbugs could have been introduced with them.
ILLUSTRATION

Map of Manila, showing districts where plague cases occurred.

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Heine: Plague in Manila.

MAP OF MANILA, SHOWING DISTRICTS WHERE PLAGUE CASES OCCURRED.
THE PLAGUE OUTBREAK IN ILOILO ¹

By CARROLL FOX ²

(From the Bureau of Health, Manila, P. I.)

One map

The outbreak of plague in Iloilo was a small circumscribed epidemic, occurring in the absence of demonstrable rat infection.

A campaign against the disease was inaugurated upon the theory that infected rats had been introduced and an epizootic had developed among the rodents of Iloilo as a result. All the precautions usually taken under such circumstances were immediately observed, such as rat catching and poisoning, rat-proofing and the elimination of rat-breeding and rat-feeding places. In addition to this, a large amount of general sanitary work was performed, such as general cleaning up, repairs and alterations to old structures, and the vacating of unsanitary dwellings. Before the work had sufficiently advanced to expect any reduction in the number of plague cases, the disease suddenly ceased. In the meantime, no infected rats had been found nor have any been found at any time since, notwithstanding the continuance of rat catching, with laboratory examination.

Upon a study of the map of Iloilo, it will be observed that there were two distinct foci of human infection; one focus comprised of one house in the nipa district and resulting in 4 cases with 4 deaths, all Filipinos; the other focus comprised of three closely associated houses in the hard material district, and resulting in 4 cases with 4 deaths, all Chinese. These foci are shown on the map as X and Y, respectively.

At the end of July, the first case occurred in house "A" followed by a case in house "B." Then there was a case in house "C." After a short period 2 cases died the same day in

¹ Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4–7, 1912.
² Passed assistant surgeon, U. S. Public Health and Marine-Hospital Service; Assistant Director of Health for the Philippine Islands; associate professor of hygiene, College of Medicine and Surgery, University of the Philippines.
the same house, some distance away from either focus. Investigation showed that they had been living in house "C," having left there within the week and being sick at the time of leaving. They were unquestionably infected in house "C." Following this, there developed a case at house "D," another at house "C," and another (the last) at house "D." Eight cases in all occurred within a period of less than two months.

The occurrence of a fourth case in each focus would indicate that previous disinfections had not proved efficacious. Therefore, the entire work of disinfection was repeated with petroleum and kreso. Walls, floors, beds, and chairs were thoroughly sprayed with a view toward exterminating bedbugs. In nipa houses it is difficult to reach bedbugs lurking between the layers of nipa, and, therefore, after disinfection, house "C" was vacated and closed for a period of two months. Houses in the immediate vicinity were also treated to kill bedbugs.

Double walls and ceilings in house "D" were torn out, and special effort made to exterminate the bugs.

The writer is of the opinion that this outbreak of plague was an instance of bedbug transmission, starting with a case of human plague, introduced into Iloilo from Manila, where plague had previously appeared, or possibly from a plague-infected foreign port, the reasons being as follows:

1. The absence of rat infection.
2. The decidedly circumscribed foci.
3. The sudden cessation of the outbreak after the houses believed to be infected had been thoroughly treated to kill vermin.
4. House "C" was owned by a Chinaman, who was as well one of its inmates, and who undoubtedly had at least business intercourse with the Chinamen living in the focus Y, and who could have readily carried infected bedbugs from one focus to another.

It is regretted that an effort to secure bedbugs was not made earlier in the campaign so that inoculation experiments could have been carried on to prove or disprove the validity of the theory in this case. However, that bedbugs may and often do carry plague infection has been proved beyond a doubt by various observers.
ILLUSTRATION

Map, showing plague foci in Iloilo.
MAP SHOWING PLAGUE FOCI IN ILOILO.

Scale 1: 2,500.
SOME CARBOHYDRATE REACTIONS OF THE DYSENTERY BACILLUS

By C. S. Butler

(From the Laboratory of the United States Naval Hospital, Cañacao, P. I.)

Ever since the carbohydrate reactions were elected to a wider field of usefulness in bacteriology, largely through the work of Theobald Smith,(1) there have been differences of opinion among authors as to their reliability. One need only compare the fermentation tables published by different authors to note differences as regards the same organism. In some cases the same author will give different results for the same bacterium. Thus in the fourth edition (1910) of Pathogenic Bacteria and Protozoa by Park and Williams, on page 257, we find in a chart that Bacillus dysenteriae (Shiga) ferments with acid production the carbohydrates dextrose, maltose, dextrin, and mannite. On page 275 of the same volume, the authors state that the dysentery bacillus produces neither acid nor gas in glucose bouillon, while on page 281 they state that the Shiga type of dysentery bacillus does not ferment mannite, maltose, nor saccharose. Here are two statements of error to one statement of fact, and while it would not bother one who was actually working with the subject, yet it does not better the general opinion of the carbohydrates as differential agents to find such opposing statements about them. Other instances of this kind could be mentioned, but it is only intended to indicate that at times the carbohydrates may not, from one reason or another, have received a fair appraisement of their real value, and it is the purpose of this paper to try and explain a few of these opposing results. As a purely theoretical proposition, it would seem logical to conclude that, if a pure culture of a given bacterium is introduced into a medium made always of the same materials and constant in reaction, with a given amount (say, 1 per cent) of a chemically pure carbohydrate added, the bac-

1 Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4–7, 1912.
2 Surgeon, United States Navy; and lecturer on the pathogenesis of physical agents in the Tropics, University of the Philippines.
terium should always react in a constant manner under constant conditions of time and temperature. In testing the truth of this statement, we have been led to the belief that, so far as the dysentery bacilli are concerned, the proposition is almost if not quite true, and that, so far as the practical determination of strains is concerned, it may be accepted as a fact.

Lehmann and Neumann (2) divide the dysentery bacilli into four strains according to their action upon mannite, maltose, and saccharose. The strains thus differentiated are: (1) Shiga-Kruse, (2) Flexner, (3) Strong, and (4) Bacillus Y (Hiss-Russell). The table of differentiations is as follows:

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Shiga-Kruse</th>
<th>Flexner</th>
<th>Strong</th>
<th>Bacillus Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannite</td>
<td>Blue</td>
<td>Red</td>
<td>Red</td>
<td>Red</td>
</tr>
<tr>
<td>Maltose</td>
<td>Blue</td>
<td>Red</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Saccharose</td>
<td>Blue</td>
<td>Blue</td>
<td>Red</td>
<td>Blue</td>
</tr>
</tbody>
</table>

Other writers, basing their opinions upon the carbohydrate reactions, would differentiate them still further. It is a matter of doubt whether the group would stand further differentiation than that just indicated, provided we use pure carbohydrates and properly control our results; and most bacteriologists are content to recognize two strains of dysentery organisms; namely, those which ferment mannite and those which do not. It is generally admitted that these two types, aside from their fermentative differences, also show differences in their capacities to produce toxin and to cross-agglutinate and cross-bacteriolysise. It is doubtful whether there is any practical advantage to be derived from going further than the recognition of these two types. But it is certainly a fact that, among those which produce acid in mannite solutions, there are strains which will and others which will not ferment maltose, and there are also strains which will and others which will not ferment saccharose. These last-named saccharose fermenters seem to be very rarely isolated from dysenteric stools, and their fermentation of saccharose seems much slower and less decisive than does the action of these bacteria as a group toward other carbohydrates upon which they will act. In order to get comparable results with these reactions, it is evident that the methods employed must be comparable. It is also found that the results vary within certain limits when dealing with the same set of organisms, depending upon whether solid media or liquid media are used and also upon whether or
not the reaction of the media has had to be previously arranged. Some carbohydrates suffer inversion to a lower order if subjected to heat in the presence of weak acid or alkali, and in the finished product we will then find for instance that a supposed Shiga strain of the dysentery bacillus is producing acid in the maltose medium. As a matter of fact, it is simply showing its capacity to ferment glucose inverted from maltose. The question of the indicator is also to be considered. The one generally used is litmus, and this, if subjected to long heating in the presence of organic substances, bleaches out and we are left with a desensitized litmus. The use of litmus, too, as a part of the medium rather than as a pure indicator is a matter to criticize. What chemist would expect his indicator to show a fine shade of reaction if he incorporated that indicator into a mass of organic material, sterilized it on three successive days at 100° C., and then stored it in the ice box for perhaps a month? I have found on a number of occasions that the results varied, particularly when dealing with maltose, if the organism be allowed to grow for about three days in the peptone sugar solution, and the indicator be then run in and the results read immediately, from the results obtained by growing the same organism in the same solution, but with the litmus added from the start. If, however, the tubes, to which the indicator was added at the end of the incubation, are set aside for a few hours, they will nearly always show the same results as those to which the litmus was added in the first instance. These statements are not of much importance from the standpoint of indicating variability in the carbohydrate reactions of dysentery bacilli, because with the thousands of trials to which these organisms have been subjected by different observers I believe we are in possession of the exact facts as to what the different strains will do. But they do indicate, it seems to me, that for the individual bacteriologist, identifying strains of dysentery bacilli from the stools, the media and the methods employed should be revised and subjected to standardization. On account of our lack of standards, combined with the inherent tendency of some of our differentiating carbohydrates to change during manipulation, I believe we are overlooking at least one perfectly valid variety of Bacillus dysenteriae; namely, Bacillus Y.

It is manifest that the simpler the solution into which any given carbohydrate is introduced, always provided that the bacterium will grow luxuriantly in it, the more nearly the facts will be revealed. If we could get organisms to grow in a simple
solution of the particular carbohydrate in distilled water, the reaction would be reduced to its simplest terms. But the large majority of microorganisms require proteid material in the medium, which of course introduces another factor into the equation and makes the reaction a resultant of two forces rather than the effect of one. As was intimated, the use of media for carbohydrates which require to be arranged in reaction is open to objection. It is also objectionable to use solid media for the reason that liquid media are more sensitive, and also because upon the surface of solid media where free oxygen is at hand many organisms will not ferment the carbohydrate in order to get oxygen. This latter fact is made use of in the Russell medium for identifying varieties of bacteria, but in finding out how a given organism will react toward a given carbohydrate the fluid media seem to be better. That our media should be sterile need not be emphasized. That it often is not sterile after three successive heatings for twenty minutes in the Arnold sterilizer I have found such a common experience that I have discontinued this method of sterilization of carbohydrates, and now use only the autoclave. So far, I have not found that heating for twenty minutes at 20 pounds' pressure has produced changes in any of the carbohydrates, when prepared as described below. After sterilization, the tubes are placed overnight in the incubator; such as are not sterile are discarded next morning, and the inoculations made as soon as possible. Were it not for evaporation, the 37° incubator would be a far safer place for storage of sterile media than is the ice box. It is probable that some of the varying fermentative reactions given for the dysentery bacillus have been due to contaminative organisms, and it might be added in passing that the only proper control of a "crooked fermentation" is the actual plating out and reidentification of the organism from the freshly isolated culture.

The medium which has been found to give the most constant results has been a peptone solution in distilled water—Witte's peptone, 1 per cent; the desired carbohydrate (the purest obtainable), 1 per cent; sodium chloride, chemically pure, 0.5 per cent; made up with distilled water. The medium is then distributed in test tubes, 10 cubic centimeters in a tube, and is sterilized at 20 pounds' pressure for twenty minutes. Then to each tube is added 2 cubic centimeters of a 5 per cent filtered litmus solution which has been autoclaved separately. This litmus solution is boiling at the time introduced, and the tubes are then heated in the Arnold sterilizer for fifteen minutes to ensure sterility. After
incubating overnight and culling out any infected tubes, the
medium is ready for inoculation. The objections which may
be offered to this medium relate to (1) the low content of carbo-
hydrate, (2) the high temperature to which the carbohydrate
is submitted, (3) the high temperature to which the litmus
solution is submitted, and (4) the large amount of litmus. As to
the last three objections I will only say that in my hands
this method has given better results than any other. As to the
first objection, that regarding the low content of carbohydrate,
a few words of explanation are necessary. The effects which
bacteria may produce upon carbohydrates are (1) to ferment
with acid production, (2) to ferment with acid and gas pro-
duction, and (3), the negative effect, to let the carbohydrate
alone entirely. The intestinal bacteria and practically all rapidly
growing bacteria show what they are going to do to any par-
ticular carbohydrate within five days, and most of them within
forty-eight hours. This statement does not refer to slow-grow-
ing organisms such as the tubercle bacillus. With organisms
which produce gas this can be shown by accurately marking off at
6-hour intervals the amount of gas in the closed arm of the
fermentation tube. There comes a time within three days, pro-
vided we have not used too much carbohydrate, when the gas
production stops, and if we measure at that point we shall find,
at the next reading, that the fluid has risen slightly above our
last mark. The organism has done all it will ever do to the
carbohydrate introduced, and the medium has taken up its limit
of gas at the existing temperature and pressure. If we give
it more carbohydrate than it can ferment, the organism will stop
growing when a certain degree of acidity is reached. Bacteria
do not produce alkali from carbohydrates, but if we leave our
inoculated tubes for two or three weeks in the incubator, con-
taminations, concentration of the medium, or the splitting of
proteid may result in a change of our original picture. We are
in that case, however, not reading the effect of the bacterium
upon the carbohydrate, but upon some other constituent of the
medium, or perhaps the effect of chemical reactions not connected
with the organism at all.

In this paper space does not permit a consideration of all the
qualities and peculiarities of the 8 or 10 carbohydrates with
which I have worked. My attention was so soon focused upon
the sources of error in the fermentation tests of the important
carbohydrate, maltose, that I have not attempted to study criti-
cally the other carbohydrates from a bacteriological standpoint.
Suffice it to say that some of them will stand almost any kind of treatment without change, while some specimens of maltose sent out by our best manufacturers are already partly inverted when the packages are opened.

My plan was to study the reactions of a large number of strains of the dysentery bacillus, by running along with these a considerable number of other organisms whose exact identity was known. The same set of organisms was always planted along with the dysenteries into any given lot of medium. In this way a score of trials of the bacterial action on the several carbohydrates have been made during the past four months. Sixteen strains of *Bacillus dysenteriae* have thus been tried a number of times against 16 other organisms under exactly similar conditions. Some of the dysentery strains were laboratory cultures for which I am indebted partly to Maj. P. M. Ashburn of the Tropical Board, and partly to Dr. M. A. Barber of the Bureau of Science. Other strains were isolated from the dysenteric stools of patients. The advantages of this method of multiple bacterial cross control will upon reflection be evident. If, for instance, as was frequently noted to be true, the 8 or 10 strains of Shiga's bacillus in the series were found to ferment maltose when they had refused to ferment mannite, it was hardly likely (with the precautions taken to guard against contamination) that all of these could be explained on the basis of mixed cultures. And, if all the other bacteria in the series acted in this set as they should have acted upon glucose, it is pretty evident that the fault lay in the maltose. If, however, an isolated tube in the series gave some unexpected result, it indicated an impure culture. That culture was then plated and reidentified. If the lactose had been hydrolysed in the manipulation, all the bacteria in the series would act not as upon lactose, but as they should act upon dextrose and galactose. If the sucrose has not remained intact, but is inverted, the baterial series will act, not as upon sucrose, but as upon dextrose and levulose. In working with the carbohydrates in this way it is soon evident that if we start with a chemically pure carbohydrate (which alone should be used in bacteriology) and do not add to it acids and alkalies, it will stand one decisive autoclaving better than it will heating for three successive days in the Arnold sterilizer. It is not requiring too much of any substance used in bacteriological work that it be able to stand the manipulations necessary to get it sterile, and, if it will not do this without change, it should be rejected. It was very soon
found that all the specimens of Kahlbaum's maltose, which we had in the laboratory, showed the fermentations characteristic of glucose, when used in fluid media. Specimens of maltose were obtained from several laboratories in Manila, and all those of Kahlbaum manufacture gave the same result, regardless of whether the sterilization was intermittent or under pressure in the autoclave. A sample of Merck's maltose was obtained from the Bureau of Science which gave the fermentative characters of uninverted maltose. Barfoed's test for monosaccharids was tried upon the Kahlbaum and the Merck maltoses, and this seemed to substantiate the idea that the Kahlbaum preparation had some glucose in it. A sample of Kahlbaum's maltose which had been in the laboratory for about one year was submitted to the Bureau of Science for analysis. The result is as follows:

\[
\begin{align*}
(a) \, D &= 121.73 \\
\text{Water (per cent)} &= 6.72 \\
\text{Corrected (a) \text{d}^5} &= 130^\circ.5
\end{align*}
\]

Osazone test shows the presence of a small quantity of dextrose. The sample contains other impurities, some of which are insoluble.

The specific rotation of anhydrous maltose should be:

\[
(a) \, D = 139^\circ.2.
\]

I cannot explain why Kahlbaum's preparations of maltose showed the reactions of dextrose while the one specimen of Merck's did not. The Merck's had been in the storeroom of the Bureau of Science certainly for over a year and very likely for five years. The Merck's package had not been opened, while all the specimens of Kahlbaum's except one had been opened for some time. I am inclined to explain the result, however, as due to the inherent tendency of the maltose molecule to take up water and change into two molecules of dextrose. In solid media (maltose agar) inoculated with the nonacid strain of *B. dysenteriae*, the change is hardly manifest as the small amount of glucose present is only fermented in the depths of the stab culture, and the slight reddening is confined to the line of stab and is not manifest upon the surface. But in maltose-peptone solution, where all the glucose present is changed and where any change is reflected throughout the medium, the result is a completely red culture, whereas in a parallel tube of uninverted maltose containing the same bacterium it is entirely blue. If we grant that this tendency of maltose to invert is pretty general, and I believe that

\[5\text{Analysis by R. R. Williams.}\]
it is, we can easily see the effect it would have upon the determinations in this group alone. The Hiss-Russell bacillus depends upon maltose for its differentiation, and if the maltose is partly inverted this type will not be identified. An investigator using a partly inverted maltose would be led astray so far as identifying this type of dysentery bacillus is concerned, while if he tried to determine the reactions of the dysentery bacilli as a class to the three carbohydrates, glucose, mannite, and maltose, he would find his Shiga strains fermenting glucose, not fermenting mannite, but fermenting maltose. Mannite tends to preserve its identity, while maltose is unstable and tends to change. The inclination would be to explain this upon the basis of inconstancy in the fermentative action of the bacterium, whereas the cause is to be found in the impurity of the carbohydrate. Long experience has shown that the dysentery bacilli as a class ferment the three substances named above in the following order: First most readily glucose, next mannite, and third and least readily maltose. Ohno,(3) in a study of types of dysentery bacilli in 1906, found a number of organisms which, in acting upon these three carbohydrates, skipped mannite, but fermented maltose. He makes no mention of having ascertained whether or not his carbohydrates were pure. If he had his maltose partly inverted to dextrose, it would explain why many of the sera produced by his acid types agglutinated nonacid types just as well as they did the organisms used in their production.

Castellani(4) in a recent article publishes a table of cultural characters for certain intestinal bacteria, in which he states that the Shiga bacillus may or may not ferment maltose. In the same table the Hiss-Russell bacillus is put down as producing acid in maltose. Now the nonfermentation of maltose is the one differentiating character of the Hiss-Russell bacillus, and if we remove that it becomes a Flexner type. I have found in the series of organisms with which I have worked that several which were at first thought to be Flexner strains were really Hiss-Russell strains. They could be made to give the fermentations of the Flexner bacillus by simply changing from Merck’s to Kahlbaum’s maltose. It is believed that this instability of maltose has been generally encountered and that many of the varying results obtained by different observers may be attributed to it. If we recognize this same principle as applying also to other carbohydrates in different degrees, we can easily see that the promiscuous use of carbohydrates will, when no control is used to detect impurity or inversion, lead to wide differences in
the results obtained by different men when working with the same bacteria. In the admirable article on bacillary dysentery in Allbutt's System of Medicine, Flexner(5) states that "the number of variants among this group of bacilli is considerable, and it is probably incorrect to continue the subdivision into type indefinitely." This statement is one which we would do well to take to heart. But I believe that when the cause of the apparent variability is identified, it will not all be explained on the basis of the bacilli doing one thing one day and another the next, but rather due to the media and the methods. It would be in the interest of exact knowledge and of practical bacteriology if some scientific body or laboratory took up the question of standard methods in carbohydrate work as applied to bacteriology. During the past four months I have inoculated several thousand carbohydrate tubes with the same strains of bacteria, and in only one instance have I been unable to explain variable results by a change in the sugar or else contamination, and it is my belief that the reactions of bacteria toward pure carbohydrates are as fixed and definite as any other characters used in the identification of microorganisms.

REFERENCES

(5) Allbutt and Rolleston. A System of Medicine (1907), 2, Pt. 2, 491 and 492.
ISOLATION OF DIPLOCOCCUS INTRACELLULARIS MENINGITIDIS WEICHESELBAUM FROM A CASE OF CEREBROSPINAL MENINGITIS OCCURRING IN A NATIVE OF THE PHILIPPINE ISLANDS

By David G. Willets and Otto Schöbl

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

It has been known for several years that specific cerebrospinal meningitis is present at times in the Philippine Islands. Strong and Musgrave found 12 typical cases among American soldiers at the Division Hospital, Manila, during the winter of 1899 and 1900. Several of the patients died, and the organism was isolated at necropsy. Musgrave also isolated the organism from a Filipino who came to necropsy in 1903; this case had not been diagnosed ante mortem. We are informed that Whitmore likewise recovered it from a case of cerebrospinal meningitis, and two or three additional cases have been discovered at necropsy in the Philippines by other pathologists. One of us (Schöbl) found a Gram-negative, intracellular diplococcus in a specimen of cerebrospinal fluid sent to the Bureau of Science for examination. Cultures made from the fluid were negative and no more material was available. A Gram-negative, intracellular diplococcus was also found by one of us (Willets) in the cerebrospinal fluid of a child presenting typical symptoms of cerebrospinal meningitis; this patient came to necropsy when pronounced evidences of acute miliary tuberculosis were disclosed. It is suspected that the child had an intercurrent infection with the meningococcus. However, the cultures made from the cerebrospinal fluid obtained by lumbar puncture and at necropsy were negative for diplococci; hence the case was unsatisfactory. One of us (Willets) recently saw a child in the Batanes Islands whose previous history suggested an attack of the disease. Dr. A. G. Sison of the Philippine General Hospital has seen cases in St. Paul's Hospital, Manila, and in the Philippine General Hospital, Manila, which he believes to have been specific cerebrospinal meningitis, but the organism was not isolated either from the cerebrospinal fluid or at necropsy in any one of them.

1 Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4–7, 1912.
Thus considerable evidence is adduced that specific cerebrospinal meningitis occurs in the Philippines. There is, however, no definite record of a case occurring in a Filipino in which the organism has been isolated and studied culturally. For this reason, it is considered to be worth while to report the findings in the present case, in order that this disease may be definitely added to the nosology of the Philippines.

Dr. Eleanor J. Pond, in whose service the case occurred, has furnished the clinical data.

Gregorio Pacheco, a 6-year-old boy of Malolos, Bulacan Province, was admitted to the Mary Johnston Hospital, Manila, on September 6, 1912, with a history of having had fever on September 2. On September 3, he had projectile vomiting. On September 4, vomiting was absent; the patient was semi-conscious, and he showed some retraction of the head.

On admission to the hospital the patient was restless and somewhat delirious. Opisthotonus was marked; Kernig's sign and trismus were present; the temperature was 39°.1 C.; pulse, 125; and respiration, 33. On September 7, the restlessness was greatly increased; the body was rigid; the jaws were stiff; opisthotonus, Kernig's sign, trismus, and photophobia were present; and touching the patient produced tremors. Fifteen cubic centimeters of very turbid, cerebrospinal fluid were removed by lumbar puncture. The patient improved rapidly after this operation. On the following day, the muscles were slightly relaxed, and on the second day he was even less uncomfortable, and took milk and ice by mouth. The blood picture at this time was as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hæmoglobin</td>
<td>95 per cent.</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>4,400,000</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>21,000</td>
</tr>
<tr>
<td>Polynuclears</td>
<td>74 per cent.</td>
</tr>
<tr>
<td>Small lymphocytes</td>
<td>15.5 per cent.</td>
</tr>
<tr>
<td>Large lymphocytes</td>
<td>6.0 per cent.</td>
</tr>
<tr>
<td>Transitionals</td>
<td>1.0 per cent.</td>
</tr>
<tr>
<td>Large mononuclears</td>
<td>2.0 per cent.</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>0.5 per cent.</td>
</tr>
<tr>
<td>Mast cells</td>
<td>1.0 per cent.</td>
</tr>
</tbody>
</table>

An urinalysis made on September 10 was as follows: yellow, acid, cloudy; specific gravity, 1.018; a trace of albumin; no sugar; ammonium urates in abundance; mucus; no pus and no casts. The temperature gradually subsiding became normal on September 11, the tenth day of the disease and the fourth day after lumbar puncture. On September 12 an offensive
ozāna, which persisted for two weeks, appeared. Blood examination made on September 13 resulted as follows:

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes</td>
<td>10,500</td>
</tr>
<tr>
<td>Polynuclears</td>
<td>64 per cent.</td>
</tr>
<tr>
<td>Small lymphocytes</td>
<td>20 per cent.</td>
</tr>
<tr>
<td>Large lymphocytes</td>
<td>5 per cent.</td>
</tr>
<tr>
<td>Transitionals</td>
<td>6 per cent.</td>
</tr>
<tr>
<td>Large mononuclears</td>
<td>2 per cent.</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>3 per cent.</td>
</tr>
</tbody>
</table>

On the following day, the urine showed a trace of albumin, otherwise nothing abnormal. On September 16 the patient sat up for fifteen minutes and asked for bread. Light was still displeasing. Fecal examination revealed the presence of a few ova of *Ascaris*, *Trichuris*, and hookworm, and much mucus. On September 19 an afternoon rise of temperature to a fraction below 40° C. occurred, and the patient vomited repeatedly. During the succeeding five days there was a daily rise of temperature and headache was present. The temperature then returned to normal. On October 1 profuse perspiration was present most of the morning, and for three or four days thereafter a rise of temperature occurred once in twenty-four hours; this apparently yielded to the administration of quinine and aspirin. On October 8 the blood picture was as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hæmoglobin</td>
<td>95 per cent.</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>4,200,000</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>13,000</td>
</tr>
<tr>
<td>Polynuclears</td>
<td>74 per cent.</td>
</tr>
<tr>
<td>Small lymphocytes</td>
<td>18.5 per cent.</td>
</tr>
<tr>
<td>Large lymphocytes</td>
<td>3.5 per cent.</td>
</tr>
<tr>
<td>Transitionals</td>
<td>2.0 per cent.</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>0.5 per cent.</td>
</tr>
<tr>
<td>Mast cells</td>
<td>1.0 per cent.</td>
</tr>
</tbody>
</table>

The blood was negative for malarial parasites. His appetite was poor after the rise of temperature on September 19; hence the patient became markedly emaciated. He appeared to be very homesick; therefore, his father was allowed to take him home on October 11. This change seems to have been beneficial, for his father stated on October 30 that the boy was gaining weight and walking about.

The specimen of cerebrospinal fluid was received by us on September 7, six hours after the puncture had been made by Dr. A. M. Saleeby, visiting physician at the Mary Johnston Hospital. It was very turbid, and contained numerous grayish white flakes. Smears from the sediment obtained by centri-
fuging for about twenty minutes disclosed a moderate number of biscuit-shaped, Gram-negative diplococci, which were chiefly within polynuclear cells. The polynuclears were very numerous; mononuclears, rare. Cultures on Loeffler’s blood serum showed a pure growth of a Gram-negative diplococcus on the following day. Subsequently, transplants were made to two tubes of each of the following culture media.

<table>
<thead>
<tr>
<th>Solid media.</th>
<th>Stabs.</th>
<th>Liquid media.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole human-blood acid agar-agar.</td>
<td>Lactose litmus agar + 1.</td>
<td>Peptone + 1.</td>
</tr>
<tr>
<td>Loeffler’s blood serum.</td>
<td>Glucose agar.</td>
<td>Lactose bouillon.</td>
</tr>
<tr>
<td>Agar-agar + 1.</td>
<td></td>
<td>Plain bouillon + 1.</td>
</tr>
<tr>
<td>Agar-agar — 1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerine agar-agar.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In no case was the growth luxuriant. The best results were obtained with the whole human-blood acid agar-agar, while those on Loeffler’s blood serum ranked second. The growth on acid and alkaline agar-agar and on glycerine agar-agar was very scanty, only isolated colonies developing. Potato media gave negative results. Of the stab transplants, one of each variety gave a very slight growth along the line of puncture, and one of each resulted negatively. No growth was obtained in fluid media excepting in one peptone tube, and this was unsatisfactory since it was contaminated with staphylococci.

The isolated colonies were somewhat elevated, yellowish, moist, and rather irregular in outline, regardless of the culture medium used. As they became older, they were white and dry with serrated edges. Smears from the cultures showed the diplococci lying for the greater part in single pairs. Tetrage-noid forms were not infrequent; clumps were present; but chains were absent.

The organisms behaved variously toward Loeffler’s methylene blue. Those from fresh cultures took the stain well as a rule, whereas those from a 48-hour culture took it poorly. Many of them were swollen in these smears, thus losing some of their biscuit shape. It was necessary to make transplants not less than once in forty-eight hours, in order to keep the culture alive.

Fermentation tests gave positive results with glucose and maltose; negative results with saccharose, lactose, and mannite.
Microscopic and macroscopic agglutination reactions were present. On September 13, the twelfth day of the disease, positive microscopic results were obtained in one hour in dilutions of 1 to 20 and 1 to 40 of the patient's whole blood. On the following day macroscopic agglutination was present in dilutions of from 1 to 6 up to 1 to 384 of the patient's blood serum, after incubating at 37° C. for two hours and allowing to stand over night.

Attempts to produce the disease in monkeys and recover the organism in pure culture were unsuccessful. The experiments were as follows:

On September 9, monkeys 6403 and 6404 were trephined. One cubic centimeter of a thin suspension of the diplococci obtained from a 28-hour culture on Loefler's blood serum was injected beneath the meninges of monkey 6403. Rather profuse bleeding rendered the operation unsatisfactory, since the meninges could not be clearly seen while the injection was being made. Monkey 6404 received 1 cubic centimeter of the 52-hour specimen of cerebrospinal fluid taken on September 7, the flakey material being avoided in so far as possible. The field of operation was clear in this case, the meninges being visible throughout the injection, and hence the operation was quite satisfactory.

During the following day the monkeys were nervous, uncomfortable, and each had a temperature of 40°C. On the second day they were greatly improved, and thereafter gradually returned to normal. No convulsions or tremors were noticed in either animal at any time after operation.

On September 17, 0.2 cubic centimeter and 1 cubic centimeter of a suspension of organisms from several 24-hour Loefler blood-serum cultures were injected into the spinal canal of monkeys 6405 and 6408, respectively.

Monkey 6408 was found dead at 7 a.m. the following morning. Necropsy was performed at 3 p.m. Smears from 4 different levels of the spinal cord showed Gram-negative, extracellular and intracellular, biscuit-shaped diplococci. Cultures from the heart's blood and the spleen were negative, while those from the four different levels of the cord gave a mixed growth of Gram-negative diplococci and staphylococci. We were unable to secure the diplococci in pure culture.

Monkey 6405 on the day after the operation was very uncomfortable, nervous, easily frightened, and found repeatedly lying upon its side. However, no tremors nor convulsions were observed. The temperature was 40° C. On September 19, approximately forty-two hours after injection, a small amount (0.2 cubic centimeter) of cerebrospinal fluid was removed by puncture; this gave Gram-negative diplococci in smears, but cultures on Loefler's blood serum were negative. The animal improved daily, and apparently returned to normal.

On September 27, 1.0 cubic centimeter, 0.6 cubic centimeter, and 0.1 cubic centimeter of a suspension of diplococci from several 24-hour Loefler blood-serum cultures were injected into the spinal canal of monkeys 6417, 6406, and 6407, respectively. On the following day all three animals were in good condition. The temperature of each was only slightly elevated. Cerebrospinal fluid obtained from each animal approximately eighteen
hours after injection was negative for diplococci. Each of the animals continued to be in good health.

Believing that the organism was becoming more and more attenuated, no further experiments were attempted. The failure to produce the disease in monkeys is attributed to the low virulence of the particular strain with which we were dealing. The clinical course of the disease in the patient lends itself to this belief.

The agglutination reactions obtained with the organism and the patient's blood, the location of the diplococcus, its presence within the polynuclear cells, its morphology, its staining properties, and its cultural characters, as herein outlined, all form a chain of evidence which, even in the absence of successful animal experiments, convinced us that it is identical with *Diplococcus intracellularis meningitidis* Weichselbaum.

While this article was in type the meningococcus was isolated by Dr. R. W. Hammack, College of Medicine and Surgery, University of the Philippines, at autopsy from a case of meningitis occurring in a Filipino 26 years of age. The organism fulfilled all of the cultural, morphological, and staining characters of *Diplococcus intracellularis meningitidis*. This is the only case from which the meningococcus has been isolated in 2,371 autopsies performed at the College of Medicine and Surgery.
THE DURATION OF PASSIVE IMMUNITY AGAINST TETANUS TOXIN

By E. H. Ruediger

(From the Section of Sera and Prophylactics, Biological Laboratory, Bureau of Science, Manila, P. I.)

In a previous communication I reported that in guinea pigs passive immunity conferred by antitetanic serum from horse is of short duration, perhaps not longer than three weeks.

The experiments to be reported are grouped under the following three heads:

1. The duration of passive immunity in horse, after the injection of homologous antitetanic serum.

2. The duration of passive immunity in guinea pigs after an injection of antitetanic serum from horse, preceded by repeated injections of antitetanic serum from horse.

3. The duration of passive immunity in guinea pigs after an injection of antitetanic serum from horse, preceded by repeated injections of normal horse serum.

EXPERIMENT NO. 1

In September, 1911, three large, apparently healthy horses, Nos. 1, 2, and 3, were obtained for this purpose. Horse 1 received 1,500 units of antitetanic serum from horse injected subcutaneously on September 9, 1911. Horse 2 received a subcutaneous injection of 1,500 units of antitetanic serum from horse on October 9, 1911. Horse 3 was used as control.

On October 23, 1911, two weeks after horse 2 had received the serum and six weeks after horse 1 had been inoculated with serum, each of the three horses was inoculated subcutaneously with about 50 standard doses of test toxin.

<table>
<thead>
<tr>
<th>No. of animal</th>
<th>Antitetanic serum units</th>
<th>Date of serum injection</th>
<th>Tetanus toxin test doses</th>
<th>Date of test</th>
<th>Weeks after serum</th>
<th>Result</th>
<th>Date of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,500</td>
<td>Sept. 11, 1911</td>
<td>50</td>
<td>Oct. 23, 1911</td>
<td>6</td>
<td>Lived</td>
<td>Oct. 30, 1911</td>
</tr>
<tr>
<td>2</td>
<td>1,500</td>
<td>Oct. 11, 1911</td>
<td>50</td>
<td>do</td>
<td>2</td>
<td>Lived</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>none</td>
<td></td>
<td>50</td>
<td>do</td>
<td></td>
<td>Died</td>
<td>Oct. 30, 1911</td>
</tr>
</tbody>
</table>

1 Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4–7, 1912.

Table I shows the results obtained. Horse 1 tolerated 50 standard test doses of tetanus toxin six weeks after having received the immunizing dose of serum; horse 2 tolerated 50 doses of test toxin two weeks after having received the serum; and the control horse 3 died on the seventh day after having been inoculated with toxin.

EXPERIMENT NO. 1 A

Horse 4 received 1,500 units of antitetanic serum on June 5, 1912. Horse 5 was inoculated with 1,500 units of antitetanic serum on July 5, 1912, and 1,500 units were given to horse 6 on August 5, 1912. Horse 7 was used as control.

On October 5, 1912, each horse received 50 standard test doses of tetanus toxin subcutaneously.

As is shown in Table I A, all horses in this series died. Immunity had disappeared eight weeks after the injection of the serum.

EXPERIMENT NO. 2

Of 8 guinea pigs, Nos. 1, 2, 3, 4, 5, 6, 7, and 8, each received three subcutaneous injections of antitetanic serum from horse as follows: February 27, 1911, 10 cubic centimeters; March 4, 10 cubic centimeters; March 11, 10 cubic centimeters.

From May 18, 1911, on every fourth day one guinea pig of the series received subcutaneously 250 units of antitetanic serum as follows: Guinea pig 1, May 18; 2, May 22; 3, May 26; 4, May 30; 5, June 3; 6, June 7; 7, June 11; and 8, June 15.

On June 27, 1911, twelve days after guinea pig 8 received the last injection of antitetanic serum and forty days after guinea pig 1 received the last injection of antitetanic serum, each of
the 8 guinea pigs was inoculated with 0.05 of the test dose of tetanus toxin. Guinea pig 9 served as control; it had not been treated with serum.

**Table II.**

<table>
<thead>
<tr>
<th>No. of animal</th>
<th>Anti-tetanic serum units</th>
<th>Date</th>
<th>Tetanus toxin test dose</th>
<th>Date</th>
<th>Days after serum</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>May 15, 1911</td>
<td>0.05</td>
<td>June 27, 1911</td>
<td>40</td>
<td>Died.</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>May 22, 1911</td>
<td>0.05</td>
<td>do</td>
<td>36</td>
<td>Died.</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>May 26, 1911</td>
<td>0.05</td>
<td>do</td>
<td>32</td>
<td>Lived.</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>May 30, 1911</td>
<td>0.05</td>
<td>do</td>
<td>28</td>
<td>Lived.</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>June 3, 1911</td>
<td>0.05</td>
<td>do</td>
<td>24</td>
<td>Lived.</td>
</tr>
<tr>
<td>6</td>
<td>250</td>
<td>June 7, 1911</td>
<td>0.05</td>
<td>do</td>
<td>20</td>
<td>Lived.</td>
</tr>
<tr>
<td>7</td>
<td>250</td>
<td>June 11, 1911</td>
<td>0.05</td>
<td>do</td>
<td>16</td>
<td>Lived.</td>
</tr>
<tr>
<td>8</td>
<td>250</td>
<td>June 15, 1911</td>
<td>0.05</td>
<td>do</td>
<td>12</td>
<td>Lived.</td>
</tr>
<tr>
<td>9</td>
<td>none</td>
<td></td>
<td></td>
<td>0.05</td>
<td>do</td>
<td>Died.</td>
</tr>
</tbody>
</table>

The results, as shown in Table II, were as follows: Guinea pigs 1, 2, and 9 died while 3, 4, 5, 6, 7, and 8 lived. Guinea pig 3 tolerated 0.05 of the test dose of tetanus toxin on the thirty-second day after having received the antitoxin, while guinea pig 2 did not tolerate the same dose of toxin on the thirty-sixth day after injection of antitoxin.

**EXPERIMENT NO. 3**

Guinea pigs 10, 11, 12, 13, 14, 15, 16, and 17 each received three subcutaneous injections of normal horse serum as follows: February 27, 1911, 10 cubic centimeters; March 4, 10 cubic centimeters; March 11, 10 cubic centimeters.

Beginning on May 18, 1911, every fourth day one guinea pig of the series received an injection of 250 units of antitetanic serum subcutaneously. Injections were given on the following dates: Guinea pig 10, May 18; 11, May 22; 12, May 26; 13, May 30; 14, June 3; 15, June 7; 16, June 11; 17, June 15, 1911.

On June 27, twelve days after guinea pig 17 had received 250 units of antitetanic serum and forty days after guinea pig 10 was inoculated with antitoxin, each guinea pig in the series was inoculated subcutaneously with 0.05 of the test dose of tetanus toxin. Guinea pig 18 was used as control.
The results shown in Table III were as follows: Guinea pigs 10 and 11 and the control 18 died. All others lived. The results obtained in experiment 3 were identical with those obtained in experiment 2.

CONCLUSIONS

The following conclusions seem justified:

1. The subcutaneous injection of 1,500 units of antitetanic serum from horse into horse confers passive immunity of between six and eight weeks' duration.

2. Guinea pigs subjected to repeated inoculations with antitetanic serum from horse do not acquire the power to eliminate it more rapidly; they acquire a tolerance as is shown by the longer period of immunity.

3. After repeated injections of normal horse serum into guinea pigs, passive immunity, following the injection of antitetanic serum from horse, is of longer duration than it is in untreated guinea pigs.
APPENDICITIS

By P. K. Gilman

(From the Philippine General Hospital and the College of Medicine and Surgery, University of the Philippines, Manila, P. I.)

Appendicitis is a disease confined to no particular locality, race, age, or station of life; it is of interest to each and every class; and its pathology and treatment have been developed in our own generation.

As late as 1886 only did R. H. Fitz, a Boston surgeon, clearly point out the cause of the frequent acute, and often fatal, attacks of pain and inflammation in the lower right abdomen. Since that date a long list of surgeons have, by their contributions, succeeded in building up a technique for dealing with the disease in its acute and chronic stages.

Before considering briefly some of the various types of appendicitis with their complications, we wish to make a statement that our experience has borne out. It is a safe rule to consider the possibility of appendicitis in every case of abdominal trouble not clearly due to something else. Therefore, in each case of abdominal disease the physician should endeavor first of all to rule out appendicitis. This should especially be borne in mind by those of us who have not at hand a completely organized hospital with its staff, including a thoroughly equipped clinical laboratory, to assist us in ruling out other conditions and narrowing the field of diagnostic possibilities.

A brief analysis of the last 50 cases of acute appendicitis operated upon in the clinic of the Philippine General Hospital may be of interest at this point. The ages of these patients range from 4 to 55 years, with the greatest number of cases between the ages of 20 and 30.

There were 38 male and 12 female cases. Nineteen of the patients complained of pain beginning in the lower right abdomen; 21 claimed a general abdominal pain, while in the remaining

1 Read before the Ninth Annual Meeting of the Philippine Islands Medical Association, held in Manila from November 4–7, 1912.
2 Professor of surgery, College of Medicine and Surgery, University of the Philippines, and chief of the department of surgery, Philippine General Hospital.
number no special mention was made of any marked abdominal discomfort. The initial pain due to obstruction and distension of the lumen of the appendix is often agonizing, although all grades occur. While this pain is present, the disease is confined to the appendix, and this severe pain is usually transient, reaching its height within four hours from the onset and at that time generally localized in the appendix region. Probably 75 per cent of the cases subside at this point—a few in thirty-six hours. If there is a sudden cessation of the pain, this is a danger signal indicating perforation.

We have found the temperature of recently admitted patients to be of comparatively little diagnostic value, as it is well known that the excitement dependent upon change of scene, often irregular transportation, and admission to a hospital will result in a much lower temperature than the patient exhibited before being brought to the institution.

Of the reflex gastrointestinal symptoms, nausea, unaccompanied by actual vomiting, was present in 9 instances, vomiting in 18, constipation in 9, and diarrhoea in but 3 of the series, no disturbance being recorded in the remainder of the cases.

The nausea and vomiting follow the initial pain in the acute cases and are reflex, the nervous mechanism of the appendix being connected with that of the stomach and small intestine. The primary nausea usually subsides fairly promptly.

On examination, tenderness in the region of appendix is practically always present in acute attacks. It is hard to elicit in the very stout and the very nervous. More or less rigidity of the abdominal wall in the lower right quadrant is present, although a careful comparison with the left side is needed in many instances to make this point apparent. Tenderness and rigidity in parts removed from the appendix region are encountered at times and may prove misleading. Four of our cases showed generalized abdominal tenderness, and 1 a marked area sensitive to palpation confined entirely to the left hypogastric region. Eight complained of no special tenderness, the others of soreness higher up than the classical point and often far toward the posterior portion of the body. Exploration of the rectum is often of great importance.

In this clinic where we have come to the point of immediate operation, in every case of acute appendicitis, little stress is now laid on the leucocytic content of the blood in the frank cases. It is in those cases where some doubt exists as to the exact nature of the condition that we depend more upon the result of
the blood examination, where for example a marked leucocytosis may be the most prominent feature of an otherwise masked inflammatory process. The leucocytes are of diagnostic significance when they are increasing.

The highest count met with in these cases of acute appendiceal trouble was 28,000, the operation in this instance revealing an appendix already perforated with localized peritonitis. The lowest count was 6,600 in a case presenting marked rigidity of the lower abdomen with severe pain and tenderness, in which the process, however, was limited to the appendix. One-third of the cases yielded a count below 10,000, all of these being simple acute processes confined to the appendix; another third made up of similar cases gave a count of between 10,000 and 17,000; while those with a higher count included the complicated cases with abscess and peritoneal invasion. Only 4 of this series gave a history of previous dysentery.

The clinical picture presented by the above group of acute cases is too well known to necessitate further elaboration here. It is our purpose to endeavor to emphasize in this communication some of the findings in those cases of more or less obscure nature which manifest themselves in disturbances of varying severity in the digestive apparatus. As our knowledge of the pathology of this probably functionless rudiment is increased, we are forced more and more to regard the appendix as the underlying cause of numerous subacute and chronic disturbances heretofore ascribed to other organs or groups of organs.

It appears a far cry indeed from a case of acute inflammation of the appendix, with its sudden severe pain localized in the right lower quadrant of the abdomen accompanied by nausea, vomiting, diarrhoea, increasing fever, and climbing leucocyte count, to a case of neurasthenia with slight stomach trouble, chronic constipation, and a power of assimilation varying from a little to considerably below normal; and yet we must ascribe the two conditions to the disease of the same organ.

These two apparently widely separated states are linked together by a series of intermediate cases.

To illustrate this latter group of cases, it will be well to give a brief résumé of a few typical cases, illustrating various results of chronic pathological change in the appendix:

**Case I.**—American woman, age 40 years, single. For a number of years she had been treated by various physicians for chronic constipation and general nervousness. The patient was well nourished, in spite of troublesome fullness and flatulency of the stomach after taking food,
which was relieved by bringing up considerable amounts of gas between meals. The constipation has grown progressively worse for a period of two years, in spite of various diets, exercises, and increasing doses of purgatives.

The result of a general examination was negative with the exception of a slight increase in the rigidity of the wall of the right lower quadrant of the abdomen over that of the opposite side and a slight tenderness on deep palpation over the cæcal region not due to the pelvic viscera, as determined by bimanual examination. No definite attacks of pain referable to this region of the abdomen were remembered by the patient.

Removal of the appendix was advised, and at operation an appendix nearly obliterated, patent, and of increased diameter for only the distal half-centimeter was found. The proximal portion of the organ was largely represented by a mass of fibrous tissue with evidences of the original layers occurring in but one or two places. There was atrophy of the lining of the distended distal portion. The patient recovered promptly, and within three months of the operation suffered no more from nervousness, indigestion, or constipation.

CASE II.—Physician, age 30, single, complained of recurring jaundice of very slight grade, accompanied by attacks of hyperacidity and constipation. Following one of these attacks, in which there had been a slight febrile reaction unaccompanied by any leucocytosis, the gall bladder had been explored and drained, it being thought that here lay the cause of the trouble. The patient recovered from this attack about as quickly as he had from the previous ones, but, after a period of freedom from jaundice lasting only a little longer than the previous intervals, he again became yellow and had a low fever. During this attack, the intestinal manifestations were more marked than usual, and examination showed on deep palpation a very tender appendix region. An appendix with thickened wall, well surrounded by adhesions and plastered posteriorly to the lower end of the cæcum, was removed through a low incision. During the past five years the patient has been free from all jaundice and digestive disturbances.

CASE III.—American clerk, age 28 years, single. This patient complained of a slight yellow color of the eyeballs, sour stomach, constipation alternating with diarrhoea, and increasing nervousness. The jaundice and the attacks of diarrhoea were to the patient the most annoying symptoms. At no time was there any tenderness elicited by pressure over the region of the gall bladder, although even light palpation over the appendix produced discomfort and a sickening sensation which, although never entirely absent, was most marked during acute exacerbations of the symptoms.

At the time of operation the advisability of exploring the gall bladder was discussed, and, as it was found possible completely to empty this organ with the finger introduced through a McBurney incision, the operation included merely the removal of the appendix, which was chronically inflamed, adherent, with signs of acute engorgement. It is now over three years since the operation, and the patient has had no further jaundice nor other manifestation of a gastrointestinal disturbance.

CASE IV.—American woman, aged 42, married, has had several children, and has lived in tropical countries for over twelve years. For the past three years or more the patient has lost weight slowly but steadily, and has had numerous attacks of jaundice of a mild grade with no special
pain referable to the gall-bladder region. There has been a gradually increasing discomfort and sensation of weight in the lower right side of the abdomen, which became actual pain, accompanied by soreness, when the yellow color of the eyes and skin was most marked. During the last attack, a month ago, the patient suffered some pain below the right costal margin, was definitely jaundiced, and the appendix region was very tender with increased rigidity of the superimposed walls.

At the operation the gall bladder was found rather thickened and did not empty readily. For this reason, following the removal of an adherent chronically thickened appendix, the gall bladder was opened, a tarry bile evacuated, and the bladder drained. No evidence of stone was found in this case. Already the jaundice has completely cleared, the digestion has been restored to practically normal, and a gain of several pounds has been made.

The above are but a few of a considerable series of cases showing concurrent disease of the appendix and gall bladder. Given these conditions, a simple drainage of the gall bladder without the removal of the appendix will not insure a freedom from recurring trouble with the biliary apparatus.

In looking over our records of gall-bladder disease where it was possible to follow the patients for any length of time after their operations of drainage for a simple cholecystitis, we find in some instances a fairly prompt recurrence of the jaundice and so-called "stomach trouble." Might not these cases have had enough symptoms above the umbilicus to cause the examining physician to overlook the presence of trouble in the appendix?

Another group of cases of appendicitis with which we have had a rather fortunate experience is the group made up of cases in which the mildness of the symptoms has been out of all proportion to the condition found at operation.

Case I.—American, aged 32, married, clerk. Entered the hospital complaining of stomach trouble. The patient was very stout, and examination was difficult, but a slight tenderness was elicited over the right iliac fossa in which region a slight general abdominal pain was localized. The leucocyte count upon admission was 8,000. There was practically no fever, and the patient's wife refused her consent to an operation for appendicitis. Twenty-four hours later the pain had increased somewhat, although it was not very severe, the temperature had risen to 38°.2 C., and the leucocytes were 15,000. Operation was again advised and consent given rather reluctantly, as the patient did not appear very uncomfortable. At operation, performed about thirty hours after the first complaint, the appendix was found gangrenous and free in the abdominal cavity, together with a general collection of bowel contents and pus with practically no attempt at localization on the part of the peritoneum and viscera. The caecum was found greatly thickened and in places puckered and pigmented as though from the old dysentery from which this patient had suffered two years previous to the operation. The patient fortunately recovered from the peritonitis, and is to-day well.
Case II.—American, aged 40, clerk, walked into the hospital, complained of abdominal discomfort and, as he had had several attacks of ameobic dysentery, was admitted to the medical service. The patient continued up and about the ward during the first day of his stay in the hospital; however, as the abdominal discomfort continued, the surgeon was asked to see the case, the resident physician in medicine having followed the course of events in Case No. I a few days before the present case was admitted to the ward.

This case presented a flat, easily examined abdomen, which showed nothing of interest save a thickened large bowel with general slight tenderness a little more marked below the level of the umbilicus, with practically no increased rigidity of the abdominal wall. The leucocytes numbered between 13,000 and 14,000.

Operation was advised, and, with the other case with a history of recurrent dysentery in mind, was performed at once, the patient walking into the operating room unassisted. The appendix was found perforated near the base. The entire structure, as well as the walls of the entire large bowel, was greatly thickened and leathery. The peritoneum contained a considerable amount of bowel content and fibrin, fairly well localized to the lower right quadrant. The lack of pain and the extremely slight reaction on the part of the patient to the condition found were remarkable in this case, even more so than in the previous one. From these two cases and others of a similar type it would seem that the resulting chronic changes in the bowel and peritoneum following recurring attacks of ameobic dysentery extending over a long period have exerted a definite influence. Neither of the above cases has had a recurrence of dysentery since the removal of the appendix.

We have then a group of cases of appendicitis occurring in persons, especially Europeans, who have had a previous dysentery extending over some time, in which cases the pathological processes may have progressed to a point out of all proportion to the manifestations of the disease exhibited clinically, and it is in these cases that we must exercise great care lest we make the mistake of not emphasizing sufficiently the necessity for immediate operative interference.

We have purposely laid less emphasis upon acute appendicitis, as it is not only more readily diagnosed than the various chronic forms, but its differentiation from other conditions—such as a perforated ulcer of the stomach or duodenum—is, so far as treatment is concerned, not so essential since both conditions demand immediate surgical intervention. On the other hand, it is the more or less obscure cases dependent upon a slowly progressing chronic change in the appendix, often without definite indications pointing to this organ as the underlying cause, to which we wish particularly to draw attention. If we will recall that the variety of the manifestations of these chronic changes may be innumerable and consider the possibility of appendicitis
in every case of abdominal trouble not clearly due to something else, we shall be in a position to relieve a large number of patients who heretofore have drifted from one physician to another. Too often these patients tell of various diagnoses by different physicians and of long courses of treatment for disorders attributed at different times to various portions of the alimentary canal.

Appendicitis as met with in the Philippines presents, among both the Filipinos and Europeans who have resided here for long periods, certain differences from the cases usually met with in the United States. This disease is not always a simple matter to diagnose nor, if a diagnosis has been made, is the condition of the appendix and neighboring peritoneum always an easy question to determine. In many cases pathological changes occur which are out of all proportion to the clinical manifestations.
The ninth annual meeting of the Philippine Islands Medical Association was held in the histological laboratory of the College of Medicine and Surgery, November 4 to 7, 1912, inclusive.

FIRST SESSION, NOVEMBER 4, 9 A. M.

The first session was called to order by President Newberne, who made a few remarks of welcome and introduced the Acting Governor-General, who presided at the opening session. The Acting Governor-General made some interesting remarks relative to medical men, sanitation in the Philippines, medical education, and the Philippine Islands Medical Association. The Acting Governor-General turned the chair over to the Honorable the Secretary of the Interior, who presided during the address of President Newberne on "Basic Principles of Psychotherapy and the Identity of all Mental Healing Systems."

The following scientific program was next presented and carried out in the order indicated:

Two Cases of Primary Purpura, by José Albert and María Paz Mendoza.

A Case of Microcephalus with Symptoms of Tetanus, by José Albert and María Paz Mendoza.

A Study of 100 Cases of Lobar Pneumonia in the Tropics, by C. R. Stanley. (Read by title.)


A Case of True Bilharzial Disease in Porto Rico, by U. R. Webb.

Korsakow's Psychosis, with Report of a Case, by Heber Butts.

Congenital Varicosities of the Vene Epigastrice Superiores, Paraumbilicae, Thoracoepigastricae, and Mammariae, by A. G. Sison.


Bone Lesions in Smallpox, by W. E. Musgrave and A. G. Sison. (Discussion by Major Ashburn and Dr. Heiser.)

Glandular Fever, by Almon P. Goff.

Number present, 76.

Meeting adjourned at 12.30 p. m.
SECOND SESSION, NOVEMBER 5, 9 A. M.

Status Thymico-Lymphaticus in Filipinos, by B. C. Crowell. (Discussion by Major Ashburn and Dr. Crowell.)
A Tumor of the Hypophysis Cerebri, by José Hilario.
Presentation of pathological specimens, department of pathology and bacteriology, College of Medicine and Surgery, University of the Philippines.
Polyneuritis Gallinarum—
(a) The Influence of Various Foodstuffs on its Development, by Edward B. Vedder.
(b) Early Changes in the Peripheral Nerves, by Elbert Clark.
Infant Mortality in Manila—
(a) General Considerations, by W. E. Musgrave.
(2) The papers by R. B. Gibson, Edward B. Vedder, Elbert Clark, and W. E. Musgrave, all being related from a nutritional standpoint, were discussed together by Dr. Heiser, Dr. Vedder, Dr. Shaklee, Dr. Gibson, Mr. Clark, Mr. Williams, and Dr. Yemans.)
Experimental Acclimatization of the Philippine Monkey to a Tropical Sun, by A. O. Shaklee.
Sunlight, by Paul C. Freer and Harry D. Gibbs.
(2) The paper of Dr. Shaklee and that of Dr. Freer and Mr. Gibbs were discussed by Mr. Clark and Major Ashburn.)
Number present, 69.
Meeting adjourned at 12.45 p. m.

THIRD SESSION, NOVEMBER 6, 7.30 A. M.

The surgical clinic with operations at the Philippine General Hospital was given at 7.30 a. m. by Drs. P. K. Gilman and Hans Schiffbauer.
The session convened for the following program at 10 a. m.
The Outbreak of Plague in Manila in 1912, by Victor G. Heiser.
Bacteriological Observations made during the Outbreak of Plague in Manila in 1912, by Otto Schöbl.
The Outbreak of Plague in Iloilo in 1912, by Carroll Fox.
(The above papers were discussed by Drs. Jackson, Ashburn, Crowell, Gibson, Butler, Yemans, Newberne, Heiser, Gomez, Fox, and Schöbl.)

Surgical Treatment of Gonorrhoeal Orchitis and Epididymitis, by B. L. Burdette.

Races of Dysentery Bacilli obtained by the Selection of Single Cells, by M. A. Barber. (Read by title.)

Number present, 85.

Meeting adjourned at noon.

FOURTH SESSION, NOVEMBER 6, 2 P. M.

Intra-orbital Tumors and Their Operative Consideration, by Rheinhard Rembe.

Notes on Some Recent Surgical Cases at the Naval Hospital, Cañaacao, by N. J. Blackwood. (Discussion by Drs. Burdette and Schiffbauer.)

A Review of the Literature on Cultivation of Fresh Tissues in Various Media, by R. M. Lhamon. (Discussion by Mr. Clark.)

Diagnosis of Appendicitis, by P. K. Gilman.

Treatment of Acute Appendicitis, by Hans Schiffbauer. (Discussion by Drs. Burdette, Blackwood, Lhamon, Newberne, Schiffbauer, and Gilman.)


Number present, 38.

Meeting adjourned at 4.30 p. m.

FIFTH SESSION, NOVEMBER 7, 9 A. M.

Clinic in department of diseases of the eye, ear, nose, and throat, Philippine General Hospital, by Dr. Rembe and assistants.

Some Carbohydrate Reactions of the Dysentery Bacillus, by C. S. Butler. (Discussion by Dr. Gibson.)

Isolation of *Diplococcus intracellularis meningitidis* Weichselbaum from a Case of Cerebrospinal Meningitis Occurring in a Native of the Philippine Islands, by David G. Willettes and Otto Schöbl. (Discussion by Drs. Heiser and Gentry.)

The Duration of Passive Immunity against Tetanus Toxin, by E. H. Ruediger.

Quantitative Determination of the Balantidical Action of Drugs and Chemicals as a Basis for the Treatment of Balantidial
Dysentery, by E. L. Walker. (Discussion by Drs. C. S. Butler and E. B. Vedder.)
Salvarsan in Malaria, by R. H. Goldthwaite.
Experiences in Anaesthesia at the Philippine General Hospital, by E. J. Ochsner. (Discussion by Drs. Blackwood and Heiser.)
Artificial Ears and Noses, by Louis Ottofy.
The Military Importance of Deviations of the Nasal Septum, by T. C. Lyster. (Read by title.)
Sanitation in Small Towns in the Philippine Islands, by Frank Baker. (Read by title.)
Ancient Medical Customs among the Japanese, by Dr. Yegawa. (Read by title.)
Number present, 41.

AFTERNOON

Medical clinics at the Philippine General Hospital (at 2 p.m.), by W. E. Musgrave and José Albert.

BUSINESS MEETINGS

FIRST BUSINESS SESSION, NOVEMBER 4, 4 P. M.

At the first meeting of the House of Delegates, the president called attention to a proposed amendment to the constitution which was read before the association at the eighth annual meeting, and which provided that the council consist solely of ex-presidents of the Association.

After discussion, it was decided to leave the consideration of this to a committee who was instructed to report at the business meeting. The president appointed Major Ashburn, Dr. Heiser, and Surgeon Butler a committee on nominations. This committee was also instructed to consider and report upon the proposed amendment.

SECOND BUSINESS SESSION, NOVEMBER 7, 12.50 P. M.

Roll call and reading of minutes were passed over.
The secretary made a verbal report. All of the expenses of the meeting not being ascertainable at this time, he promised a written report to be submitted to the council.

There being no unfinished business, motions under the head of "new business" were next considered.

Dr. Heiser suggested and proposed that, in view of the recent advances in the work concerning the etiology of beriberi, and in view of the confirmation of the former theory concerning the close interrelation of polished rice diet and beriberi, the Philippine Islands Medical Association propose, through the proper
channels, to the Philippine Legislature now in session, the following resolution:

Resolved, That in the opinion of this Association, sufficient evidence has been produced in support of the view that beriberi is associated with the continuous consumption of decorticated rice as a staple article of diet; that this Association strongly recommends to the Philippine Legislature the passage of suitable legislation which will have for its object the bringing about of the general use of unpolished rice among those who use it as a staple article of diet.

The proposed amendment to the constitution that all ex-presidents be considered members of the council of the Philippine Islands Medical Association, and that the council consist solely of ex-presidents was brought before the house by the president. This was proposed at the eighth annual meeting.

After discussion, the amendment was rejected.

REPORT OF THE COMMITTEE OF THE HOUSE OF DELEGATES

The committee of the House of Delegates, appointed by the president at the first meeting of the House of Delegates on Monday the 4th, submitted its report.

Dr. R. P. Strong was recommended by the committee as a delegate to the next annual meeting of the American Medical Association to be held in Minneapolis, June 15, 1913.

The committee further recommended that the incoming president appoint an alternate, it being impossible to determine at this time what member of the Association will be in America at the time of the meeting of the American Medical Association.

The secretary was instructed to cast a vote for Dr. Strong, and this part of the report of the committee was adopted.

The same committee recommended that the same method of procedure be employed in the selection of a delegate to the next annual meeting of the Far Eastern Association of Tropical Medicine to be held in Saigon. After motion, the suggestion was adopted.

The same committee submitted the following ballot for officers for the ensuing year:

Vice-presidents: Dr. N. M. Saleeby and Dr. A. G. Sison.
Councillor for five years: Dr. R. E. L. Newberne.

Upon motion, which was seconded and passed, the secretary was instructed to cast the ballot for this ticket.

On the suggestion of the president, it was unanimously moved that a vote of thanks be extended by the Philippine Islands
Medical Association to Dr. Woodbury, of the Philippine General Hospital, for furnishing plants for decoration; to Dr. Alvin J. Cox, Acting Director of the Bureau of Science, for the erection, free of charge, of a platform; and to Mrs. S. E. Waddington, of the University of the Philippines, for decorating the platform and arranging bouquets for the ninth annual meeting of this Association.

On motion of Major Ashburn, which was amended by Dr. Heiser and the secretary, a vote of appreciation was extended by the association to the president for his keen interest in the ninth annual meeting; to the Honorable Dean C. Worcester for furnishing entertainment for the social evening, and to such other officers and committees which took an active interest in making the ninth annual meeting a success.

President Newberne expressed his appreciation of the work of the other officers and of the committees, and for the good attendance and interest which was manifested in the various sessions.

The secretary was instructed to inform each of the above persons of this action by the Association.

There being no further business, the ninth annual session of the Philippine Islands Medical Association adjourned.

After the annual meeting had adjourned, the members and their guests gathered at the roof garden of the Manila Hotel and were favored by a most interesting illustrated lecture on Wild Tribes in northern Luzon by the Honorable the Secretary of the Interior, Dean C. Worcester.

Secretary Worcester showed some interesting motion pictures, and told many interesting personal details of the plan of civilization and his experiences in northern Luzon.

After the lecture, a buffet lunch was served, and the members enjoyed a pleasant social evening.

The committee on exhibits arranged the exhibits in the physiological laboratory, and the pathological museum was open to inspection. Exhibits were shown by the department of physiology and of pathology and bacteriology of the College of Medicine and Surgery, by Dr. Louis Ottofy and by Dr. Mariano V. del Rosario. Exhibits were also arranged by Parke, Davis & Co., the German Dispensary, Philippine Education Publishing Company, Pacific Commercial Company, Nestle Anglo-Swiss Milk Company, and by the PHILIPPINE JOURNAL OF SCIENCE.

ELBERT CLARK,
Acting Secretary, Philippine Islands Medical Association.
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MEDICINE

REPORT OF THE INTERNATIONAL PLAGUE CONFERENCE
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483 pages, 18 plates (2 colored, 4 half-tones, 12 charts and maps)
Order No. 416. Paper, $2.50; cloth, $3.50; postpaid.
The proceedings of this International Conference and information gained therefrom, together with the results of certain bacteriological investigations, constitute the present report.
The Bureau of Science of the Government of the Philippine Islands has been appointed sole agent for the distribution of the printed proceedings of the International Plague Conference.

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INTESTINAL PARASITES ENCOUNTERED IN FIVE HUNDRED AUTOPSIES, WITH REPORTS OF CASES

By B. C. CROWELL and R. W. HAMMACK

(From the Department of Pathology and Bacteriology, University of the Philippines, and the Biological Laboratory, Bureau of Science, Manila, P. I.)

INTRODUCTION

The incidence of intestinal parasites in the inhabitants of the Philippine Islands has been the subject of investigation by numerous workers since the American occupation of the Islands. These investigations have been made largely from the clinical standpoint, using the microscopic examination of the faeces as the standard of the incidence. Among the works on the subject may be mentioned those of Garrison, Garrison and Llamas, Garrison, Leynes, and Llamas, Rissler and Gomez, Chamberlain, Bloombergh, and Kilbourne, Willets, and Musgrave and Clegg.

No report on any large series of cases in the Philippine Islands has been made solely from autopsy investigations with the aim of establishing the percentage incidence of intestinal parasites. The present report is based on the findings in 500 consecutive autopsies performed in the department of pathology and bacteriology, College of Medicine and Surgery, during the last twelve months. The compilation in reality entailed the examination of 583 records, 83 of which were not utilized; the excluded cases were those of infants under three months of age and those in which the intestine was not examined on account of advanced post-mortem changes or for other reasons. While the previous clinical investigations have been performed for the most part in certain selected regions or on certain selected classes of individ-

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1 Associate professor and chief of the department of pathology and bacteriology, College of Medicine and Surgery, University of the Philippines; pathologist, Bureau of Science.

2 Instructor in pathology and bacteriology, College of Medicine and Surgery, University of the Philippines.
uals, as, for instance, the male prisoners in Bilibid Prison, the present series includes all cases autopsied within a year with the exceptions already noted. There were 353 males and 147 females; 116 were below the age of fifteen and 384 were above this age.

The autopsy service from which this report is compiled includes persons dying in a large general hospital, in a hospital for contagious diseases, in a large prison hospital, and those who were the subjects of medico-legal investigations in the city of Manila. The overwhelming majority of these cases are Filipinos, but the list also includes Americans, Europeans, Chinese, Japanese, and East Indians. The findings embodied in this report are not based on any special investigation conducted with the view to determine the absolute incidence of intestinal parasites, but have been compiled from the routine autopsy records which are on file.

In as far as our report attempts to be statistical, its interpretation should be made with certain restrictions. While, as above indicated, the class of cases on which it is based is more comprehensive than some of the series of cases previously investigated in the Islands as to age, sex, and distribution, the fact that a large percentage of these cases comes from a hospital where routine treatment for intestinal parasites is in vogue may diminish the number of parasites found post mortem and somewhat invalidate the percentage values. Also, the readiness with which *Ascaris* is recognized on account of its size and the difficulty encountered in detecting the smaller *Trichuris*, hookworm, and *Oxyuris* may have some bearing on the subject in as much as the autopsies were not performed with this investigation in view. Routine microscopic examination of the feces for ova has not been performed by us.

The pathogenicity of some of the intestinal parasites has been the subject of so much discussion and the conclusions which have been accepted are based on such uncertain grounds that reports will be included here of certain cases where definite pathological conditions have been found, which in some cases certainly bear relation to the infesting parasite and in other cases may with a certain amount of probability be definitely associated with them.

The situation with regard to the influence of the parasites on the human host is stated by Braun (8) as follows:

In a great many cases we are not in a position to state anything regarding any marked influence exercised by the parasite on the organism and on the conditions of life of the host. Most animals and many persons exhibit no signs of such influence. As a general rule, the parasite, which
is always smaller and weaker than its host, does not attempt to endanger the life of the latter, as simultaneously its own existence would be threatened. The parasite, of course, robs its host, but usually in a scanty and sparing manner, and the injuries it inflicts can hardly be taken into account. There are, however, numerous cases in which the situation of the parasites or the nature of their food, added to their number and movements, may cause more or less injury, and even threaten the life of the host.

For purposes of easy comparison, the tables compiled and published by Willets,(6) bearing on the parasites found by clinical examination of feces, are inserted.

**Table I.—Summary of findings.(6)**

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<td>Persons examined</td>
<td>4,278</td>
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<tr>
<td>Persons infected</td>
<td>3,536</td>
<td>85.46</td>
</tr>
<tr>
<td>Persons infected with—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascariis</td>
<td>2,653</td>
<td>62.04</td>
</tr>
<tr>
<td>Hookworm</td>
<td>2,326</td>
<td>54.37</td>
</tr>
<tr>
<td>Trichuris</td>
<td>342</td>
<td>7.39</td>
</tr>
<tr>
<td>Oxyuris</td>
<td>64</td>
<td>1.50</td>
</tr>
<tr>
<td>Taenia</td>
<td>59</td>
<td>1.38</td>
</tr>
<tr>
<td>Hymenolepis</td>
<td>5</td>
<td>0.12</td>
</tr>
<tr>
<td>Strongyloides</td>
<td>4</td>
<td>0.09</td>
</tr>
<tr>
<td>Trematodes a</td>
<td>1</td>
<td>0.02</td>
</tr>
<tr>
<td>Total infections</td>
<td>5,454</td>
<td>127.49</td>
</tr>
</tbody>
</table>

*a Possibly *Fasciolletta silocana* Garrison, 1908.

**Table II.—Percentages of persons infected and total infections in various parts of Luzon.**

<table>
<thead>
<tr>
<th>Authority and date.</th>
<th>Place.</th>
<th>Sex.</th>
<th>Exam.</th>
<th>Infect.</th>
<th>Per cent.</th>
<th>Total infections</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garrison, 1908 (1)</td>
<td>Manila</td>
<td>Mostly males...</td>
<td>4,166</td>
<td>3,447</td>
<td>84.00</td>
<td>5,812</td>
<td>142.00</td>
</tr>
<tr>
<td>Garrison and Llam-</td>
<td>Las Pinas, Rizal</td>
<td>Women and children...</td>
<td>335</td>
<td>342</td>
<td>90.00</td>
<td>533</td>
<td>138.70</td>
</tr>
<tr>
<td>mas, 1909 (2).</td>
<td>Maytay, Rizal...</td>
<td>Males and females...</td>
<td>1,000</td>
<td>959</td>
<td>95.30</td>
<td>1,726</td>
<td>172.60</td>
</tr>
<tr>
<td>Rispler and Gomez,</td>
<td>Los Piñas, Rizal</td>
<td>do...</td>
<td>6,018</td>
<td>5,406</td>
<td>89.83</td>
<td>8,996</td>
<td>149.43</td>
</tr>
<tr>
<td>1910 (3).</td>
<td>Tuguegarao, Caga-</td>
<td>do...</td>
<td>2,594</td>
<td>1,932</td>
<td>74.13</td>
<td>2,887</td>
<td>111.30</td>
</tr>
<tr>
<td>Do</td>
<td>Santa Isabel, Il-</td>
<td>do...</td>
<td>802</td>
<td>602</td>
<td>75.00</td>
<td>927</td>
<td>114.34</td>
</tr>
<tr>
<td></td>
<td>lagan, Isabela...</td>
<td>Adult males...</td>
<td>119</td>
<td>110</td>
<td>91.00</td>
<td>209</td>
<td>174.00</td>
</tr>
<tr>
<td>Chamberlain, Bloom-</td>
<td>Baguio, Benguet</td>
<td>Adult males...</td>
<td>119</td>
<td>110</td>
<td>91.00</td>
<td>209</td>
<td>174.00</td>
</tr>
<tr>
<td>ergh and Kilbourne</td>
<td>San Antonio and</td>
<td>Males and females...</td>
<td>4,278</td>
<td>3,656</td>
<td>85.46</td>
<td>5,454</td>
<td>127.49</td>
</tr>
<tr>
<td>1911 (5).</td>
<td>Maluno, Ilagan,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isabela...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>19,302</td>
<td>16,355</td>
<td>85.66</td>
<td>26,544</td>
<td>137.52</td>
</tr>
</tbody>
</table>

*a Protozoan findings included.  
*b Intestinal worms only.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Garrison (1)</td>
<td>1908</td>
<td>Manila, Bilibid Prison.</td>
<td>4,106</td>
<td>1,052</td>
<td>26.00</td>
<td>2,426</td>
<td>59.00</td>
<td>2,136</td>
<td>52.00</td>
<td>32</td>
</tr>
<tr>
<td>Garrison and Llamas (2)</td>
<td>1909</td>
<td>Manila, hospitals, etc...</td>
<td>385</td>
<td>132</td>
<td>51.22</td>
<td>300</td>
<td>87.60</td>
<td>46</td>
<td>13.45</td>
<td>2</td>
</tr>
<tr>
<td>Garrison, Leynes, and Llamas (3).</td>
<td>1909</td>
<td>Taytay, Rizal Province.</td>
<td>1,000</td>
<td>839</td>
<td>82.90</td>
<td>770</td>
<td>77.00</td>
<td>116</td>
<td>11.60</td>
<td>4</td>
</tr>
<tr>
<td>Rissler and Gomez (4).</td>
<td>1910</td>
<td>Las Piñas, Rizal Province.</td>
<td>6,018</td>
<td>4,647</td>
<td>77.21</td>
<td>3,241</td>
<td>53.40</td>
<td>671</td>
<td>11.14</td>
<td>298</td>
</tr>
<tr>
<td>Do.</td>
<td>1910</td>
<td>Tuenegarao, Cagayan.</td>
<td>2,594</td>
<td>1,907</td>
<td>73.55</td>
<td>672</td>
<td>25.90</td>
<td>208</td>
<td>8.01</td>
<td>68</td>
</tr>
<tr>
<td>Do.</td>
<td>1910</td>
<td>Santa Isabel, Iigan, Isabela.</td>
<td>802</td>
<td>486</td>
<td>60.59</td>
<td>50</td>
<td>6.23</td>
<td>364</td>
<td>45.38</td>
<td>10</td>
</tr>
<tr>
<td>Chamberlain, Bloomergh, and Kilbourne (5).</td>
<td>1911</td>
<td>Baguio, Benguet.</td>
<td>119</td>
<td>87</td>
<td>73.00</td>
<td>72</td>
<td>60.00</td>
<td>35</td>
<td>29.00</td>
<td>0</td>
</tr>
<tr>
<td>Willets (6)</td>
<td>911</td>
<td>San Antonio and Malano, Iigan, Isabela.</td>
<td>4,288</td>
<td>2,633</td>
<td>62.04</td>
<td>342</td>
<td>7.99</td>
<td>2,236</td>
<td>54.37</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>19,302</td>
<td>11,845</td>
<td>61.36</td>
<td>7,873</td>
<td>40.79</td>
<td>5,901</td>
<td>30.87</td>
<td>478</td>
</tr>
</tbody>
</table>
Our findings with reference to the more frequently encountered intestinal parasites in the series of 500 autopsies are as follows:

### Table IV.—Incidence of intestinal parasites in 500 autopsies.

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Number of cases</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris</td>
<td>206</td>
<td>41.2</td>
</tr>
<tr>
<td>Hookworm</td>
<td>83</td>
<td>16.6</td>
</tr>
<tr>
<td>Trichuris trichiura</td>
<td>172</td>
<td>34.4</td>
</tr>
<tr>
<td>Oxyuris</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>Tenta</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Entamoeba</td>
<td>25</td>
<td>5.0</td>
</tr>
</tbody>
</table>

As to other parasites we may mention the occurrence of malaria in 5 per cent of the cases, liver flukes (*Clonorchis sinensis*) in 2 cases, and at least 1 case of schistosomiasis.

Our figures are lower than the averages compiled by Willets, the incidence of *Entamoeba* being especially lower than the results published by many authors. The incidence of the various parasites will now be taken up in greater detail, and reports will be added of the cases which we desire to place upon record.

**ASCARIS**

Our figures (41.2 per cent) on the incidence of *Ascaris* are lower than those of Willets(6) and higher than those of Garrison.(1) The number of *Ascaris* found in any one case varied from one to nearly two hundred, and while the worms have not been individually identified they have all been classed as *Ascaris lumbricoides*. It has been stated by Braun(8) and other authors that the *Ascaris* leaves the human host in the course of febrile diseases. In order to study this question in our series, the febrile cases were studied with the following result:

<table>
<thead>
<tr>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 cases of malaria, with <em>Ascaris</em> 9</td>
</tr>
<tr>
<td>15 cases of typhoid, with <em>Ascaris</em> 4</td>
</tr>
<tr>
<td>46 cases of plague, with <em>Ascaris</em> 17</td>
</tr>
<tr>
<td>25 cases of lobar pneumonia, with <em>Ascaris</em> 14</td>
</tr>
<tr>
<td>14 cases of bacillary dysentery with <em>Ascaris</em> 9</td>
</tr>
</tbody>
</table>

This table shows that in our series the febrile cases did not have a lower percentage of infestation with *Ascaris* than the nonfebrile cases.
As has been emphasized by many clinicians and pathologists dealing with this subject, in by far the greater number of cases the individuals harboring these parasites present no derangements which can be attributed to the presence of the parasites. That they can in some cases produce harmful action, either mechanically or by wandering from the intestine to other parts of the body, has been definitely established. Their toxicity is a matter still under active discussion.

Flury(9) in a chemical and toxicological study of the horse- and pig-ascaris found numerous substances both in the body substance and in the excretions which caused local irritation or necrosis. He was able to demonstrate volatile aldehydes of fatty acids and even the free acids, the principal ones being valerianic and butyric acids, and in smaller amounts formic, acrylic, and propionic acids, alcohols and esters of the ethyl, butyl, and amyl series. All of the symptoms of irritation of the mucous membranes noted by zoologists and the digestive disturbances in ascariasis he attributed to these substances. All of the disturbances of the central nervous system attributed to Ascaris (hallucination, hysteria, chorea, epilepsy, cramps, tetanus, delusions, psychic disturbances) may be explained on the basis of chronic aldehyde poisoning. A nitrogenous compound was found which caused death with severe hæmorrhages in the intestine after subcutaneous injection in the dog. This was ascribed to its action as a capillary poison. Flury concludes that Ascaris produces not a single poison, but a number of pharmacologically active substances which may produce very different and at times very severe symptoms.

Cases encountered in our series exemplifying the wandering of the Ascaris both before and after the death of the host and one where there was a possible toxic action will be recorded.

CASE 1

Ascaris in the vermiform appendix.—This case came to us from the operating room of the Philippine General Hospital. A 21-year-old Filipino was operated upon to relieve symptoms due to a prolapse of the uterus. In the course of the operation the appendix was found to be enlarged and congested and was removed by the routine method, a ligature being placed about its base. When received at the laboratory the following record was made: "The specimen consists of an appendix 7.5 centimeters in length and 1 centimeter in diameter; it is stiff and blue. The superficial vessels are rather prominent. On section through the proximal end the mucosa bulges, and a small amount of thick turbid substance exudes. On opening the appendix it is found to contain a large living Ascaris, the body of which has been cut, apparently a small part having
been left in the cæcum. The mucosa of the appendix is reddened, and shows one minute ecchymosis. Microscopic sections show some congestion of the mucosa, some fibrosis of the submucosa, œdema of the muscular and serous tunics, and infiltration of the muscularis by polymorphonuclear leucocytes and lymphocytes. Two ova of the *Ascaris* were found overlying the tissue in one section of the appendix." Examination of the faeces of this patient previous to operation had shown the presence of *Entameba, Trichuris, Ascaris*, blood cells, and mucus. No history was given of symptoms which were interpreted as indicative of disease of the appendix.

CASES 2 AND 3

*Ante-mortem infestation of the liver by Ascaris.*—Two cases* have been encountered in which numerous ascarides had passed through the bile ducts into the liver. In the first case several of the worms in the liver were surrounded by abscesses, one of which had ruptured into the peritoneum. In the second case there was abundant evidence of inflammation about the bile ducts in the liver, indicating the presence of the worms before death of the host.

**Case 4**

*Post-mortem wandering of the Ascaris.*—One case worthy of note occurred in a child, whose middle ear was found to contain a live *Ascaris* after the death of the host.

Numerous cases have been encountered in which the ascarides were found in the bile ducts, pancreatic duct, stomach, oesophagus, and larynx.

**Case 5**

*Case of possible toxic action of the Ascaris.*—This case, which occurred in our series, was reported by Albert and Mendoza at the ninth annual meeting of the Philippine Islands Medical Association. An 8-year-old Filipino, six days before death, was seized with an attack of vomiting, followed on the next day by pain in the chest and knee joints. There soon developed external œdema, jaundice, melæna, haemorrhage from the gums, dyspnœa, prostration, and ecchymoses on the right upper eyelid and over the lower extremities. Physical examination on the day before death showed the above conditions, hæmorrhage, cardiac murmurs, and evidence of severe anaæmia. Blood examination showed:

<table>
<thead>
<tr>
<th>Leucocytes</th>
<th>11,500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphonuclears</td>
<td>88 per cent.</td>
</tr>
<tr>
<td>Small lymphocytes</td>
<td>9 per cent.</td>
</tr>
<tr>
<td>Large lymphocytes</td>
<td>2 per cent.</td>
</tr>
<tr>
<td>Transitionals</td>
<td>1 per cent.</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>1,080,000</td>
</tr>
<tr>
<td>Hæmoglobin</td>
<td>30 per cent.</td>
</tr>
</tbody>
</table>

The Widal reaction with *B. typhosus* was negative, and a blood culture remained sterile.

There was no fever.

The autopsy report in full follows.

*These cases will be reported in detail by Dr. V. L. Andrews.*
ANATOMIC DIAGNOSIS

Purpura hæmorrhagica; ecchymoses, cutaneous, epicardial, pleural, retroperitoneal, retropleural, gastric, and intestinal; partial atelectasis of lungs; anæmia of the viscera; hæmorrhage into intestines; ascariasis; trichuriasis; ankylostomiasis.

BACTERIOLOGIC DIAGNOSIS

Cultures made from the spleen and the hæmorrhagic area behind the left knee joint on agar-agar, in bouillon, and on blood serum remain sterile forty-eight hours.

PROTOCOL

Body is that of a fairly well-nourished Filipino child, weighing 17.10 kilograms and measuring 110 centimeters in length. Rigor mortis is present. Over the right outer canthus is a minute bluish area, and there are two or three isolated, small bluish areas over the lower extremities. (These during life were bright red, ecchymotic areas.) The cornea and conjunctivæ are clear. Ears and nose normal. The margins of the teeth are blood-stained, but the gums themselves are pale.

On section there is a small amount of subcutaneous fat. Muscle tissue is moist and brown. On stripping muscles from the thorax, the right fourth intercostal muscle near its sternal attachment shows considerable infiltration of blood. The peritoneum contains a small amount of blood-stained fluid. That covering the anterior abdominal wall is smooth and pale. The serosa of the intestine is smooth, but there is extensive subperitoneal ecchymosis over the left kidney extending on to the mesocolon and mesentery. This is also extensive in the tissues at the lesser curvature of the stomach and along the vertebrae surrounding the abdominal aorta. Diaphragm at the fourth interspace on the right, fifth interspace on the left. On removal of the sternum, the tissues on its under surface show extensive ecchymosis. Lungs are considerably retracted, there being some blood-tinged fluid in both pleural sacs. The pericardium contains some excess of clear fluid. The structures of the neck and thorax were removed en masse.

Thorax; organs of neck.—The tongue is blood-stained on its superior surface throughout its anterior half. Tissues at the base of the tongue are not blood-stained, and the lymphoid tissue is not unduly prominent. Tonsils are of normal appearance. Æsophagus, larynx, and trachea are normal. Surrounding the trachea and æsophagus in the posterior mediastinum, the tissues are infiltrated with blood to an area of about 2 centimeters peripherally from the æsophagus in all directions. Thymus gland is small, and contains very abundant fat.

Heart.—There are numerous minute ecchymoses in the epicardium. The heart itself contains fluid blood. Its myocardium and endocardium are intact. It is of about normal size, and the musculature is extremely pale. Heart weighs 85 grams.

Lungs are retracted and of small volume, the right lower lobe being adherent to the tissues about the vertebrae at the extreme lower portion, that is, near the median line. The lungs present numerous, dark, depressed, firmer areas which have regular outlines, and the lower portion of the right lower lobe which was adherent along the median line is still firmer than these areas. On section of the lungs they are pale, except
for the firmer areas which are darker and contain practically no air, being rather leathery in consistence. The portion at the lower part of the right lower lobe on section cuts readily and is made up of an intensely hemorrhagic mass in which no structure can be differentiated. Peribronchial nodes are not enlarged. The larger pulmonary vessels are free.

Spleen is not enlarged and has a normal appearance, except for marked pallor. The lymphoid tissue is not prominent. Spleen weighs 42 grams.

Adrenals are normal in size and appearance.

Kidneys are imbedded in a small amount of fat. Ecchymosis over the left kidney has already been referred to. Kidneys are of normal size. On stripping the capsules, minute hemorrhages are visible on the internal surface of the capsules. The surfaces are smooth and very pale. On section they are of normal appearance except for very marked pallor. The pelves and ureters are free. Both kidneys weigh 101 grams.

Urinary bladder is considerably distended with clear urine. Its mucosa is pale and smooth.

Intestine.—There are very numerous ascarides in the small intestine and stomach. These number about 150. A few ankylostomata and many trichurides are also present. There is considerable blood-stained fluid throughout the intestine and numerous ecchymoses, especially in its upper portion.

Stomach contains some ascarides and few ecchymoses on its mucous surface.

Pancreas is practically surrounded by hemorrhagic tissue, but on section is of normal size and appearance, except for very marked pallor. Gall bladder and bile ducts are normal.

Liver is of about normal size with a thin capsule, and on section the cut surface is smooth, showing rather prominent bile ducts. The color is pale yellowish brown. Lobular markings are fairly distinctly visible. Liver weighs 458 grams.

Along the vertebral column, extending through the diaphragm at the oesophageal opening and continuing retroperitoneally to the bifurcation of the aorta, is a very extensive hemorrhagic mass which is firm, 2 to 3 centimeters in radius, its center being about the aorta. The mesenteric lymph nodes are enlarged, pale, and firm.

Structures of the scalp and calvarium are normal. Dura mater is adherent to the inner surface of the calvarium. There is no excess of fluid in the meninges over the hemispheres. The brain itself is firm and very pale.

On section into the right knee joint, it is found to contain clear fluid, and there is one minute petechia anteriorly in the capsule of the joint. On section into the left knee joint, it is also found to contain clear fluid, and all the muscle tissue posterior to the joint shows extensive hemorrhagic infiltration.

TRICHURIUS

Our figures (34.4 per cent) on the incidence of Trichuris are a little below the average (40.79 per cent) established by Willets(6) in his compilation of the statistics of investigators in the Philippine Islands, but are higher than those of three of the investigators. Our records in this case probably represent the true incidence of the condition in the cases examined
by us, as these worms are notably extremely difficult to dislodge by medication.

In our autopsy series no instance has occurred where any pathological condition was encountered which could be traced to Trichuris even though very large numbers of the parasites have been found in some of the cases. They are not infrequently attached rather intimately to the mucous membrane of the cæcum, and the sole exception to the above statement of the absence of pathological conditions has been the occasional finding of minute petechiae in the mucoæa of the cæcum which have been attributed to Trichuris. However, this relation has not been established by serial microscopic sections.

On account of the frequent assertion that Trichuris is more prevalent in females and children than in males and adults, our records were examined to ascertain the incidence in our series with respect to sex and age. The following record shows our findings.

**Table V.—Incidence of trichuriasis according to age and sex.**

<table>
<thead>
<tr>
<th></th>
<th>Total males</th>
<th>Males under 15 years</th>
<th>Males over 15 years</th>
<th>Total females</th>
<th>Females under 15 years</th>
<th>Females over 15 years</th>
<th>Total under 15 years</th>
<th>Total over 15 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases examined</td>
<td>333</td>
<td>78</td>
<td>275</td>
<td>147</td>
<td>38</td>
<td>109</td>
<td>116</td>
<td>384</td>
<td>500</td>
</tr>
<tr>
<td>Cases trichuriasis</td>
<td>120</td>
<td>14</td>
<td>106</td>
<td>52</td>
<td>4</td>
<td>48</td>
<td>18</td>
<td>154</td>
<td>172</td>
</tr>
<tr>
<td>Per cent trichuriasis</td>
<td>33.9</td>
<td>17.9</td>
<td>38.5</td>
<td>35.4</td>
<td>10.5</td>
<td>44.0</td>
<td>11.5</td>
<td>40.1</td>
<td>34.4</td>
</tr>
</tbody>
</table>

Examination of this table shows that in our series the occurrence of Trichuris in females is very little more frequent than in males, and that the cases over 15 years of age had the parasites nearly three times as frequently as those under that age.

Metchnikoff(10) was the first to ascribe a rôle to Trichuris in the etiology of appendicitis, and the literature on the whole subject of trichuriasis up to 1908 had been carefully reviewed by Musgrave, Clegg, and Polk,(11) who say that "very little is given about the clinical findings, but a priori there is no reason to assume that they would be materially different from appendicitis of other etiology."

Castellani and Chalmers(12) in discussing the appendicular variety of trichuriasis say "the symptoms of this variety are the same as those for appendicitis arising from other causes. Operative treatment reveals the nature of the malady."

Cecil and Bulkley(13) have reported finding trichurides in two cases of appendicitis, 1 catarrhal in type and 1 gangrenous.
The establishment of direct etiological relationship seems rather arbitrary, and the pathological changes described by them do not seem pathognomonic of trichuriasis.

Among the very large number of appendices received from the surgical clinic, 2 have been examined which contained Trichuris. The total number of cases examined is not given for the reason that not all were opened before fixation. However, microscopic sections of none of the appendices showed anything which led to the suspicion of the presence of Trichuris, and in the two cases mentioned the presence of Trichuris would not have been suspected from the sections made.

**CASE 1**

*Trichuris in the vermiform appendix.*—A 21-year-old Filipino was admitted to the hospital with symptoms pointing to disease of the appendix and giving a history of two previous similar attacks within four months. The symptoms were some pain over the region of the appendix, tenderness on slight pressure, nausea, some vomiting, and slight rigidity. The temperature was not above 38°.6 C. before operation. He was in the hospital for twenty-six days before operation; his leucocytes, which had ranged from 12,600 to 14,800 per cubic millimeter of blood previous to this, numbered 28,000 on the day of operation. At the operation the appendix was removed, and it was noted that there were adhesions about the intestines. The examination of the faeces previous to operation was unsatisfactory, but 3 examinations at varying periods after operation showed the presence of the ova of hookworm but none of Trichuris.

When received at the laboratory the following record was made of the appendix: "The specimen consists of an appendix which is 6 centimeters long and has some attached mesenteric fat. It is about 7 millimeters in diameter, and the vessels of the serosa are prominent. On opening the appendix, it is found to contain a small amount of soft brown fecal material, and the mucosa is somewhat swollen and moist, and shows a few injected areas. Two trichurides are found in the lumen, rather closely attached to the mucosa. Microscopically there is seen some loss of epithelium with the passage of lymphocytes into the lumen, and the epithelium of the glandular tubules shows a well-marked catarrhal state. There is active proliferation of the lymph follicles and some fibrosis of the submucosa. Marked round-celled infiltration of the submucosa is seen as well as of the outer muscular and serous coats. The outer muscular coat is very oedematous, and the vessels just beneath the serosa are much dilated and surrounded by dense masses of round cells. Eosinophiles in large numbers are present throughout all parts of the sections."

**CASE 2**

*Trichuris in the vermiform appendix.*—In the course of a gynecological operation on a 38-year-old Filipina the appendix was removed. It was a small, somewhat distorted appendix, from the cut proximal end of which an intact Trichuris protruded. Microscopic examination of this appendix shows a very thin muscular tunic and an intact serosa. The lymphoid tissue is scanty. In the mucosa there is some congestion and
some leucocytic infiltration and oedema, the cells of the glandular tubules being largely converted into goblet cells. At the point where the Trichuris was in contact with the mucosa several ova are found in the crypts of the mucosa.

HOOKWORMS

In considering the incidence (16.6 per cent) of hookworms in our series, it must be borne in mind that the majority of our cases are derived from a hospital where careful examination of the faeces is a routine clinical procedure and active treatment of this disease is the rule. Willets' percentage based on the work of different investigators in the Philippine Islands was 30.57, while his own clinical examination of 4,288 cases gave him a percentage of 54.37.

Chamberlain, (14) as a result of his own investigations, concludes that "Uncinariasis is found among the Filipinos in probably not over 15 per cent of the general population and is mild in type and of small economic importance."

While anæmia, the chief symptom in hookworm disease, has been a prominent feature in a large number of our cases, no case has been encountered in which the hookworm was considered an important factor, and the death of the patient has always been ascribed to other causes. However, a not infrequent finding in our cases has been punctate haemorrhages in the mucosa of the upper part of the small intestine sometimes with slight erosion or, if not actual haemorrhages, minute pigmented spots. These latter have been interpreted as evidence of residual blood pigment from previous haemorrhage. One case which exemplifies the condition we have encountered, and in which the number of hookworms found was unusually great, is here reported. In this case the bacillary dysentery was the cause of death, and the previous malarial infection along with the dysentery may have accounted for the severe anæmia. Nevertheless, neither of these caused the condition described in the small intestine, and the case does serve to illustrate our findings, at the same time emphasizing the presence of other diseases as being the ones to cause death.

CASE REPORT

A 24-year-old male Japanese was admitted to hospital four days before death with symptoms of bacillary dysentery, marked anæmia, and an enlarged spleen. The temperature range was between 37.5 and 39.5 C. Pulse 110 to 140. Faeces contained ankylostoma ova, blood, mucus, and pus. The urine contained a trace of albumin and numerous casts. The
blood was not examined. At autopsy the following anatomical diagnosis was made:

Acute colitis (bacillary dysentery); acute lymphadenitis, mesocolic; subacute splenitis, malarial; infarcts of spleen; acute parenchymatous degeneration of liver and kidney; malarial pigmentation of liver, pancreas, and lungs; congestion and edema of lungs; hookworms; and hemorrhages and erosions in mucosa of small intestine.

*Bacillus dysenteriae* was isolated from the mucosa of the large intestine.

The description given of the intestines was as follows:

In the duodenum are found a few hookworms, one being attached within 5 centimeters of the pylorus. In the jejunum they are found in great numbers both attached and free. At the points of attachment of many, and also between them, are small hemorrhagic areas which are sometimes visible from the serous surface. Over a number of these there occur shallow erosions of the mucosa. In the lower 10 centimeters of the ileum there is marked hyperemia of the mucosa. The mucosa of the ileoceleal valve is swollen and reddened. Throughout the large intestine the mucosa is greatly swollen, edematous, and hyperemic. The greatest congestion occurs in the ascending and sigmoid portions. In many places small ulcers have formed, most frequently on the tips of the folds of the mucosa. The swelling and congestion extends into the appendix.

Sections taken through the areas in the small intestine mentioned as hemorrhagic show a destruction of epithelium and glandular tubules. The mucosa and submucosa immediately beneath are edematous, and many leucocytes and erythrocytes are present. Extending for a short distance each way from this, situated deep in the submucosa, there is a thin layer of extravasated blood.

The lesions in the small intestine in this case can with a very great degree of probability be ascribed to the hookworms, while the lesions of the large intestine are those of bacillary dysentery.

**OXYURIS**

The incidence (1 per cent) of *Oxyurus* has been so small and its presence is of so little pathologic importance that no further comment will be made upon it. It is possible that it may have been overlooked in a number of our cases.

**ENTAMOEBA**

Our figures on the incidence of *Entamoeba* (5 per cent) refer only to those cases in which there was a recognizable amebic colitis. Routine examination of the feces at autopsy has not been performed. Musgrave and Clegg(7) found 26 per cent of 587 individuals harboring amebae, from an examination of the feces, and Garrison(1) found 23 per cent in 4,106 prisoners. These authors did not differentiate between pathogenic and nonpathogenic amebae, and according to Walker(15) individuals may harbor amebae without suffering from amebic colitis.
TAENIA

Only one case has been encountered in which a Taenia was found. This occurred in a 57-year-old Filipino who died as the result of cerebral hemorraghe dependent on arteriosclerosis. This adult worm was found in the intestine and was identified as Taenia saginata. Three other tapeworms obtained from the human host which are in the museum of this department are also Taenia saginata.

No example of the adult of Taenia solium has been encountered, but one case of widespread infestation with Cysticercus cellulosae occurred in a 28-year-old Filipino, a resident of Manila, who died suddenly.

Cysticerci in hogs are a very frequent finding in the Philippine Islands, and these, on account of their occurrence in this host and on account of their scolices bearing 4 suckers and a rostellarum with hooklets, are presumably Cysticercus cellulosae. This would seem to presuppose the frequent presence of the adult parasite in the human host. However, the adult worm has not been found in our series, and only one example of Cysticercus cellulosae has occurred in a series of over 2,200 autopsies performed in this department.

The autopsy report of our case will be given in full.

ANATOMIC DIAGNOSIS

Cysticercus cellulosae of brain, hypophysis, and muscles; dilatation of right ventricle; congestion of lungs; passive congestion of liver and kidneys; chronic fibrous pleurisy; persistent thymus; hookworms; ascariasis; trichuriasis.

Body is that of a large, well-developed and fairly well-nourished Filipino, weighing 55.65 kilograms and measuring 172 centimeters in length. Post-mortem suggillation is marked on the back. There are no external evidences of violence. Left knee is slightly flexed. Rigor mortis is marked. The body is twisted slightly to the right above the pelvis.

A small elevation is noticed on the left arm over the biceps muscle; this feels firm, and the skin moves freely over it. On incising the skin there is found just beneath the fascia of the biceps muscle a small, oval, translucent cyst, measuring 15 millimeters in length by 6 millimeters in thickness. The long axis is parallel to the muscle fibers. The capsule of the cyst is thin but moderately resistant. Another cyst is found near this, and others are found just beneath the fascia of other muscles, one in the right biceps, one in the left extensor digitorum communis, one in the lateral head of the left gastrocnemius, and one in the subscapularis. Most of these are somewhat smaller than the one described, particularly in the long diameter. The deeper muscles were not investigated. Subcutaneous fat is present in moderate amount.

Peritoneum is smooth and pink. The peritoneal cavity contains a small amount of clear, light-reddish fluid. Appendix appears to be normal.
There is a slight fibrous adhesion between the colon and the inferior surface of the liver. Diaphragm stands at the fifth interspace on the left side and the fourth interspace on the right.

Thorax.—The pericardial sac is large, and contains a few cubic centimeters of clear fluid. The thymus is long and narrow, measuring 12 by 4 centimeters. It is pink, both externally and on section. Thymus weighs 30 grams.

Lungs.—Left lung is bound to the chest wall by a few fibrous adhesions. The bronchi are pink, and contain a little frothy fluid but no mucus. On section the lung is slightly moist, but on pressure very little serous fluid can be expressed. However, the blood vessels are well filled with dark fluid blood. Its surface is smooth, except near the anterior border where there is a fibrous tag. Color of the surface is purplish red. The lung is everywhere soft and crepitant, and pits slightly on pressure. On section the right lung is similar to the left, although the surface is slightly more moist.

Heart.—On the anterior surface of the right ventricle are 2 slightly thickened, grayish patches. The right ventricle is very prominent, but the left also seems large. The tricuspid ring admits easily four fingers. The auricle is slightly larger than normal. The right ventricle is markedly dilated; the wall, however, is thin, measuring 3 to 5 millimeters in thickness and the muscle is soft. The tricuspid ring measures 13 centimeters in circumference. The left auricle is normal in size. The left ventricle is fairly well contracted, the chamber being small, and the muscle rather soft and light reddish grayish brown in color. The papillary muscles are rather thick. The wall measures 15 millimeters in thickness. The mitral ring measures 10.5 centimeters. Pulmonary ring measures 7.2 centimeters. Heart weighs 353 grams.

Spleen is of normal size. On section it is found to be rather firm. The surface is dark red, and the organ contains considerable blood. The Malpighian bodies are barely visible. Spleen weighs 187 grams.

Kidneys are large. The capsules strip easily, leaving a smooth, purplish red surface. On section the cortex is bluish red in color; the glomeruli are barely visible; striations are regular. The pyramids are purple. The organs are moderately firm. Both kidneys weigh 272 grams.

Adrenals are thin, and the medullary substance is scanty.

Pancreas is rather firm. On section it is pink.

Liver is enlarged. The surface is smooth, and the organ is fairly firm. On section it is uniformly dark red in color and friable, the lobules being easily seen. Liver weighs 1,815 grams.

Stomach contains a large amount of partially digested food consisting principally of rice.

Intestines.—There is slight hyperemia of the upper part of the small intestine, otherwise no lesions are noted. Intestines contain hookworms, ascarides, and trichurides.

Genitalia are rather small but otherwise normal.

Urinary bladder is normal.

Brain.—Meninges are rather moist, especially about the base where they seem slightly thickened. On the surface of the brain in, or in most cases beneath, the pia-arachnoid and embedded in the cortex are found a number of small cysts. These have a slightly thicker capsule, and are in most cases more nearly spherical than those of the muscles. Some
lie upon the gyri, while others are deep in the sulci. On the left hemisphere, one is found on the superior frontal, one on the middle frontal, one on the posterior central gyrus, one on the temporal pole, two on the middle temporal gyrus, one on the external surface of the occipital lobe, near the anterior end of the occipito-temporal gyrus, and one on the precuneus. On the right hemisphere one is found on the inferior frontal, two on the inferior temporal, one in the collateral sulcus, and one in the lateral occipital sulcus. There is one also on the right precuneus. On section of the brain, one is found on the left lateral wall of the third ventricle, projecting slightly into its lumen. The lateral ventricles are not dilated; the cerebrospinal fluid is clear. On section of the basal ganglia one cyst is found in the lower lateral portion of the right thalamus and one in the head of the right caudate nucleus. On removing the hypophysis, a small cyst similar in appearance to those of the brain but containing soft, gelatinous material is found on its inferior surface.

ADDENDUM

Closer inspection of a number of the cysts reveals the following: Near the middle is seen a white spot which, on opening the cyst, is seen to correspond with a small rounded mass projecting from one side into the lumen. Under the microscope this projection resembles the head of a tapeworm. There are 4 suckers beyond which is a circular row of hooklets, 26 in number.

CLONORCHIS

Two cases have occurred in which liver flukes were found. In the first case they were present in the cystic, common, and intrahepatic bile ducts of a 27-year-old Chinaman who died of an acute dilatation of the heart, complicating chronic nephritis. The second case in which they occurred was also a Chinaman, 29 years of age, who had died as the result of a perforation of the intestine in the course of typhoid fever. In this case they were found only in the intrahepatic bile ducts. In neither case did the bile ducts or liver show any changes attributable to the presence of the parasites. The flukes in both cases have been identified by Doctor Willets of the Bureau of Science as Clonorchis sinensis Cobbold, 1875.

The identification is based upon the presence of dark-brown, granular pigment in the body parenchyma and the excretory apparatus and the following anatomical characters:

Size and shape.—Flat; elongated, 18 by 3.5 millimeters; another, 18 by 4 millimeters.

Cuticular spines.—Absent.
Anterior end.—Rather pointed. Head cone absent.
Oral sucker.—Small.
Acetabulum.—Small; well developed; near oral sucker.
Pharynx.—Small; well developed.
Esophagus.—Rather short.
Intestinal ceca.—Unbranched; extending to near the posterior extremity of the body.
Excretory vesicle.—Tubular; contains considerable, fine dark-brown, granular pigment.
Testes.—Two; posterior; branched with irregular swellings.
Receptaculum seminis.—Very well developed; relatively large; immediately anterior to anterior testis.
Ovary.—Lobate; anterior to receptaculum seminis.
Uterus.—Full of yellowish brown, operculated ova; between acetabulum and ovary.
Genital pore.—Mid-line; immediately anterior to acetabulum.
Vesicula seminalis.—Present; well developed; long.
Cirrus pouch.—Absent.
Yolk glands.—In lateral fields; extend from about the level of the acetabulum to about that of the ovary; several follicles are underdeveloped in each set.
Ova.—Small, yellowish brown, operculated; average size, 30 by 16 microns.

SCHISTOSOMA

One case* of schistosomiasis has been encountered in an 18-year-old Filipino. The case showed the characteristic changes, and the parasite has been identified as Schistosoma japonicum.

SUMMARY

In a series of 500 consecutive autopsies on people of all ages in Manila:
Ascaris lumbricoides occurred in 41.2 per cent, Trichuris trichiura in 34.4 per cent, hookworm in 16.6 per cent, Taenia saginata in 0.2 per cent, Cysticercus cellulosae in 0.2 per cent, Oxyuris in 1 per cent, Clonorchis sinensis in 0.4 per cent, Schistosoma japonicum in 0.2 per cent, malaria in 5 per cent, and amoebic colitis in 5 per cent.
The manifestations of ascariasis have been the presence of Ascaris in the liver as the result of both ante-mortem and post-mortem wanderings, and its possible action as the exciting factor in one case of widespread haemorrhages. One clinical case of Ascaris in the appendix has been encountered. Ascaris has not been found less frequent in febrile than in other cases.
Trichuriasis occurred in 33.9 per cent of the males and 35.4 per cent of the females; 15.53 per cent of the cases under 15 years of age and 40.1 per cent of the cases above that age harbored Trichuris. Two clinical cases of appendicitis in which Trichuris was found in the appendix have been encountered.
One case has been described exemplifying the frequently encountered haemorrhages in the submucosa of the intestine associated with the presence of the hookworm.

*This case will be reported in full by Dr. V. L. Andrews.
One *Taenia saginata* was the only tapeworm encountered. The infrequency of *Taenia solium* in human cases as compared with the great frequency of *Cysticercus* in hogs in the Philippines has been deemed noteworthy.

One case of *Cysticercus cellulosae* in the human cadaver has been reported in detail.

REFERENCES

CONCERNING THE BERIBERI-PREVENTING SUBSTANCES OR VITAMINES CONTAINED IN RICE POLISHINGS

A SIXTH CONTRIBUTION TO THE ETIOLOGY OF BERIBERI

By Edward B. Vedder and Robert R. Williams

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It has been claimed at various times that beriberi has resulted from the use of an undermilled rice which had been stored for some time or which had been kept in a damp place. Thus Braddock (1) says:

I have had an extensive experience with beriberi, and never saw it except when the rice used for food had been stored in a damp place; when care was taken to store the rice in a dry place, there was no beriberi.

An interesting case was reported by Bréaudat, (2) where there were two neighboring settlements. In the first the rice was always polished by hand daily, and not a single case of beriberi has ever been reported. In the second settlement the rice was polished every ten or twelve days and stored in wooden boxes, and beriberi occurred every season with an incidence of from 60 to 90 cases. He advised the manager to use rice polished by hand daily, and not a single case occurred after that procedure was adopted. Bréaudat says: "Therefore I come to the conclusion that even hand-polished rice, if 10 or 12 days old, may produce Beriberi." These statements cast grave doubts on the protective value of stored rice, particularly as it is well known that the protective substances in rice polishings are readily destroyed by certain agencies; for example, moist heat and strong alkalies.

In view of the practical importance of this question, an experiment was performed to test the keeping qualities of undermilled rice.

Experiment 38.—A sack of the undermilled rice furnished the Philippine Scouts was obtained from the commissary. This rice

1 Read before the Manila Medical Society, April 7, 1913, and published with the permission of the Chief Surgeon, Philippine Department.
2 Edward B. Vedder, captain, Medical Corps, United States Army, member of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.
was then stored for one year in a tin can without any cover, in the ground floor of a building not specially protected from dampness. It will thus be seen that this rice was exposed to the dampness throughout an entire rainy season and was freely accessible to roaches, weevils, and other insects. At the conclusion of this period all the rice was musty, and actually moldy in spots, and absolutely unfit for human consumption.

Four fowls were now fed exclusively on this rice. All four fowls remained in perfect health for a period of four months when the experiment was discontinued. The fowls fed on this rice did not lose their appetite as do fowls fed on polished rice, but ate every grain of their ration up to the very last. It is believed that this experiment imposed a far more severe test on the keeping qualities of undemilled rice than will ever be required in the case of rice used for human consumption. Before the experiment was concluded, this rice was fully a year and a half old, since it must have been several months old before it was obtained from the commissary. This experiment shows conclusively that an undemilled rice does not lose its protective power because of dampness or long storage. We might add that in a former communication(3) we stated that we had confirmed Shiga's experiment that undemilled rice which had been allowed to ferment in the incubator still retained its protective properties, and that we have found that an extract of rice polishings, prepared according to the method previously described and kept in an ice box to prevent decomposition,(4) has retained its protective and curative powers for a period of at least seven months.

Soon after describing the preparation of this extract(4) we had reason to believe that all of the protective substances in the polishings were not extracted by this method because of their slight solubility in cold 95 per cent alcohol. Therefore, in all our later work we extracted the polishings three times with successive portions of fresh 95 per cent alcohol, using 3 liters of alcohol to each kilogram of polishings for the first extraction and 1.5 liters of alcohol for each of the two following extractions. The extract so obtained was combined. It was found, in fact, that this successive extraction with increased quantities of alcohol increased the protective and curative action of the extract.

It will be remembered that Strong and Crowell(5) used extract prepared according to our method together with a polished rice in feeding one group of men in their experiment, but that these men were not fully protected, and some of them developed symptoms of beriberi. Since Strong and Crowell extracted each 5
kilograms of polishings with 14 liters of 95 per cent alcohol in three successive macerations, while we have for some time been using 30 liters of alcohol to extract each 5 kilograms of polishings, it seemed possible that the extract used by Strong and Crowell was only partially successful in preventing beriberi because insufficient alcohol was used completely to extract the protective substances from the polishings. In order to obtain more definite information on this point, the following experiment was performed.

Experiment 39.—Five kilograms of polishings were extracted with 15 liters of 95 per cent alcohol. The polishings were pressed, and the dry residue again extracted twice in a similar manner, using 8 liters of fresh alcohol each time. The extracts obtained from these three processes were kept separate, the alcohol evaporated off, the fat removed, and the residue diluted with water according to our usual method.

(a) Four fowls were fed on polished rice plus the substances obtained on the first extraction. An amount equivalent to 10 grams of polishings was given each fowl daily. All 4 fowls remained well for a period of three months when the experiment was discontinued.

(b) Four fowls were fed on polished rice plus the substances obtained on the second extraction. An amount equivalent to 10 grams of polishings was given each fowl daily. All 4 fowls remained well for three months when the experiment was discontinued.

(c) Four fowls were fed on polished rice plus the substances obtained on the third extraction. An amount equivalent to 10 grams of polishings was given each fowl daily. All 4 fowls remained well for three months when the experiment was discontinued.

(d) Four fowls were fed on polished rice plus a daily dose of 10 grams of the polishings that had been extracted three times with alcohol. All 4 fowls remained well for a period of three months when the experiment was discontinued.

This experiment shows that even three successive extractions with 95 per cent alcohol, using a total of 6 liters of alcohol to each kilogram of polishings, are insufficient to remove all of the protective substances from rice polishings. We may, therefore, conclude that the protective substances are only slightly soluble in cold 95 per cent alcohol, and that it is very probable that the extract used by Strong and Crowell in the experiment already quoted failed to confer complete protection because the polishings were incompletely extracted by the quantity of alcohol they used.
The method of extracting by alcohol was originally adopted by us because it produced an extract that was chemically much simpler than that obtained by other methods and thus afforded a better opportunity for identifying the protective substances, and we have continued to use this method in preparing extracts for the treatment of beriberi cases in spite of the disadvantage of the comparative insolubility of the protective substances in alcohol, because we have found that extracts obtained in several other ways are distinctly poisonous, a fact to which we shall refer again later. We have found, however, that a more powerful extract may be obtained by using alcohol of only 90 per cent strength, or by using alcohol at a temperature of from 60° to 70°.

In a previous paper(3) we stated that sodium hydroxide destroyed the neuritis-preventing substance. We have also found that it is destroyed by ammonia.

Experiment 40.—In the course of our experiments to determine the solubility of the protective substances in ether, extract of rice polishings which had previously been tested and found active was rendered strongly alkaline with ammonia, and was then extracted by shaking with ether. The ether was then removed and evaporated at a low temperature and the residue diluted with water.

(a) Four fowls were fed on polished rice, and in addition were given daily a quantity of the substances extracted by the ether, equivalent to 10 grams of polishings. One fowl developed neuritis in twenty-eight days and one in twenty-nine days, when the experiment was discontinued.

(b) Four fowls were fed on polished rice, and in addition were given daily 10 cubic centimeters of the aqueous extract of rice polishings remaining after the extraction with ether. One fowl developed neuritis in thirty-eight days and one in forty-five days, when the experiment was discontinued.

Since this extract originally protected fowls completely, while, after treatment with ammonia, both the ether extract and the residual portion failed to protect, it appears that the protective substances were destroyed by the ammonia. The significance of this fact will appear later in discussing the action of barium hydroxide.

In one of his papers(6) Funk stated that while allantoin had no effect in curing pigeons that had already developed neuritis, it appeared to prolong the life of the birds. As we had found (see experiment 46) that the administration of a mixture of
purine bases undoubtedly exerted some protective influence, we tried a feeding experiment with allantoin.

Experiment 41.—Five grams of allantoin were dissolved in 1,000 cubic centimeters of water. Four fowls were fed on polished rice and in addition were given daily 10 cubic centimeters of this solution of allantoin or 50 milligrams daily. One fowl developed neuritis in twenty days, one in twenty-five days, and one in twenty-nine days.

From this experiment it is concluded that allantoin does not possess any protective action.

Having used basic lead acetate as a precipitant in a previous experiment, (3) the result of which was in doubt, we performed the following experiment.

Experiment 42.—A quantity of extract of rice polishings was precipitated by basic lead acetate. The precipitate was separated from the filtrate and the lead was removed from both by hydrogen sulphide.

(a) Four fowls were fed on polished rice, and in addition were given a daily dose of the substances precipitated by basic lead acetate equivalent to 10 grams of polishings. One fowl developed neuritis in twenty-six days, one in thirty days, and one in thirty-two days, when the experiment was discontinued.

(b) Four fowls were fed on polished rice, and in addition were given a daily dose of the substances not precipitated by basic lead acetate equivalent to 10 grams of polishings. One fowl developed neuritis in thirty days, but the remaining 3 fowls continued in good health for sixty days, when the experiment was discontinued.

From this experiment it is concluded that the neuritis-preventing substance is not precipitated by basic lead acetate, but remains in the filtrate. However, since one of the fowls (probably highly susceptible to this disease) fed on this filtrate developed neuritis, it appears that a portion of the neuritis-preventing substances is lost as a result of the chemical manipulation. After we had performed this experiment, we received the paper of Edie, Evans, Moore, Simpson, and Webster (7) in which they report obtaining a similar result after using lead acetate in isolating the antineuritic substances from yeast.

Before any of Funk’s papers had reached us, we had tried (3) precipitating our extract with phosphotungstic acid and had decomposed the phosphotungstes with barium hydroxide. We did not succeed in obtaining any protective action from these decomposed phosphotungstes. Funk’s work, whose es-
sential accuracy we could not doubt, was therefore apparently contradictory to our own results. After receiving Funk's papers, we repeated the feeding experiment several times, using Funk's method on our extract as we had always prepared it. In each case the result was a failure, for the fowls developed neuritis, both in the group receiving the filtrate from phosphotungstic acid and in the group receiving the decomposed phosphotungstic acid.

Being convinced that Funk's work was correct, and that our previous work was also correct, it became certain that, if an adequate explanation of this apparent discrepancy could be afforded, further progress would be made. This explanation we are now prepared to make. Funk(8) tried only curative experiments, while we were relying on feeding experiments. Further, Funk had extracted his rice polishings with alcohol containing 2.5 per cent of gaseous hydrochloric acid, while the preparation with which we worked was extracted by alcohol alone. These differences in method which may appear trifling are in reality crucial.

The therapeutic action of these two extracts is totally different. Funk reported that large doses of his extract were poisonous, but that, if doses small enough to avoid this poisonous action were administered to pigeons that had developed polyneuritis as a result of rice feeding, the birds were immediately cured. Our extract, on the other hand, was never poisonous, although given in enormous doses, equivalent to several kilograms of polishings; nor did it produce an immediate cure when given to birds that had already developed polyneuritis; but if the birds were given small doses of this extract daily (equivalent to 10 grams of polishings), their lives were saved, although they remained paralyzed. Further, if the administration of our extract was continued for several months, the paralysis also was thereby cured. Funk's extract was therefore poisonous, but smaller doses promptly cured the paralytic symptoms, while our extract was nonpoisonous and only slowly curative. It appeared probable that this difference in therapeutic action resulted from the fact that Funk used hydrochloric acid in the preparation of his extract while we did not. In order to test this hypothesis, the following experiment was performed.

Experiment 43.—A quantity of our extract was divided into two portions. One part was untreated and the other part was mixed with 5 per cent hydrochloric acid and allowed to stand at room temperature for twenty-four hours.
(a) A normal fowl was given the untreated extract from 2 kilograms of polishings. It was entirely unaffected by this dose.

(b) A normal fowl was given the extract from 300 grams of polishings, treated with hydrochloric acid, and subsequently neutralized with sodium bicarbonate. The fowl was quickly prostrated and died within fifteen minutes.

(c) A fowl that had developed neuritis as a result of feeding on polished rice, and whose legs were completely paralyzed, was given untreated extract from 2 kilograms of polishings. No improvement.

(d) Another fowl that had developed neuritis, and whose legs were completely paralyzed, was given the extract treated with hydrochloric acid from 100 grams of polishings. This fowl was greatly improved and within twenty-four hours was able to walk like a normal bird; in fact, it was completely cured.

This experiment demonstrated that the difference in therapeutic action between Funk's extract and our own was undoubtedly due to the hydrochloric acid used in one case and not in the other, and, further, that the properties of our extract could be changed so that it resembled the action of Funk's extract by simple treatment with 5 per cent hydrochloric acid.

It was found that 5 per cent sulphuric acid effected the same transformation. It is apparent that strong mineral acids break up the protective substance which is present in the rice polishings and the alcoholic extract, reducing it to a more active and probably simpler form. This may be regarded as a hydrolysis which, however, is not produced by the weak organic acids normally present in the extract. Funk's(6) later results in working with yeast amply confirm our observations as to the effect of hydrolysis with strong mineral acids.

Having this clue, we again prepared an extract of rice polishings according to our previous method, hydrolyzed it with 5 per cent sulphuric acid, and precipitated it with phosphotungstic acid and silver nitrate and baryta, following Funk's procedure closely. We thus obtained a small quantity of a crystalline base, 30 milligrams of which promptly cured fowls suffering from polyneuritis. This base was undoubtedly the same as that isolated by Funk. The next step was to try a feeding experiment with this base.

Experiment 44.—A quantity of this base was dissolved in distilled water in such proportion that each 10 cubic centimeters of the solution represented the amount of the base extracted from 10 grams of polishings.
Four fowls were fed on polished rice, and were given a daily dose of 10 cubic centimeters of this solution. One fowl developed neuritis in thirty-five days, one in thirty-seven days, and one in forty-five days, when the experiment was discontinued. It appears from this experiment that the fowls may have received some protection, since the incubation period was somewhat prolonged. (Average incubation period of our experiments twenty-six days.) The protection afforded was but slight.

The explanation of the failure completely to protect these fowls undoubtedly lies in the fact that sufficient amounts of this base were not used. In the curative experiments relatively large quantities of this base were used; that is, 30 milligrams. Funk obtained only 0.4 gram of this base from 50 kilograms of polishings, and the amounts we have obtained from several hundred kilograms of polishings have been proportionately even smaller. It is, therefore, apparent that to cure one fowl a quantity of the base extracted from about 5 kilograms of polishings was used, or almost five times the total quantity used to feed 4 fowls. But if this base were the only protective substance contained in the rice polishings, and were completely extracted, it is evident that the equivalent of 10 grams of polishings should have fully protected. Since this quantity did not protect completely in a feeding experiment, we now considered the following possibilities:

1. That there are other protective, if not curative, substances in the polishings besides Funk's base.
2. That Funk's base is incompletely extracted by Funk's method.
3. That both of the above hypotheses are correct.

Experiment 45.—An extract of rice polishings was prepared according to our usual method and without hydrolysis was precipitated by phosphotungstic acid. The washed precipitate was then mixed with distilled water in such proportion that 10 cubic centimeters of the suspension represented the substances precipitated by phosphotungstic acid from 10 grams of polishings.

Four fowls were then fed on polished rice, and were given a daily dose of 10 cubic centimeters of this suspension. One fowl died of avian diphtheria after fifty-one days, and the other 3 birds remained in perfect health for seventy-one days, when the experiment was discontinued. This experiment showed that the total phosphotungstic acids precipitated from an unhydrolyzed extract of rice polishings were sufficient to prevent polynarthritis in a dosage corresponding to 10 grams of polishings,
and even when fed as phosphotungstates, without any further treatment.

Experiment 46.—The hydrolyzed extract obtained from 10 kilograms of polishings was completely precipitated by phosphotungstic acid. The precipitate so obtained was extracted by repeated and prolonged shaking with 50 per cent alcohol. The phosphotungstic acid was then removed from this alcoholic solution of the phosphotungstates by barium hydroxide. A sufficient quantity of this alcoholic extract of phosphotungstates was reserved to feed 4 fowls (group a). The remainder of the solution was carefully neutralized with sulphuric acid, and silver nitrate added until a drop of the clear solution gave a brown precipitate of silver oxide with cold barium hydroxide. The flocculent precipitate so obtained was filtered off and the silver removed by hydrogen sulphide. The filtrate from the silver sulphide so obtained was used to feed 4 fowls (group b).

The filtrate obtained from this first precipitation with silver nitrate was then rendered distinctly alkaline with baryta. The precipitate so produced was filtered off and the silver removed by hydrogen sulphide, and the filtrate from the silver sulphide was used to feed 4 fowls (group c). The filtrate remaining after these two precipitations by silver nitrate was used to feed 4 fowls (group d).

Sixteen fowls were fed on polished rice, and each group of 4 received one of these solutions in a daily dose equivalent to 30 grams of polishings, as follows:

Group a: Four fowls receiving alcoholic extract of total phosphotungstates. All 4 fowls remained well for sixty days.

Group b: Four fowls receiving silver nitrate precipitate in neutral solution (purine bases). One fowl developed neuritis in thirty-six days and died. A second fowl developed neuritis in forty-six days. A large dose of the solution was given this fowl and the remaining 2 birds. The sick fowl improved in health and ended the experiment on the sixtieth day almost entirely cured, and the other 2 birds remained in health for the full period of sixty days.

Group c: Four fowls receiving silver nitrate precipitate in alkaline solution (Funk's base). All 4 fowls remained well for sixty days.

³ This method was adopted, because in this way the phosphotungstic acid can be removed without subjecting the solution to a great excess of baryta, as must necessarily be done when the dried precipitate is broken up by barium hydroxide. The use of strong alkaline solutions is to be avoided, as they undoubtedly decompose a part of the protective substances.
Group *d*: Four fowls receiving filtrate remaining from the above double precipitation. All 4 fowls remained well for sixty days.

The experiment was discontinued after sixty days because the solutions prepared were exhausted.

Group *a* in this experiment simply served as a control, showing that the alcoholic solution of the total phosphotungstates sufficed to confer protection in the amount used. But groups *b*, *c*, and *d* all contained substances that differed from each other chemically. Group *c*, which contained Funk's base, might be supposed to confer protection, as it did; but group *b*, containing purine bases which have in the past been supposed to be devoid of protective action, and group *d*, presumably containing choline and other similar bases, also both conferred protection. The protection conferred in group *b* was not complete, it is true; but we have reason to believe, from the fact that the second bird to be affected by neuritis improved when larger doses were given, that the amount of protection is simply a question of the size of the dose of these purines given. If these purines had been fed to these fowls in large amounts from the first, complete protection might have been secured.

It is important to note that, of these three groups of substances which conferred protection, only one was promptly curative; namely, group *c*, which contained Funk's base. The substances in the other two groups were tried repeatedly on fowls suffering from polyneuritis and absolutely failed to produce prompt recovery from the paralyses, although the lives of the birds appeared to be prolonged by this treatment.

Therefore, we have evidence that there is in our extract of rice polishings:

1. A substance (Funk's base) which, used in sufficient doses, will both protect fowls from developing polyneuritis and promptly cure fowls that have already developed the disease.
2. Two other groups of substances which will protect fowls from developing polyneuritis, but which are incapable of promptly curing fowls already suffering from the disease. The latter groups of substances, therefore, have entirely escaped previous discovery, because all the other investigators who have so far attempted to isolate these vitamins have relied exclusively upon curative experiments. Therefore, it appears certain that there are several groups of chemical substances that are capable of protecting fowls against polyneuritis gallinarum. Unfortunately we are unable at this time to state positively that these substances do not contain Funk's base. For it is
possible, of course, that these different chemical compounds may all contain Funk's base as a nucleus. This question can be solved only by obtaining complete information as to the constitution both of Funk's base and of these several compounds. We had hoped to begin this work, but have been greatly hampered owing to the extremely minute amounts of Funk's base which so far we have been able to obtain. This leads us naturally to the consideration of the second point outlined above; namely, is Funk's base completely recovered by Funk's method?

From what has already been said with regard to the action of alkalies on the protective substances, it appears probable that Funk's base is not completely recovered by his method, but that the greater part of it is destroyed when the precipitated phosphotungstates are broken up by barium hydroxide, which is a powerful alkaline reagent. This seems the more probable from several observations that we have made. In the first place, the curative action of the simple hydrolyzed extract is very powerful, the extract from 200 grams of polishings being usually sufficient to produce a complete cure. It is evident from experiment 46 that precipitation with phosphotungstic acid does not injure the protective substance; but, when the phosphotungstates are broken up by barium and the decomposed product used for curative experiments, it is found that a quantity from twenty-five to fifty times greater than the original 200 grams of polishings is required to cure a fowl. Manifestly the greater part of the curative substance is lost in the manipulation, and it can only be the result of the use of the alkaline barium hydroxide. In the second place, during this decomposition of the phosphotungstates by barium a strong methylamine smell is given off as was previously noticed by Funk. In our experiments we have observed that the loss of curative properties appears to be proportional to the strength of this methylamine odor given off, and we deduce from this fact that the methylamine odor indicates the amount of decomposition of nitrogenous bases to which class the protective substances belong.

In the course of our attempts to discover a better method for the isolation of this base, we tried a method used by Suzuki, Shimamura, and Odake. This method consisted in precipitating the unhydrolyzed extract by tannic acid, treating this precipitate with 3 per cent sulphuric acid, and subsequently removing the excess of sulphuric acid and tannic acid by barium hydroxide. By following this method exactly we succeeded in curing fowls by the extract so obtained, and therefore confirmed the above authors' observation. We also found that if the
extract is hydrolized before precipitation with tannic acid, the curative substance is not then precipitated by the tannic acid, but remains in the filtrate. We did not succeed in isolating any greater amount of the curative base by this method than by Funk’s method, doubtless because the former also depends upon the use of barium hydroxide to remove the tannic acid.

We had further hoped that the method described in experiment 46 might yield larger quantities of this curative base, since the excessive use of barium hydroxide in breaking up the precipitated phosphotungstates was thereby avoided; but after repeated attempts we were obliged to abandon this method also, because we found that the greater part of the curative base was lost during the final precipitation with silver nitrate in the presence of baryta. This loss may be caused by the action of the free barium hydroxide which is present during the precipitation, or may possibly be caused by the oxidizing action of the traces of free nitric acid remaining when the silver is removed by hydrogen sulphide, and the last traces of barium by sulphuric acid prior to evaporation. It appeared probable to us that larger yields could be obtained only by devising some method in which a prolonged treatment with barium hydroxide would not be employed.

In selecting more suitable precipitants, barium acetate was used for the following reasons. Funk believed that the curative substance is a pyrimidine base similar to uracil or thymine, and since pyrimidine bases are found only in nucleic acid it is, therefore, probably a constituent of nucleic acid. Several reactions which have been obtained with this base strongly confirm this view. Thus, the precipitation by silver nitrate in the presence of barium hydroxide, but not in neutral solution, places this base definitely in the pyrimidine rather than the purine group. The neutral reaction of the free base and its precipitation by phosphotungstic acid as a free base as well as in the unhydrolyzed form are in perfect agreement with this theory.

Now, if this base exists as a constituent of nucleic acid, it should be possible to obtain it from the basic barium nucleinate precipitated by barium acetate from the unhydrolyzed extract previously neutralized with barium hydroxide.

Accordingly the following experiment was performed:

Experiment 47.—A quantity of unhydrolyzed extract of rice polishings was carefully neutralized by barium hydroxide. An excess of barium acetate was then added and the precipitate so obtained was broken up or hydrolyzed by treating with 5 per cent sulphuric acid at 60° C. for three hours and filtered. The
filtrate from this mixture, after the removal of the excess of sulphuric acid with barium carbonate, was used to treat fowls suffering from advanced polyneuritis, and we succeeded in promptly curing 3 fowls by administering to each quantities of this mixture corresponding to the extract from 200 grams of polishings. This is approximately the quantity of the original extract which, after hydrolysis, is required to cure such a fowl. From this it is clear that little or none of the curative power of the extract was lost during this process. This is a far better result than we have obtained by any other method we have used.

Moreover, this result is a strong confirmation of the belief that the curative base exists in food as a constituent of nucleic acid, and it may be well at this point further to consider this possibility. Schaumann(10) originally thought that the active principle of such foodstuffs as rice polishings, katjang idjo, etc., was probably nucleic acid, but he supposed that the active constituent of the nucleic acid consisted of phosphorus in some form. This has been shown to be incorrect. Grijns(11) in testing this hypothesis isolated the nucleins in an impure state from katjang idjo. His dried preparation contained 1.43 per cent of phosphorus pentoxide, and another preparation in watery suspension contained 3.2 per cent of phosphorus pentoxide. He was unable to protect fowls fed on polished rice by either of these two preparations. Grijns concluded that the nucleins did not contain the therapeutic principle of katjang idjo, a conclusion with which de Haan agreed in 1910. Schaumann obtained similar results; for in a later publication he stated that in the case of pigeons suffering from polyneuritis he could produce no effect by giving nucleins prepared from yeast. This would seem to be conclusive experimental evidence that the protective substance is not contained in nucleins. Chamberlain and Vedder in 1911(12) fed 4 fowls on polished rice plus 0.2 gram nuclein daily. They state:

As 2 fowls out of 4 developed neuritis, it is not believed that the nuclein used had any decided power to prevent polyneuritis gallinarum. Since the incubation period for the 2 fowls which did develop neuritis is perhaps slightly above the average, and since 2 fowls remained well for fifty-six days, it can not be denied that there may have been a small amount of neuritis-preventing substance in the nuclein, a quantity sufficient to retard the onset of the disease.

Hoping that this result could be improved upon, we obtained a chemically pure nucleic acid from yeast (Merck's) and fed 4 fowls with polished rice plus large quantities (0.5 gram daily)
of this nucleic acid. All 4 fowls developed polyneuritis within thirty days.

We believe that these confusing results are satisfactorily explained by the fact that the methods commonly used for the extraction of nucleic acid from proteid substances depend upon the use of fixed alkalies. Thus Neumann's(13) much-used method for the preparation of thymo-nucleic acid involves the use of 33 per cent sodium hydroxide, and Slade's(14) method for the preparation of yeast nucleic acid requires that the yeast be treated with 1.1 per cent of its weight of sodium hydroxide. Alkalies are also frequently used in the preparation of nucleins. We have already said enough concerning the loss of the curative base when treated with alkaline reagents to indicate that such methods would surely destroy it.

It seems probable to us, therefore, that the curative base exists in foodstuffs as a constituent of a nucleic acid, as is indicated by the chemical reactions of this base, but that it is not present in the nucleic acid or nucleins obtained from yeast or other substances by the use of alkalies. This accounts for the failures which have resulted so constantly when nucleins or nucleic acids were used as preventive or curative agents. The partial protection reported by Chamberlain and Vedder above may well have been due to the use of a nuclein in which the protective substances had not been completely destroyed, for the origin and method of preparation of this nuclein is unknown.

It is further known that the nucleic acid obtained from different sources differs in chemical composition. Thus the nucleic acid obtained from the thymus gland contains cytosin, thymine, and uracil, while the tritico-nucleic acid obtained from the wheat embryo contains uracil and cytosin, but not thymine. Therefore, it is very probable that this curative base exists as a constituent of certain nucleic acids, but does not occur in every nucleic acid.

The solution resulting from the acid hydrolysis of the barium acetate precipitate was much too complex to give crystals of the base by simple evaporation. Various methods have been tried to resolve this solution into its constituents, but so far without succeeding in isolating much larger quantities of Funk's base than had been obtained previously. Experiments in this direction are being continued.

We now wish to report certain experiments which we have performed in the treatment of human cases of beriberi. As a natural result of the great success obtained by the treatment of cases of infantile beriberi with the extract used by Cham-
berlain and Vedder(4) we proceeded to administer this extract to adult cases of beriberi.

Case I.—A woman who had been delivered about a month previously and was nursing her infant. She had been in good health until about a week before the time she consulted us, when her legs began to swell and became heavy. This became steadily worse and gradually extended to other parts of her body, and finally induced her to seek treatment. When first seen, ödemata was pronounced and very general, having extended even to her face. Heart, lungs, and kidneys were apparently normal. A careful examination of the urine showed no albumin, casts, nor other abnormality. Her muscles were somewhat tender to the touch, and her reflexes were slightly impaired. She informed us that she had been living almost exclusively on polished rice. A diagnosis of beriberi was made and the case was kept under observation for a few days, during which the ödemata became steadily worse, and finally the child developed symptoms of infantile beriberi, consisting of slight ödemata and attacks of cardiac insufficiency. The case was now considered to be surely beriberi, and the patient was given the unhydrolyzed extract from 1 kilogram of rice polishings daily for three days. The ödemata at once began to clear up, and had entirely disappeared by the end of the three days; the woman was able to walk better and the tenderness of the muscles was greatly relieved. The infant was not treated at this time.

Case II.—A woman in the Philippine General Hospital who was very kindly referred to us for treatment by Dr. A. G. Sison. This woman had been in the hospital for some time and was suffering from typical dry beriberi. Her muscles were much wasted and exquisitely tender, and she was completely bedridden. This woman was given the unhydrolyzed extract from 1 kilogram of polishings daily for two weeks with no improvement except possibly a slight alleviation of the tenderness of the muscles. She remained completely paralyzed. It may here be stated that we have been informed by several physicians that they have been similarly disappointed in the treatment of cases of dry beriberi with the extract of rice polishings.

Case III.—A man, who had suffered from dry beriberi for some time, was admitted to the Philippine General Hospital on September 6 suffering from an acute cardiac crisis. Through the courtesy of Doctor Sison we were invited to see this case and treat it. When first seen on the morning of September 9, the man was sitting up in bed gasping for breath, and the resident
The physician stated that he had been momentarily expecting his death during the preceding night. His pulse was 150, respiration 45, and his heart was palpitating violently. He presented the typical history and appearance of a case of chronic beriberi suffering from an acute cardiac exacerbation. The muscles of the entire body and limbs were wasted and extremely painful to touch, and in addition he had been unable to take any nourishment for several days because of persistent vomiting. The patient was at once (11 o’clock, the morning of September 9) given the unhydrolyzed extract obtained from 1 kilogram of rice polishings. This was retained, and his symptoms at once began to improve, and by the evening of the same day his pulse had dropped to 80, his respiration to 30, and the vomiting had ceased. On the following morning another acute exacerbation occurred, and he was again given the extract from 1 kilogram of polishings. Again the symptoms improved, and thereafter he was given the extract from 1 kilogram of polishings daily for about two weeks. The cardiac attacks never returned, the hyperesthesia of the muscles was greatly relieved, the vomiting ceased, and his appetite returned; but he still suffered from paralysis and was unable to walk.

About one month was now allowed to elapse, during which time the condition of this man remained practically the same. He had had no recurrence of cardiac attacks, but he was still unable to walk. He was then given Funk’s base, which we had extracted by Funk’s method, from 10 kilograms of polishings. The crystalline base was dissolved in a small quantity of water for purposes of administration. The next day this man showed marked improvement, and for the first time since he had been in the hospital he made attempts to hobble around. This dose was repeated on the next day, and was followed by still further improvement, so that in a few days the man was able to walk about by holding on to the bed, chairs, etc. He was not completely cured, in the sense of being able to walk easily without assistance, but this we attribute to the fact that his muscles were so completely atrophied that they did not have sufficient power to support him. A similar result is seen after any disease which necessitates a long period in bed. We believe that the paralysis in this case was greatly relieved, if not cured, by the administration of Funk’s base.

We were unable to treat other cases with this base owing to the minute quantities at our disposal.

It will be seen from these cases that the administration of
extract of rice polishings, prepared according to our usual method, is capable of dissipating the dropsy in cases of wet beriberi, and of promptly relieving the attacks of cardiac insufficiency, but that this extract is incapable of curing the paralysis in cases of so-called dry beriberi. On the other hand, Funk's base isolated from this extract after hydrolysis is capable of promptly relieving the paralysis in dry beriberi. This closely parallels the results obtained in treating fowls suffering with polyneuritis. Ödema is not present in these fowls, and the disease is apparently similar to human dry beriberi. The untreated extract when given to these fowls fails to cure promptly the paralysis, but Funk's base obtained from this same extract will promptly cure the paralysis.

The exact relationship existing between dry beriberi and wet beriberi has never been satisfactorily explained. For many years clinicians considered them to be distinct diseases, but since it has been found that cases of dry beriberi sometimes develop öedema or become "wet," while cases of wet beriberi sometimes lose this öedema, and thereby become "dry," it has been generally accepted by clinicians of the present day that the two conditions are manifestations of the same disease, beriberi. However, this belief has never been definitely proved, and we are still in the dark as to why some cases of beriberi should be "dry" and some "wet." In a previous paper(15) the hypothesis was suggested that rice polishings and other foods contain two substances or vitamines that are essential for proper metabolism, one of these being a neuritis-preventing vitamine, and the other a substance which affects metabolism in such a way that its absence results in öedema and cardiac failure. It is believed that the results just detailed in the treatment of cases of beriberi afford some support for this hypothesis. Thus it is clear that, since cases of wet beriberi and cardiac beriberi may be promptly relieved by the administration of the untreated extract, while cases of dry beriberi receive little or no benefit from its use, the extract must act differently in these different classes of cases. It seems probable that this different effect may be due to the fact that there are two different chemical substances or vitamines in the extract, one of which acts directly in cases of wet beriberi, while the other occurs as a constituent of some substance (nucleic acid) in such chemical form that it is only available for immediate curative effects when it has been broken up.

Realizing that these results, while suggestive, are not complete proof of this hypothesis, experiments were planned to afford
more satisfactory evidence. Thus we intended to treat cases of wet beriberi with Funk's base. If our hypothesis that there are two vitamines is correct, the œdema should not be relieved by this treatment, since Funk's base has been shown to be the neuritis-preventing and curing vitamine.

We were unable to try this experiment, because we have been unable to obtain cases of wet beriberi in Manila at this time.

It is known that the neuritis-preventing and curative vitamine (Funk's base) is completely precipitated by phosphotungstic acid. It seemed that, if the vitamine of wet beriberi actually exists as a separate chemical substance, it might be possible to effect a separation by this method, if it should happen not to be precipitated by phosphotungstic acid. Accordingly, we took a portion of the extract of rice polishings which had been used in the treatment of the above cases and precipitated it with phosphotungstic acid. This precipitate was filtered off and the filtrate used to treat a case of infantile beriberi. Infantile beriberi belongs most frequently to the wet or cardiac types of the disease. This child suffered from œdema and from attacks of cardiac insufficiency. The child was promptly relieved as the result of this treatment, the œdema disappeared, and the heart returned to a normal condition, although it still continued to nurse its beriberic mother. This case is a strong confirmation of our hypothesis.

We realize fully that we cannot claim to have proved this hypothesis upon the basis of such a small number of cases; but, as we are at present compelled to discontinue this work, the facts so far obtained are reported in order that others may continue these experiments and obtain a definite proof or disproof of this hypothesis, which we are led to believe is the correct explanation of the difference between wet and dry beriberi.

CONCLUSIONS

1. Undermilled rice may be stored for one year in a damp place without losing its protective powers against polyneuritis gallinarum. It is improbable therefore that a rice which originally affords protection against beriberi will lose this property by storage even in damp places.

2. The neuritis-preventing substances or vitamines contained in rice polishings are only slightly soluble in cold 95 per cent alcohol, since three successive extractions, using a total of 6 liters of alcohol to each kilogram of polishings, fail to remove all of the neuritis-preventing substances from rice polishings.
3. Strongly alkaline reagents, such as sodium hydroxide, ammonia, and barium hydroxide, destroy the neuritis-preventing vitamine in its free or unhydrolyzed state, and the use of these reagents must be avoided in endeavoring to isolate this substance.

4. Basic lead acetate does not precipitate the neuritis-preventing vitamine, and a considerable portion of this substance may be recovered from the filtrate.

5. The therapeutic properties of an alcoholic extract of rice polishings are greatly altered by hydrolysis (treatment with 5 per cent hydrochloric or sulphuric acid). The unhydrolyzed extract is not poisonous and is only slowly curative. The hydrolyzed extract is exceedingly poisonous in large doses and promptly curative in small doses.

6. We have confirmed Funk's observations by isolating a crystalline base from an extract of rice polishings by Funk's method. This base in doses of 30 milligrams promptly cured fowls suffering from polyneuritis gallinarum.

7. Funk's base or vitamine is present in rice polishings in considerable amounts, and only a very small portion of it can be obtained by Funk's method.

(1) Because the polishings themselves are incompletely extracted.

(2) The greater part of this base is lost during the chemical manipulations required by Funk's method as shown by the facts:

(a) The curative action of this base, isolated, is from twenty-five to fifty times weaker than the curative action of the original hydrolyzed extract.

(b) When fowls are fed on polished rice and given a daily dose of this base in amounts corresponding to 10 cubic centimeters of the original extract, these fowls are not protected. Ten cubic centimeters of the original extract or 10 grams of polishings daily are amply sufficient fully to protect fowls.

(3) Because Funk's method depends upon the use of barium hydroxide, and we have shown that this reagent destroys this base.

8. Two groups of substances (purine bases, choline-like bases) may be isolated from rice polishings in addition to Funk's base and are capable of partly or wholly protecting fowls fed on polished rice against polyneuritis gallinarum, but are incapable of curing fowls that have already developed the disease. The chemical nature of these two groups of bases requires further investigation.
9. We have confirmed the observation of Suzuki, Shimamura, and Odake, that Funk's base may be precipitated from unhydrolyzed extract by tannic acid, but did not succeed in obtaining large amounts of this substance by this method.

10. It is probable that this base or vitamine exists in food as a pyrimidine base combined as a constituent of nucleic acid, but that it is not present in the nucleins or nucleic acids that have been isolated by processes involving the use of alkalis or heat.

11. The administration of unhydrolyzed extract of rice polishings to cases of adult wet beriberi, or to cases suffering from acute cardiac insufficiency, results in the prompt dissipation of oedema and relief of the cardiac symptoms.

12. The administration of unhydrolyzed extract of rice polishings to cases of dry beriberi is followed by little or no improvement in the paralytic symptoms.

13. The administration of Funk's base to cases of dry beriberi is followed by an immediate improvement in the paralytic symptoms. This should remove the last doubt that dry beriberi is caused by the deficiency of this substance in the diet. It also finally proves that dry beriberi of man and polynneuritis gallinarum are essentially the same disease.

14. We have succeeded in curing a case of infantile beriberi (of the wet type) by administering that portion of the extract of rice polishings represented by the filtrate from the phosphotungstic precipitate. Since this filtrate does not contain Funk's base, this is evidence that wet beriberi is cured by some other substance.

15. Conclusions 11, 12, 13, and 14 are striking confirmatory evidence for the hypothesis previously stated by Vedder and Clark that wet beriberi and dry beriberi are two distinct conditions, each being caused by the deficiency of a separate vitamine.

REFERENCES


(2) BRÉAUDAT. Discussion on beriberi. Trans. of Second Congress of Far Eastern Association of Tropical Medicine. Hongkong (1912), 72.


(4) CHAMBERLAIN and VEDDER. The cure of infantile beriberi by the administration to the infant of an extract of rice polishings and the bearing thereof on the etiology of beriberi. Bull. Manila Med. Soc. (1912), 4, 26.

(6) Funk. The preparation from yeast and certain foodstuffs of the substance the deficiency of which in diet occasions polyneuritis in birds. *Journ. Physiol.* (1912), 45, 80.


THE BIOLOGY OF TABANUS STRIATUS FABRICUS, THE HORSEFLY OF THE PHILIPPINES

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Seven plates

The importance of investigating the bionomics of insects capable of transmitting disease need not be emphasized. However, the paucity of literature on this subject leads one to suspect that insects as pathogenetic factors are not sufficiently appreciated by the entomologist. Too often this negligence of the entomologist has to be corrected by the overburdened research worker in medicine, in order to supply important links in the etiology and transmission of infectious diseases.

Tabanus striatus Fabricus is the most prevalent horsefly of the Philippines. It has a possible economic bearing relative to trypanosomiasis, in as much as it is found wherever surra is abundant, and the seasonal distribution of the fly and the disease are in a measure coincident throughout the principal regions of the Archipelago. In Manila and within a radius of 50 kilometers this Tabanus is found rather continuously from October to March. It is during this period that surra outbreaks in this region are the most prevalent. It is during this period also that other species of bloodsucking flies have their seasonal preponderance. Species of Tabanus have been experimentally incriminated in the transmission of surra in India, in North Africa, and elsewhere. In the Philippines Tabanus has never been proved to be a carrier of trypanosomiasis. At the present time this fly is the subject of an experimental investigation to determine its relation to the spread of surra. With this purpose in view, it was found essential first to breed the fly preparatory to use in transmission experiments, and the data collected and presented here are the results of the rearing of thousands of

1 Archibald R. Ward, chief.
2 I am indebted to Professor James S. Hine of Ohio State University and to Mr. Austin of the British Museum for this identification.
flies under laboratory conditions in the veterinary research laboratory at Alabang, Rizal.

The writer had been unsuccessful in locating the eggs or larvae in their natural habitats. Special efforts were made during three months, prior to the appearance of the imago, to find the young forms by searching plants and stones and by using a water net along the course of the neighboring creek and along the shores of the lake within a kilometer of the laboratory. Except for the absence of this material, the local conditions were ideal for the complete study of the bionomics of this species of fly. At the foot of the hill upon which the research laboratory is located, a lane of rain trees (Enterolobium saman Prain) divides a pasture land from a low, wet area which drains into the adjoining creek. The pasture land was used by a herd of about 50 cattle which passed back and forth along one side of the rain trees. The Tabanus when present used the cattle for feeding and the rain trees adjoining for resting when engorged or to escape from the sun's heat. During the early part of the day, probably until 2 o'clock, the cattle were disturbed by the attacks of many of these gadflies, which during the hottest portion of the day and through the night rested on the trunks of the convenient rain trees. Had the creek served as a harbor for the eggs and young forms of the fly, the ecological relations for a biological study of this fly would have been complete.

To obtain the eggs for study it was found necessary to keep the flies in captivity in a structure built for this purpose. This consisted of a capacious fly-proof building with brass-gauze sides and top and cement flooring, sufficiently large for from 4 to 6 big animals. One section was provided with a concrete water tank and growing plants. Here 2 carabaos were placed with thousands of flies, which were gathered while resting on trees along the road. In a short time females were observed feeding on the hosts provided, and several were found ovipositing in various places about the inclosure.

**OVIPOSITION**

The time selected for egg laying under the conditions provided was invariably during the early afternoon, never later than 2 o'clock. This was observed in nearly 50 instances.

Subsequent to the writing of this paper, larvæ and pupæ have been discovered in large numbers buried in sand at many points on the shore of Laguna de Bay.
The eggs are laid in a compact mass either extended on a flat surface or surrounding various attached objects, usually of small diameter, such as projecting splinters of wood, suspended fibers of jute sacking, fine brass wire, a single animal hair, and coarse iron wire. Upon these materials the eggs are laid in an ellipsoidal form sometimes surrounding the objects completely or nearly so. On one occasion 2 egg masses were found upon a small splinter of wood which they entirely enveloped. The surfaces of the egg masses were continuous, so that the double mass resembled a single large one. When eggs were found deposited on a flat surface, on two occasions a leaf was the object selected. These were leaves of an ornamental plant which was used for shade purposes in the breeding cage. The plant in question grew close to the cement water tank in the breeding cage. In all other instances the eggs were deposited upon woodwork on the sides and ceiling of the cage, invariably upon the shaded portions, as the underside of beams and partitions. In egg laying upon flat surfaces there was a strikingly constant geometric form. Usually the form assumed was roughly a pentagon with a biconvex center.

At the beginning of oviposition usually 2 eggs are deposited in the position of an inverted V. Three to 4 eggs are then laid on either side of the apex of this V, and then one side and then the other is built up, rather irregularly at first, until the sides of the pentagon are completed. The eggs are laid cleanly and definitely, each line slightly overlapping that preceding. When the eggs are laid in the extended order, they are deposited 3 or 4 layers in depth, but usually as many as 6 layers are required to complete the mass when the eggs surround a convex object.

In the process of laying, the body is held away from the egg mass, the legs being planted firmly. When the eggs are attached to an object above, the insect stands with head downward, the forelegs suspended alongside the head, the hind and middle legs supporting the weight of the body. At the first movement, the anal end of the body is bent toward the thorax under the abdomen, and with a slight jerk the egg is laid, while the brush-like appendage of the ovipositor exudes a tiny drop of liquid coating the egg as it is deposited. The movement of deposition is very much like squeezing a bit of pasty material from a collapsible metal tube.

In several counts that were made, the fly was observed to lay with clock-like precision at the rate of 10 eggs per minute. This did not vary, whether the attached object was above or below the fly. In three instances observed, the process occupied
from forty to forty-five minutes. Both the beginning and comple-
tion of the performance were without deliberation, the insect
walking away from the mass of eggs and flying off as soon as the
last egg was deposited.

When disturbed during oviposition, the insect does not fly and
can readily be carried without attempting to escape. While in
the act of laying, if interrupted and dislodged from the position,
it immediately begins to deposit a new egg mass. This was
twice repeated with one female, and three distinct egg masses
were deposited, all of them identical in geometrical arrangement.

The eggs of this species of Tabanus are laid with very little
cementing material. The cement used is a transparent sub-
stance and not dark and opaque as found to exist in the species
described by Hine.(1) The cement provided by this species was
tested and found to be waterproof, as well as insoluble in various
grades of alcohol and xylene.

The eggs when laid are a pale clay yellow, but within twenty-
four hours become slightly darkened with an ashy gray tinge.
Microscopically fine black striations can be seen running length-
wise for nearly 0.5 millimeter from the end opposite the
micropyle.

The shape of the individual egg is that of the muscid type
with more sharply pointed ends; it is not quite spindle-shaped.
Several eggs were measured and found to average in size 1.6 by
0.4 millimeters. The size of the mass varies from 9 to 12 milli-
meters in length by 6 to 9 millimeters in breadth.

The number of eggs laid in a mass varied greatly. In 4
masses counted, there were respectively 270, 340, 417, and 425.
Ten masses dissected from the bodies of killed flies were found
to average 405; the greatest number found in any female was 495.

THE HATCHING PROCESS

Two egg masses were observed microscopically during the
entire process of hatching, and 14 egg masses were noted as to the
length of the incubation period. The minimum period
observed was three days and the maximum five days. Four
days is probably the average length of time required for in-
cubation. It was observed that the degree of temperature and
moisture influenced the time of hatching. Slight changes in
either of these factors can be used to control the time of
emergence from the egg. The following is a series of observa-
tions on the hatching of an egg mass.

Twenty-two hours previous to the hatching of the embryo,
certain unmistakable activities were discernible in the egg. The
first signs of these were seen in the two eggs which formed the nucleus for the egg mass and which are the first eggs laid. These movements, as indicated by either of the dark eye spots, could be seen with a hand lens at intervals of a few seconds; their action was similar to that of the bubble in a spirit level. In about an hour the movement was seen to be rather general in the egg mass, accompanied in the eggs first laid by an alternate collapsing and distending of the exchorion. This action is the result of the torpedo-like movement of the head capsule of the embryo in the direction of the micropyle of the egg. The movement is effected by the piston-like action of the apophyses of the cephalopharynx, which appear to work alternately, bringing the saw-toothed mandibles in contact with the micropyle canal. These movements proceeded uninterruptedly during the hours of the night, the only change observable being that the body segments of the embryonic larva became better defined. At 4.25 the next morning the segments of the embryo could easily be counted through the chorion. The dorsal surface of the exochorion was seen to be slightly shrivelled.

Fully one hour and thirty minutes intervened during which there was no action worth noting. This quiescence was interrupted by a sudden remarkable activity of all of the visible eggs of the mass. At 6.08 there was a general upheaval of the surface of the egg mass, an agitation within the eggs, and an alternate collapsing and distending of the eggshells. At 6.10 the first layer of eggs gave birth to a silvery horde of young larvae, which at 6.12 had crawled from view. Then ensued another spasmodic agitation giving birth to another lot of larvae, which crawled from the mass of empty eggshells. The emergence which is effected by the head structures is aided by the posterior protuberances, which functioning as prolegs push the body of the larva clear of the eggshells.

MORPHOLOGY AND HABITS OF THE YOUNG LARVAE

Immediately after emerging from the egg, the young larvae seek concealment. In nature, no doubt, resort would be had to the convenient water course where aquatic plants, drift wood, and stones would be the probable hiding places. The larvae under observation became very active and crawled out of the stender dish, a height of 9 centimeters, and tumbled into the water of the basin provided. When collected and placed in a deep glass vessel with some water, the entire mass took refuge behind the filter paper in the glass. Here they crowded side to side with their siphons projecting from the upper edge of the
paper. When disturbed and forced to take to the water, they were found in thirty minutes reassembled in the characteristic gregarious fashion behind the filter paper against the glass.

For convenience in study, a majority of the larvæ were transferred when 1 day old to individual glass jars one-third filled with clean wet sand from the lake shore and provided with strips of filter paper soaked in muck from the creek bottom. The jars, which were the common half-pint jelly glasses recommended by Hine, were kept covered with filter paper, held in place by the tin lid which had a disk cut from its top to admit air. By renewing the moisture on the strip of filter paper in the jar, the filter-paper cover serves ideally to control the humidity.

A considerable number of the larvæ were not separated, but were left together for observation in a glass dish with a few strips of paper saturated with muck from the creek.

The larva one hour after hatching is 1.5 millimeters in length. The following day several were found to measure 1.8 millimeters. The general color is a dirty white with a tracheal system of waxy white, the abdominal contents pale green, and the Malpighian tubules of a lilac color. There are 2 black eye-spots located midway on the head capsule. The latter tapers to a sharp-pointed mouth with a prominent pair of great hooks or mandibles. The segments are provided with typical, conical, truncated prolegs, each armed with a chaplet of medium long, brown hairs. The siphon which is carrot-shaped at this stage is a prominent feature.

Food in a variety of forms was furnished the larvæ. They thrived from the start on minute crustacea, larvæ of Stomoxys, mosquito larvæ, and young angleworms. Full-grown angleworms were found unsuitable, and larvæ of the blowfly and flesh fly were not satisfactory unless killed previously, as they were capable of killing or injuring even well-developed Tabanus larvæ. As soon as the insect becomes aware of the presence of food, the claw-like mandibles are protruded from the head capsule, and bury themselves in the live food like meat hooks. With a slight curve dorsally, the larva's body is brought forward, and a small portion of the food is lacerated. This is aided by a twisting of the head and a pulling with the extended jaws. The mandibles are brought together with a rapid clawing action, the parts working in apposition. When prehension is effected, the jaws move alternately upward and downward and laterally, and the bolus is swallowed in fibrous strands.

Seeking and devouring food is not a continuous operation as
it is in the case with *Stomoxys* and the dung flies. The *Tabanus* larva requires a long rest after a sufficient meal is taken. A 2-day-old *Tabanus* is capable of devouring 2 half-grown larvae of *Stomoxys* in twenty-five minutes. In one instance a full-grown *Stomoxys* larva was destroyed in exactly twenty minutes. Here the attack on the *Stomoxys* was made through accidental collision, the *Tabanus* instinctively thrusting out its mouth and tentatively taking a bite. It apparently became greatly excited (this was its first meal), and, thrusting its head into the body of the *Stomoxys* larva, commenced to probe by twisting its head rapidly. In less than a minute the cuticle was broken through and an ample slit was made through which the entire head was buried in the body of the victim, whereupon an energetic gouging took place. The *Tabanus* worked through the cephalic third of the body upward to the head, then worked in the other direction on the lower two-thirds. This gouging was continued until the *Stomoxys* had become completely eviscerated, during which time the head of the *Tabanus* kept steadily probing, twisting its pharynx from side to side, and pushing forward with its rostrum until the *Stomoxys* larva was completely devoured with the exception of the cuticle.

The full-grown *Tabanus* larva does not wait for its food, as is the tendency in the young stage, but actively pursues its prey. When an angleworm is seen, perhaps 2 millimeters distant, the elastic head capsule of the larva darts forth, curves its claw-like hook about the worm’s body, and, with its head curled under its struggling prey, retreats quickly into the sand until all but its cephalic end is hidden. It begins to feed then, devouring in twenty minutes an angleworm fully four times its own length.

The intestinal tract seen through the hyaline cuticle soon partakes of the color of the food ingested. The color is pale brown when the food consists of the wet muck in which crustacea and minute forms are sought. As a result of feeding on blowfly larvae and angleworms, the young *Tabanus* assumes a variegated coloration. The intestinal tract then appears tinted with green, yellow, brown, and red particles of the food.

In one set of larvae the origin of cannibalism as an acquired habit was observed. This was seen in larvae which had been kept together for four days since their birth. Until that day no food was offered them except that which they might have obtained from the surrounding creek water. Apparently they lived together amicably with their bodies compressed against the glass dish and the bit of filter paper. A
live angleworm was placed in the glass dish while the resting larvae were observed with a lens. The worm was not placed in the immediate vicinity of the mass of larvae, but nearly 4 centimeters distant. The presence of the food appeared to act as a stimulus. No movement was made toward the worm, but each larva appeared to become greatly excited and began to prod the larva nearest to it and to nip its neighbor's appendages; several very marked instances of laceration were noted. This doubtlessly marked the beginning of systematic cannibalism. From this cause, 39 of the 265 larvae kept in a large glass dish were destroyed within four days. Four dead bodies were recovered. Upon another occasion the extent of cannibalism was very much more marked. A lot of 415 larvae which hatched on November 12, 1912, was placed in a deep glass dish with moist lake-beach sand, and fed daily on angleworms. Each morning it was observed that only about one-half of the worms supplied the previous day was eaten, so that with the daily fresh supply more than enough food was present. Another lot of 300 larvae, the same age as the preceding, was kept in individual glasses under similar conditions. On December 6, counts were made of the survivors in the large glass dish. Thirty-five larvae remained, of which 18 were the maximum size, 11 were a little more than one-half this size but equal to the largest found in the individual jars, and the remaining 6 larvae were so small as to be easily overlooked. The census taken of the larvae from the individual jars showed a loss of 12, or less than 5 per cent. Allowing 5 per cent for loss from other causes, it appeared that above 85 per cent of the larvae kept together in the large jar was destroyed through cannibalism.

It has been observed by Hine in other species that a *Tabanus* larva is enabled to survive for a few days in the absence of food. In this species likewise there seems to be a decided resistance to starvation, two instances showing periods of ten and twelve days.

The movements of the body are in general similar to those of larvae of the muscid type. There is a general progressive peristaltic movement, invariably accompanied by a decided telescoping of the segments. The head is raised as the prolegs of the anal end push the body forward, then it is lowered. The mouth is projected when the head capsule is extended, but recedes quickly when the glass sides of the container or any obstacle is encountered. The larva can easily move backward for a considerable distance. This it does if wedged in a
tight place or in capturing food when it retreats into a channel previously made in the sand.

The larve readily adapt themselves to a watery medium. They can remain submerged for several minutes at a time without apparent discomfort. When placed in deep water the movements of the body are a general struggling without apparent definite purpose. At any rate, there is little or no progression, the body doubles like a bow, the head and tail meeting, then straightens with a whipping action. In swimming, the body is held along the surface of the water and the siphon is extended toward the air in a manner very suggestive of the larva of an anopheline mosquito. The principal movement observed is that of simple telescoping of one segment into another. When speed is required or an obstruction is to be passed, there is a vigorous whipping movement of the siphon laterally, toward and away from the head. This latter movement is also noted when the insect is disturbed.

When a young larva is placed in water containing entomos-tracans or other minute animals, a barely perceptible churning of the liquid occurs in the region of the mouth. This disturbance is no doubt caused by the movements of minute tentacles which assist in procuring food. These tentacles form the armature of the stomal disk, consisting of a process arranged like a turnstile mounted on a pitted chitinous plate at the base of the great hook or mandible. In the very young larva the stomal disk appears as a chaplet of delicate chitinous rods. When a larva is treated with strong caustic potash, the stomal disk appears to be the only structure which resists its action, the other chitinous structures, even the heavy pharyngeal apophyses, are bleached. In common with the other chitinous portions of the head capsule the stomal disk is shed at each of the three ecdyses.

GENERAL DEVELOPMENT OF THE LARVA

The young larva shows in its form and behavior its adaptability to an aquatic life. This is well illustrated when a larva is turned adrift in an aquarium containing mosquito wrigglers. The *Tabanus* has no difficulty in keeping afloat with them and foraging at will upon the active culicid larvae. *Tabanus* larvae have been observed capturing wrigglers, holding them by their jaws under the water, and actually killing the culicid through drowning. In one instance a *Tabanus* larva held its victim, which was fully five times its size, suspended beneath it in such a manner that the culicid was unable to project...
its siphon for breathing purposes, while that of the Tabanus was functional. The Tabanus, obtaining a secure perch by dragging itself and the prey above the water, devoured the mosquito wriggler in a few minutes. In another instance the weight of the culicid pulled its captor under the water to the sandy bottom a distance of nearly 30 centimeters. Here the Tabanus showed its superior vitality by remaining attached for nearly two minutes until apparently assured of the immobility of its prey, then, releasing its hold, the Tabanus larva struggled to the surface where it rested with siphon extended. The mosquito larva meanwhile moved feebly several times, and succumbed within a few minutes.

This adaptability is lost, however, in the developed larva which becomes more slothful in movement and grub-like in superficial appearance. Both extremities, the head and the siphon, become obtuse in form, and the ventral protuberances functioning as prolegs become more truncated. Growth after the second molt becomes noticeably less in length and more in thickness. The greatest growth observable was shown to be between the periods of the first and the second molts.

The following table is given to show the normal growth of a larva. The measurements and the critical stages of life are indicated:

**Table I.—Progress of development of a larva.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Length</th>
<th>Stage of development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
<td></td>
</tr>
<tr>
<td>Sept. 15</td>
<td>1.5</td>
<td>At birth.</td>
</tr>
<tr>
<td>16</td>
<td>1.8</td>
<td>1 day old.</td>
</tr>
<tr>
<td>20</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>11.0</td>
<td>After first molt.</td>
</tr>
<tr>
<td>30</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td>Oct. 5</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>25.0</td>
<td>Second molt.</td>
</tr>
<tr>
<td>12</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>29.5</td>
<td>Mature larva.</td>
</tr>
<tr>
<td>21</td>
<td>27.5</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>17.5</td>
<td>Pupa, third molt.</td>
</tr>
</tbody>
</table>

In all biological accounts of the Tabanidae there appears to be one phenomenon which is uniformly noted. This is the remarkable difference in growth shown by individual flies of the same species. The only process in the development which
seems to be synchronous is the hatching of the eggs. After that the variations in time of development are extreme. In \textit{Tabanus striatus}, for example, some larvae twelve days old measured 3 millimeters, while others under precisely the same conditions measured fully 11 millimeters. In another instance 2 flies emerged as well-developed imagoes October 31, while 27 of the same brood still remained apparently healthy in the larval stage December 20.

THE ECDYSES

In the very meager literature available I have been unable to find any reference to the molting process in Tabanidae. It is referred to indirectly by King (2) at Khartoum, who found in \textit{T. biguttatus} the shed larval skin adhering to the puparium.

The process of shedding the skin was observed in a great many instances. The time of molting of a brood of larvae is extremely variable, which is consistent with the great variations noted in the time of development in general. The process has been accurately noted in two individual larvae, although observed superficially in numerous others. The three molts are similar in their general aspects, the main distinction being the more profound changes produced in the insect at the later molts.

The usual preparations for molting were observed in this species. The premonitory signs were the refusal of food, uneasiness when exposed to light, desire to find a remote corner, and finally the stiffening of the cuticle. In one instance the larva was found in one spot pressed against the glass for three days. Here, between the sand and the glass of the jar, an excrementous cement was used to fasten the end of the abdomen. This material holds the end of the body very securely, although the remainder of the body requires free lateral movement. By the time the ecdysis is completed, the head has moved 3 millimeters from the spot where preparations for the process are made, while the anal end has retained its original position.

The shedding of the skin usually requires several hours; in one instance, due no doubt to interference on the part of the observer, the time was nearly twenty-four hours. In the first and second molts, splitting of the cuticle begins at the thorax, resulting in the tearing out of the entire head capsule which adheres to the molt during the remainder of the process. The anal segments are molted finally and the larva, emerging in its new skin, crawls its length on the cast skin and rests alongside it for two or more hours.

The first molt begins with larvae 7 days old, the majority
molting before the tenth day. The second molt usually occurs after an interval of at least four days, and in some larvae as late as eight days, that is, when 15 to 18 days old. The time of the third molt precedes immediately the appearance of the puparium. This period, as has been noted, shows the greatest diversity among individuals of the brood. The third molt in 12 instances was shed between the ninth and twelfth days of life. In other individuals the process was not completed within three months, yet the adult fly was an apparently healthy insect.

Certain unimportant changes in morphology, dependent on the molting process, are noticeable. The loss in size due to contraction of the cuticle preparatory to ecdysis is usually compensated by a substantial extension immediately following the process. The extent of shriveling of the cuticle is represented by 1 millimeter in the first molting, 1.5 to 2 millimeters in the second stage, and 2 to 3 millimeters preparatory to the third stage. There is a notable increase in length resulting from the second ecdysis. A larva, measuring 22 millimeters on the day previous to the shedding of the skin, measured fully 25 millimeters the following day. In measurements of this sort one must make allowance for the extraordinary amount of telescoping of segments. As much as 5 millimeters may be involved in this process.

The structures mainly involved in the ecdysis are the tracheal system and the appendages of the head. The anal wing of the trachea constituting the siphon is drawn off in each molt in a perfectly cylindrical form. The body trachea is torn from its connections in irregular strands. The entire head capsule, including the chitinous pharyngeal framework, the great hook, and other mouth structures, are found in perfect form in the various exuviae. These parts upon renewal in the larva become more heavily reinforced. The exuvia is usually in a good state of preservation; crumpled to be sure, but it can be extended in alcohol to three-fourths the length of the larva. Following each ecdysis, the larva is invariably leaden gray with tracheal strands of waxy white. Three anal segments including the siphon become lead colored and stiffened in structure. They are at this stage more truncated, with an anal band of cuticle 1 millimeter in depth, making the siphon appear somewhat atrophied. This is no doubt consistent with its restricted function. The color of the viscera has changed from the brilliant red and yellow to an indeterminate white, and the lilac tint of the Malpighian tubules has changed to a salmon color. These latter
changes are due probably to a clearing process, in which the larva indulges during the somnus preceding each ecdysis.

After the second molt the fleshy protuberances functioning as prolegs become reinforced with a slight cuticular ring at their bases. The mouth parts at this stage are heavily chitinized. The great hooks or mandibles show a marked serration of the biting edge. The head projects more, exposing the dark brown ocelli, which prior to the second molt are seen only through the cuticle of the thorax situated nearly on the middle of the concealed head capsule.

The signs characteristic of the final molt are refusal of food, restlessness, attempted migration, and finally burial in the sand at the bottom of the jar. The body decreases slightly in length, but the thickness remains the same.

On the extremity of the abdomen tiny tubercles appear which project more from time to time, becoming tapering and spike-like. Near the caudal end of the abdominal segments, roots of hairs appear. These at first resemble brown spots of pigment and gradually lengthen into stiff brown hairs. The cuticle on the body becomes stiffened and shingle-like at the joints of the segments. The latter telescope less, and one can see numerous particles of sand embedded in the joints of the segments. These sand particles have been carried in during the telescopic movements of the abdomen.

After the fully developed larva passes through a period of semidormancy buried in the sand, the skin is seen to be ridged with cuticular plates. The head region is reinforced by stiffened cuticle, and the mouth orifice is closed by a plug of hard rose-colored cuticle. This pigmented material lines the entire pharyngeal sinus, plugging the mouth and the cephalopharynx. The cuticular plug has a substantial fold which forms a slit for the passage of the molting mouth. Caudally a similar impervious mass closes the opening of the siphon. A cuticular collar strengthens the base, and the connective tissue surrounding the trachea of the tract of the siphon tends to contract. Then the supports of the central trachea are gradually cast loose by a gentle wriggling of the insect’s body. About this time there is a general wrinkling of the epidermis, the folds telescoping upon each other, and the surface becomes parchment-like.

Synchronous with the primary contraction of the segments, a light pea-green suffuses the last 3 segments of the body. The remainder of the larva changes to this color overnight. By morning the abdominal segments have changed from green to
ocher, when the molting of the cuticle ensues. The shedding takes place in sections. The chitinous framework of the head is thrown off like a hood. This portion is everted upon the body, and remains dangling from the exuvia during the process. One-half the length of the skin is loosed on the side opposite to that to which the chitinous framework of the head is attached. This is shed by a peculiar auger-like movement of the tail end which is not attached to the glass or other object in the container, as in the previous molts. The skin is virtually unrolled from the detached head to the anal end, where it lies in a crumpled heap. Then the skin of the other side of the body begins to be shed. The chitinous framework constituting the former head capsule of the larva becomes rolled up in the exuvia, while the skin is torn slowly from the new membrane. When the first half of the skin is peeled off to the anal tip, the cast skin becomes attached to some object. In this instance the glass of the jar served as an anchorage during the remainder of the ecdysis.

The upper half of the body of the newly molted larva is encased as in an armor in pouches and pads of integument, outlining in a gauzy film the future appendages of the fly.

GENERAL DESCRIPTION OF THE FULL-GROWN LARVA

The length is 28 to 29.5 millimeters; the width, 3 to 4 millimeters. The anterior half of the body is a greenish yellow, the remainder is a dirty white. At this stage the form is grub-like.

The head capsule, which occupies one-fifth the length of the larva, is a cylindrical bulb, formed by the invagination of the thoracic ectoderm. It supports the eyes, the antennae, and the mouth parts. It is bound by a framework of chitinous rods, the cephalopharyngeal apophyses. This structure, observed through the thorax when the insect is in action, is composed of 4 black, medium-thick skeletal pieces running the length of the 3 cephalic segments in the form of a pyramid, with its apex provided with the external mouth parts. It terminates in the claw-like mandibles which are similar in color and texture.

The mandibles are heavy, powerful structures, slightly serrated on their inner surfaces. The musculature of these appendages permits the two elements working in apposition. At rest they are held horizontally, and can be projected suddenly and thrust vertically downward, which is obviously of great assistance in grasping the prey.
The palpi and antennæ in this species are silvery white, and usually found glistening with moisture.

The eyes are oval in shape, with the long axes parallel. When the larva is prepared to molt, the pigmented spots are usually distorted. In this species the eye spots or ocelli are very prominent, especially in the younger stages of the larva. They can be first seen in the embryo where they appear as dark beaded structures through the chorion of the well-developed egg. In the young stage of the larva the eyes appear in the pharyngeal cavity midway between the mouth and the cephalopharynx, and as growth continues they are located nearer the distal end of the head capsule; so that when the larva is full-grown and the mouth structures protrude in locomotion or prehension the eye spots are seen to project on the head capsule with the mouth parts.

The trachea which terminates in the conical tubular siphon is lead gray in contrast to the dense white of that portion anterior to the anal segment. Anterior to the siphon there is a cuticular collar of a slightly darker shade.

The prolegs are formed by truncated projections, 6 in number, 3 on each side of the midventral line and extending laterally. Each protuberance is provided with a tuft of short, fine, brown hairs. These hairs appear to be surrounded by a secretory substance, which is slimy in character.

At the base of the siphon, beneath the cuticle on the dorsal side opposite the anal capsule, is a tiny structure which attracts attention on account of its movements and peculiar arrangement. In the newly hatched larva it is a process composed of 4 lustrous black disks arranged in two pairs, one in front of the other, and set in a mass of fat bodies. The larger of the disks, the anterior pair, is less than 0.1 millimeter in diameter. The movement of the process is similar to that of a pendulum, and is active only when the larva moves. With each molt these disks become smaller and increase in number. In the full-grown larva the process becomes a triangular mass of loosely arranged beaded disks. They appear to be mere specks of pigment beneath the skin, but their structure and action are so constant that either the process is characteristic of the species or investigators have overlooked or ignored them in other species.

DESCRIPTION OF THE PUPARIUM

The average length is 18 millimeters, and width, 3.5 millimeters. The color is pale brown, the last 2 segments of the abdomen being slightly darker. The head tubercles are not
clearly defined; color, dark brown. The prothoracic spiracular tubercle is slightly elevated, oblique; rima, salmon colored and crescentic in form.

The first abdominal spiracle is perfectly round and larger than the others, which are slightly ovoid; the rima of all the spiracles curves from above posteriorly.

The terminal abdominal segment shows a sexual distinction in the arrangement of the short spines midway on the ventral side, anterior to the terminal teeth. In the male 10 to 12 of these spines form a continuous serrated border. In the female the spines occur in two groups of 4 to 6 spines similar to those of the male, but separated by a space equal in width to that of one of the groups.

The terminal teeth of the posterior segment are arranged with 2 pairs close together on the dorsal side and 1 pair on the ventral side. These teeth are black-tipped and acute; all of them are directed slightly outward. The lateral teeth of the 2 dorsal pairs are the longest. The ventral pair is smaller and is set slightly in from the periphery of the segment.

After the final ecdysis which results in the formation of the puparium, the nymph, at first a light green, gradually changes to yellow. Upon the second day, the eye spots change from yellowish to pale brown, then to a chocolate color. Beginning with the third day the pads of the wings and the legs, at first light brown, assume the same color as the eyes. The chitinous pad enveloping the wing is densely opaque, so that only the plications of the developing wing can be discerned. Upon the penultimate day, the fifth or sixth usually, the abdomen, which heretofore has been a uniform yellow-brown, becomes striped with light orange and brown, which colors gradually deepen until the time of emergence.

EMERGENCE FROM THE PUPARIUM

In emergence, the puparium which lies buried to some depth in the sand is invariably dragged to the surface where the final acts of emergence are completed. Two to three days prior to the act of emergence, the puparium shows considerable mobility when disturbed by handling or stimulated by light. Certain movements, which one learns through numerous observations to be characteristic, can be considered as actually premonitory. These occur usually from ten to twenty minutes prior to the breaking of the cuticle, and serve the observer as warning signs. If during this interval a low-power lens be focussed on the compound
eye, the epidermis of the fly separating from its connective-tissue fastening of the puparium can easily be seen. This action resembles strikingly a wave of water moving between the walls of the puparium and the epidermis of the fly. It may be considered as the movements of a semiliquid layer between the fly and its puparium. Another movement, which can be observed within a few minutes after that previously described, is the momentary contraction and expansion of the sides of the abdomen between the two lateral ridges. This too, no doubt, is effective in tearing the connective-tissue lining to facilitate emergence. A few minutes later the anal end of the abdomen is torn loose from its fastening, and emergence of the fly begins.

Since the puparium is unrolled from the head, the compound eyes are soon exposed to view, so that the sex of the fly may be distinguished. The appendages, antennæ, palpi, and mouth parts are dimly visible. The head appendages are freed primarily by the spasmodic wriggling of the abdomen, but the labellum, which is seen to become turgid and flaccid in turn by the injection of air into, and withdrawal of air from, the “extensive tracheal sacs which lie in its cavities,” and the erectile stomal disk through its pressure downward against the walls of the puparium, assist also in the process. That these head appendages assist effectually in the emergence is evident from the lines of cleavage in the enveloping membranes.

The puparium splits on the median line of the thorax; simultaneously the hood enveloping the head drops by a sternal hinge. The labellum can be seen still pressing upon the interior of the hood as the head emerges. Within a minute the wings are rent from their envelopes by the sturdy pressure of the legs, which have slid out of their sheaths simultaneously with the cleavage of the thorax. The legs directly assume their normal position, and the fly walks forth bodily, spreading its plicated wings. The liberated wings show a clear expanse of un- wrinkled membrane which at first is soft in texture and clear lead-colored throughout. Finally, the inflated abdomen appears in the dorsal slit, and at once is drawn clear of the encumbering puparium.

The time from the appearance of the head to the evacuation of the puparium requires less than two minutes. This time is increased a minute or two whenever the wing sticks to the lining of the puparium, resulting usually in a torn wing.

Directly after emergence the wings are shorter than the body, but, constantly vibrating, they gradually lengthen, whereupon
they become hardened and prepared for flight. The fly does not
spend any time preening itself as is the case with some of the
Muscidae at this stage. The time prior to flight is spent, how-
ever, in a clearing process. This begins with a copious dis-
charge of meconium within three to five minutes after emerging.
At first the defecation is performed at least five times per minute,
then once per minute for a period of twelve minutes. At the
end of this time the excretions become more watery in char-
acter. In the meantime the fly walks about in a restless man-
ner, constantly vibrating the balancers and flapping its wings,
while the distended abdomen becomes reduced to more normal
proportions.

The meconium, which is deposited in large quantities, is in
color pale brown, rapidly changing to amber, then becomes clear.
The primary, heavier excretion appears decidedly oily in nature,
when examined with the microscope.

In from fourteen to twenty minutes, voluntary flight takes
place. This is at first tentative, the insect alighting upon the
floor about a meter distant. After a minute of rest, flight is
resumed, the fly escaping through the open window.

The puparium left behind shows certain points of cleavage
which prove to be very constant. There is a dorsal slit on the
median line of the thorax which extends nearly the length of the
notum. Another slit extends midway across the orbital region
through the genae to the wing pouches. A third slit extends
between the two wing envelopes, and a slight one behind the
prothoracic spiracular tubercle.

In the 32 emergences recorded, the males preceded the females
by an average of half a day. The males spent from three to
seven days in the pupal stage, averaging five and one-half days,
while this period required four to nine days with an average of
six days in the female flies.

GENERAL DESCRIPTION OF THE IMAGO

MALE

The male is very distinct from the female, being smaller and
having a larger head and different color markings.

The distinctly clavate palpi are shorter than in the female,
only two-thirds as long as the labium; they are dirty white and
fringed with moderately long black hairs.

The abdominal color markings take the form of a T of pale
cadmium yellow in a field of burnt sienna, bordered with pale
clay yellow. The area of the large facets of the eyes is colored
Roman sepia surrounded by an elliptical band of ultra ash gray. The field of small facets has a mauve fringe bounding an area of iridescent mauve and Prussian green.

Size: 14 to 15 millimeters.
Wing expanse: 25 to 28 millimeters.

**FEMALE**

The front is narrow, converges slightly anteriorly; the color is golden, marked with a black callosity of irregular form. The head is considerably smaller than that of the male; eyes iridescent mauve and Prussian green.

The palpi are prominently conical, as long as, or slightly longer than, the labium; the color is the same as in the male, mottled with short black hairs.

The abdomen is alternately striped with Cologne earth and pale clay yellow. The median stripe is pale clay yellow. In both sexes the thorax is indistinctly striped with pale clay yellow and pale brown, and the wings are transparent except the costal and subcostal cells which are pale brown.

Size: 15 to 17 millimeters.
Wing expanse: 26.5 to 29 millimeters.

**FEEDING HABITS OF THE IMAGO**

The males in nature appear to derive the greater portion of their subsistence from gum exuded from trees, particularly rain trees; they can be found upon these trees at all hours, feeding whenever a gummy excrescence is present. In addition to the gum they lap the moisture found on the leaves in the early morning. In this connection Baldrey(3) found that *Tabanus* in India has a fondness for chestnut leaves, and sucks greedily any fluid thereon.

The females spend much of their time on trees in company with the males, feeding in a similar fashion. There is no doubt that this is the primary source of the female's food, but whether it is sufficient to stimulate egg laying is undetermined. Judging from the longevity of females kept under experimental conditions, it appears that a diet of blood is essential for the development of the eggs. For example, 200 flies kept individually in large flasks were fed daily on fresh gum of the rain tree. The records show that females outlived the males several days, and that the former lived a maximum of fourteen days. Females fed on monkeys and guinea pigs daily and permitted to feed as often as they desired were kept in similar flasks and under similar conditions as those in the first experiment. The average
longevity of these proved to be ten days, and the maximum eighteen days. Captive flies were used in the first experiment and laboratory-bred flies in the second.

In considering the feeding habits of the female, it is deemed profitable to discuss the notes taken from observations made at close range with laboratory-bred flies fed on experimental animals. The behavior of flies bred in the laboratory indicates that the female does not feed on the day of its emergence. Thirty individuals tested in this regard refused to feed on the first day of emergence, 3 fed upon the second day, and the remainder took the initial feed upon the third and fourth days.

The insertion of the proboscis seems to be not the only means of obtaining blood, for, when abrasions of the skin of the animal exist, the fly may actually fill itself to the engorging point by means of its spongy labellum. In this process the latter organ is unassisted by the other mouth parts. The proboscis is a distinct appendage never coöperating with the lapping organ. The labellum is situated posterior to the piercing structures; it is held in this relation either when at rest or when active. When the labellum is in action, the stomal disks which constitute the lapping structure are unfolded downward and outward, bringing the pseudotrachea of the lapping area in contact with the moist surface of the skin. The female laps the blood offered in the same manner as it would any desirable liquid. This does not appear to be a usual method of obtaining blood, since it has never been observed under natural conditions. If it did occur in nature it is obvious that it might prove of importance in the mechanical transmission of animal diseases. This is at once apparent when one considers the area of the exposed surface of the lapping organ of this fly as compared with that of other flies infesting domestic animals.

Under experimental conditions *Tabanus striatus* bites usually not oftener than once in two days. A few instances have been noted of biting on consecutive days, but this is considered abnormal.

The process of inserting the various elements of the proboscis is difficult to ascertain since the large head conceals the biting parts when the vertex is lowered. In the process there are no movements which might be considered as premonitory. The fly when applied to the host quickly lowers its head, braces itself on its widely spread legs, and stabs its victim. The end of the abdomen is scarcely inclined, but settles in position parallel
to the host's skin. The palpi, the position of which may be seen when the parts are withdrawn, are directed alongside the proboscis bow-like on either side of the puncture. These parts bend as the labium is thrust into the skin, and they probably aid by their elasticity in the withdrawal of the proboscis.

The head raises with a jerk in the withdrawal of the proboscis, and the punctured site is marked by a distinct blooddrop. Only a few seconds intervene prior to a repetition of the stab in an adjoining spot, and as many as 7 punctures may be made in a period of ten minutes. The biting may continue for as long as twenty-three minutes. The distention of the abdomen, only the anterior part of which is affected, gives no indication of the extent of satiation of the fly. They have been seen to feed with the abdomens bulging laterally with food, which bulging sometimes persists for several days, after which time the fly may bite once or even twice. The bites appear to be very painful to the host. The insect is never satisfied with a single bite, but makes several punctures before a complete meal is obtained. No doubt this is due in part to the interruptions caused by the tormented animal, and the fly may have acquired the habit of making short swift stabs in order to facilitate its escape from the host.

Tabanus striatus, although commonly termed a "horsefly," is found biting cattle and carabao as well as horses. During over two years of personal observation, this fly has never been known to annoy man in the Philippine Islands. Reports of attacks from this fly can usually be attributed to the biting of Stomoxys, which is frequently annoying to the native caretakers of draft animals. Perhaps the carabao may be accepted as the host of choice of the Philippine horsefly. This is at once apparent when flies are very abundant. Many times I have seen sparsely haired carabao with their bodies actually covered with droplets of dried blood resulting from the bites of tabanids. I have collected from a single carabao 61 specimens of Tabanus striatus in less than fifteen minutes.

THE OCCURRENCE OF TABANUS STRIATUS

The time of day when this species of fly is most active is usually the same as that of other species of Tabanus. The fly begins its activities usually between 7 and 8 o'clock in the morning, and during the greatest heat of the day is found on the shady sides of houses and trees. Between the hours of 3.30 and 6 in the afternoon it is again active, resting for the night on the convenient rain trees. These trees have been observed to harbor
Tabanus striatus of both sexes at all hours of the day and far into the night. During the cooler part of the day the flies assemble on the trunk and main branches, but when it is sunny the upper limbs and leaves are resorted to; on windy days they may be found at all hours perched on the side of the trunk of the tree shielded from the wind. During their greatest prevalence they are found in large numbers indoors in the lightest portions of the room, usually perched on the windows.

The flies obtained for this biological study were collected from rain trees in the vicinity of the laboratory. These when gathered were identified and turned loose in the large breeding cage where two carabaos furnished the food required for further development. The daily supply of flies varied from 25 to 300 according to their prevalence and the skill of the Filipino collectors. Insect nets were used for trapping.

In 1912 the first appearance in this locality of horseflies in appreciable numbers was on August 15. From that time tabanids were collected daily at the rate of 25. August 15 to September 4 males predominated in the proportion of 2 or 3 to 1 female; and shortly afterward the female count increased to the proportion of 1 male to 1.7 female. There was a constant preponderance of females to the extent of 3 to 1 in October and 4 or 5 to 1 in December.

Beginning September 4, there was observed the first appearance of mammalian blood in flies collected from rain trees. The source of this blood, which showed itself in the dejecta and dissected stomachs of the females, was presumably a herd of 50 cattle which had been grazing on land along the lane of trees which harbored the flies. Although the cattle were present for eight days previously, blood was not taken until September 4. From September 4 to September 6 every lot of females collected was found to contain a preponderant number of individuals containing blood.

The first occurrence of oviposition was observed in a female collected in a lot of flies from this locality. It was removed from a tree September 10, and found laying eggs the following day, September 11. These eggs proved fertile, and were subjects of the life-history study described in this paper.

SUMMARY

1. The eggs of Tabanus striatus Fabricus have been found in masses of from 270 to 425, laid mainly on particles of wood.
The incubation period has been determined to be from three to five days.

2. The behavior of the larva indicates that it is essentially an aquatic form. The insect in this stage has been found to be extremely cannibalistic. In some instances as high as 85 per cent of the brood has been destroyed by this means. They apparently prefer their kind to any other food; at least, there is no diminution of the practice even when an abundance of other food is present. There is shown to be a marked diversity in the development of larvae of the same age. The larval period covers six weeks or longer.

3. In the ecdyses of Tabanus striatus there are 3 distinct molts; the final one, coming a considerable time after the larva is full-grown, results in the unveiling of the puparium. The puparium is formed beneath the molting skin of the full-grown larva. This stage lasts from three to seven days, with an average of five and one-half days, in the male; and from four to nine days, with an average of six days, in the female. In this period the male can be distinguished by the arrangement of the short spines anterior to the terminal teeth of the abdomen. These form a continuous serrated border of from 10 to 12 short spines. In the female these spines occur in two groups of from 4 to 6 each. Evidence of development of the adult fly is had in the changes of coloration visible through the puparium.

4. The imago emerges through an opening formed by a dorsal slit of the thorax and the fractured hood of the orbital region. There is a definite clearing process upon emerging. In from fourteen to twenty minutes after emergence the imago takes flight.

5. The process of feeding in the female fly is described. Both sexes have a lapping organ, in addition to which the female possesses a distinct piercing organ. In the latter sex two methods of procuring food are indicated.

6. All of the draft animals of the Philippines serve as hosts for Tabanus striatus. The carabao appears to be the host of choice.

7. The rain trees of this locality serve to harbor great numbers of resting horseflies. In considering methods of eradication, this fact should be considered.

8. The following table gives an outline of the minimum days required for the life cycle of flies of this species. From the time the eggs are laid to the emergence of a female fly a period of fifty-two days elapses.
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TABLE II.—Life-history chart of Tabanus striatus Fabricus.

First observation of flies in nature, August 15.
First appearance of mammalian blood in the flies, September 4.

<table>
<thead>
<tr>
<th>Date</th>
<th>Stages</th>
<th>Period of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 11</td>
<td>Beginning of egg laying</td>
<td></td>
</tr>
<tr>
<td>Sept. 15</td>
<td>Hatching of eggs</td>
<td>4</td>
</tr>
<tr>
<td>Sept. 25</td>
<td>First molt</td>
<td>15</td>
</tr>
<tr>
<td>Oct. 9</td>
<td>Second molt</td>
<td>28</td>
</tr>
<tr>
<td>Oct. 17</td>
<td>Larva full-grown</td>
<td>36</td>
</tr>
<tr>
<td>Oct. 24</td>
<td>Third molt</td>
<td>43</td>
</tr>
<tr>
<td>Oct. 24</td>
<td>Puparium formed</td>
<td>43</td>
</tr>
<tr>
<td>Oct. 31</td>
<td>Emergence of male fly</td>
<td>50</td>
</tr>
<tr>
<td>Nov. 2</td>
<td>Emergence of female fly</td>
<td>52</td>
</tr>
<tr>
<td>Nov. 10</td>
<td>Date of death of male</td>
<td></td>
</tr>
<tr>
<td>Nov. 20</td>
<td>Date of death of female</td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES

ILLUSTRATIONS

(Plate I: Plate IV, figs. 3 and 4; and Plate V, figs. 5 and 6, are from photographs by Boynton. Plates II, III, and VI; Plate IV, figs. 1 and 2; and Plate V, figs. 1 to 4, are from photographs by Cortes; Plate VII is from a colored drawing by Dimayuga.)

PLATE I

Fig. 1. *Tabanus striatus* Fabricus depositing eggs on a flat surface. × 1.4.

2. A group of egg masses attached to bits of wood. × 1.9.

3. A mass of eggs photographed during the process of hatching. A few white bodies are seen emerging through the eggshells. The tracks made by escaping larvae can be traced. × 5.

4. A mass of empty eggshells. × 6.6.

PLATE II

Fig. 1. A group of *Tabanus larvae* one hour after hatching. × 7.9.

2. An individual larva one day old. × 30.

3. The head capsule of a developed larva, showing the great hooks, stomal disk, and pharyngeal apophyses. × 15.3.

4. Full-grown larvae. × 1.2.

PLATE III

Fig. 1. The exuvium of the initial molt. × 8.5.

2. The molted skin of the second ecdysis. The shed head capsule is at one end and the cuticle of the anal siphon opposite. × 8.

3. The shed skin of the final process of molting. × 8.

PLATE IV

Fig. 1. The last segment of the male puparium, showing the terminal teeth. × 12.4.

2. The same of the female. × 13.3.

3. The puparium just prior to the act of emergence. × 2.2.

4. The fly emerging from its puparium. × 2.7.

PLATE V

Fig. 1. Head of the male fly. × 6.3.

2. Head of the female fly. × 6.3.

3. Mouth structures of the male. × 12.

4. Mouth structures of the female. × 10.5.

5. Male fly with wings spread, showing body markings. × 2.

6. Female fly with wings spread. × 2.

PLATE VI

Fig. 1. A group of male and female *Tabanus* on a branch of the rain tree, feeding on the gummy excrescence. × 0.48.

2. Female flies infesting a native pony, showing their characteristic position and attitude of feeding.

3. Flies on the abdomen of a work bullock.

PLATE VII

Figs. 1–2. *Tabanus striatus* Fabricus in resting position. × 1.5.

3–4. Male and female flies with wings spread. × 2.4.
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE I.
PLATE III.
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

PLATE IV.
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

PLATE V.
PLATE VII. TABANUS STRIATUS FABRICUS.
THE MECHANICAL TRANSMISSION OF SURRA BY TABANUS STRIATUS FABRICUS

By M. Bruin Mitzmain

(From the Veterinary Division, Bureau of Agriculture, Manila, P. I.)

The Tabanidae have hitherto not been investigated in connection with the transmission of surra in the Philippine Islands, as Stomoxys calcitrans has been the only carrier generally suspected. Recent work has, however, practically eliminated the latter fly from further consideration as an important factor in surra dissemination. In no place has the transmission of any trypanosome infections through the bite of tabanid flies been proved with flies bred in the laboratory.

The geographical and seasonal distribution of Tabanus striatus have been recorded in the preceding article, and the status thereof marks this species as preeminently the most formidable bloodsucking fly in the Philippines.

The investigation of which this is a preliminary note has been conducted during the past year in the veterinary research laboratory at Alabang, Rizal Province, Luzon, with tabanid flies, which were for the most part bred from the egg; in some instances the flies were obtained from larvae taken from their aquatic habitats; and in a few instances captured adult flies were employed.

In the present series Tabanus striatus was used in experiments on the direct transmission of surra. The flies were first allowed to bite an infected guinea pig or horse for not more than one minute, usually forty-five seconds; they were then transferred to a healthy animal and allowed to complete the meal without interruption. An interval of from five seconds to three minutes intervened during the transfer from the infected to the healthy animal.

1 To be published as Bulletin No. 28, Bureau of Agriculture of the Government of the Philippine Islands.
2 Archibald R. Ward, chief.
3 This Journal, Sec. B (1912), 7, 475.
In every instance the healthy animals used in the experiments were proved to be free from trypanosomes by rigorous quarantine and frequent microscopic examination of their blood. The mule and the horses employed in these experiments were kept for from six to eight months in the screened stable prior to their use for exposure to fly bites or blood inoculation to test the pathogenic nature of the trypanosome involved. The temperatures of these animals were registered morning and evening during the period of quarantine, preceding and following each experiment.

Monkeys, guinea pigs, and rabbits utilized in the experiments were quartered previously in fly-screened cages and declared surra free after ninety days, during which time blood examinations were made regularly once or twice weekly prior to the experiments.

Table I gives the data of these experiments.

**TABLE I.—Experiments on the mechanical transmission of surra by Tabanus striatus.**

<table>
<thead>
<tr>
<th>Date of experiment</th>
<th>Trypanosomes in the infected host</th>
<th>Flies employed</th>
<th>Healthy animal used</th>
<th>Result of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1912</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov. 21</td>
<td>Swarming</td>
<td>1 Guinea pig 87</td>
<td>Negative.</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Moderate</td>
<td>1 Guinea pig 92</td>
<td>Negative.</td>
<td></td>
</tr>
<tr>
<td>Dec. 12</td>
<td>do</td>
<td>1 Guinea pig 111</td>
<td></td>
<td>Negative.</td>
</tr>
<tr>
<td>26</td>
<td>do</td>
<td>2 Guinea pig 93</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>Nov. 21</td>
<td>Numerous</td>
<td>1 Monkey 5</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Swarming</td>
<td>1 Monkey P</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Numerous</td>
<td>1 Monkey D</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>Dec. 1</td>
<td>do</td>
<td>1 Monkey S</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>1913</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan. 1</td>
<td>Scanty</td>
<td>2 Monkey D</td>
<td>Negative.</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Numerous</td>
<td>2 Horse 66</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>do</td>
<td>2 Horse 69</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Swarming</td>
<td>1 Horse 277</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Numerous</td>
<td>1 Horse B 120</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>Feb. 2</td>
<td>Swarming</td>
<td>2 Horse 50</td>
<td>Positive on eighth day. One mule, 2 monkeys, and 2 guinea pigs reacted to inoculation of blood of horse 50.</td>
<td></td>
</tr>
<tr>
<td>6 to 10.</td>
<td>Scanty to moderate, b</td>
<td>6 Horse 342</td>
<td>Positive on ninth day. Disease reproduced by blood inoculation into 2 monkeys and 2 guinea pigs.</td>
<td></td>
</tr>
</tbody>
</table>

a Infected guinea pig used.  
b Infected horse used.
The three positive experiments are described in detail as follows:

The experiment in which monkey L became infected was conducted with flies bred from eggs. The source of this strain of surra was carabao 3182 which had been infected with surra for nearly one year previous to this experiment. Blood of this animal was inoculated into guinea pig 119 which showed infection in eight days, and was used for the present experiment, December 28, when its blood showed numerous trypanosomes. Three flies were applied individually in tubes to guinea pig 119 and allowed to feed from forty-five seconds to one minute and thirty seconds. They were then transferred to monkey L, after intervals of from twenty seconds to three minutes, and allowed to feed until satisfied. The flies fed on the latter animal five, sixteen, and twenty-one minutes, respectively.

From December 28 to January 8 no reaction was noted. The first high temperature, 40°.1, occurred on the evening of January 8, accompanied by a few trypanosomes in the peripheral circulation. The presence of trypanosomes continued daily, moderate to swarming in numbers, with several febrile periods until the animal's death on December 22, 1912.

Blood from the heart of monkey L was inoculated into horse 343 and guinea pigs 101 and 102. The latter showed infection upon the eighth and ninth days, respectively. Horse 343 had an abnormal temperature and a moderate number of trypanosomes in its blood upon the seventh day. The animal died March 1 with surra. At autopsy there was observed a general emaciated condition and enlargement of the spleen. The splenic pulp contained enormous quantities of trypanosomes as did the heart blood.

Horse 343 was also used as the blood donor in transmission experiments in which tabanids infected two other horses, namely, 50 and 342. The latter experiments were made in order to verify the previous one, in which a monkey contracted the disease. In only the first of the latter experiments were bred flies used. Two flies were permitted to bite horse 343 at a time when its blood was swarming with trypanosomes. The flies were interrupted in their biting in from forty to forty-five seconds and transferred to healthy horse 50. The infected flies bit after intervals of from five to fifteen seconds and were permitted to complete the feeding on horse 50. The latter was replaced in the fly-screened stable and examined daily. The initial rise of temperature was noted upon the eighth day, February 10, when a few surra organisms were found in the animal's blood. On
the day following, horse 50 showed numerous trypanosomes in its blood and high temperature. Blood from this horse was inoculated into a mule, 2 monkeys, and 2 guinea pigs. The mule reacted with the usual symptoms after an incubation period of six days. Both monkeys had an incubation period of five days, and died of surra on the fourteenth and the fifteenth day, respectively. The 2 guinea pigs likewise became infected.

A second experiment was conducted with captured flies, surra horse 343 being used in this experiment. From February 6 to February 10, six flies in all were allowed to bite healthy horse 342 after they contaminated their proboscides with the blood of surra horse 343. The blood of the latter contained numerous trypanosomes upon only one occasion during the experiment; at other times the trypanosomes were scanty or moderate in numbers. The interval between the biting of the infected and the healthy horse was never more than twenty seconds, and the meal was completed in from four to eleven minutes on horse 342. An incubation period of nine days following the last bite elapsed before the healthy horse showed evidence of infection from the bites of the contaminated flies. On the evening of February 19 the temperature of horse 342 rose to 41°.1 C., and the blood showed a moderate number of trypanosomes. Upon the following day, when the trypanosomes were more numerous, blood from this animal was inoculated into 2 monkeys and 2 guinea pigs. The monkeys showed the first signs of infection on the seventh day and the guinea pigs on the eighth and ninth days. The 2 monkeys and the 2 guinea pigs were alive, but still infected on March 3, 1913.

BITING EXPERIMENTS WITH ANIMALS IN A LARGE CAGE

An effort was made to induce flies to feed on a healthy and on infected animals kept together in a large screened cage. The results were negative, the flies dying in a few days when kept within the inclosure. The animals used were 2 surra-infected and 1 healthy carabao. The latter was separated from the others by a coarse-meshed wire partition. The flies were introduced daily into the common inclosure and were given ample opportunity to bite the animals exposed. From November 9 to December 22, 1911, 2,087 female tabanids were liberated in the cage. The animals were examined daily, and after the experiment the healthy animal was removed and observed. Fourteen months have elapsed and the exposed carabao remains normal. Two guinea pigs inoculated with its blood were alive and negative on April 12, 1913.
HEREDITARY TRANSMISSION

As a precautionary measure it was thought advisable to eliminate the possibility, however remote, of the existence of hereditary transmission of trypanosomes in these flies.

In one experiment of this nature 74 flies were tested during two weeks after the emergence of the lot by allowing them to bite a healthy monkey. The eggs from which these flies developed had been laid August 14 by a fly which had fed twice on a monkey infected with surra and whose blood was swarming with trypanosomes. The following table contains the data resulting from allowing flies of this lot to feed on a healthy monkey. Monkey 5, which was examined daily during the experiment, showed no signs of infection and remained healthy until April 12, 1913.

**Table II.—Experiments to test the hereditary transmission of surra infection in Tabanus on monkey 5; results negative.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Flies tested</th>
<th>Average time of feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>14</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

AN ATTEMPT TO TRANSMIT SURRA BY MEANS OTHER THAN BITING

It was observed that many flies of both sexes in feeding supplemented the sucking of the labium with the lapping of the spongy labellum. Usually these processes are independent, but not infrequently the female in attempting to bite the host will lap up any moisture present preparatory to inserting its proboscis. When an abraded surface is presented the majority of flies of this species are capable of nearly filling the stomach with blood without the aid of the proboscis. This has been observed in numerous instances in flies used in biting experi-
ments, but has never been seen in flies attacking animals in the natural state.

Considering that there might be a remote possibility of conveyance of infection through this peculiarity in feeding habits, five experiments, based on this hypothesis, were attempted. The technique was the same in all of the 5 guinea pigs used. A highly infected guinea pig was used to contaminate the flies. A portion of the skin of infected and healthy animals was abraded with a razor and the flies applied individually in tubes. The fly was permitted to lap the blood from the abrasion on the infected animal for a minute or less and then transferred immediately to the healthy animal, where it was induced to apply its labellum for from five to ten minutes.

Table III.—Attempts to transfer infection by the fly’s labellum. Results negative.*

<table>
<thead>
<tr>
<th>Date</th>
<th>No. of guinea pig</th>
<th>Flies applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 21</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>Nov. 22</td>
<td>91</td>
<td>2</td>
</tr>
<tr>
<td>Nov. 23</td>
<td>110</td>
<td>2</td>
</tr>
<tr>
<td>Nov. 24</td>
<td>104</td>
<td>4</td>
</tr>
<tr>
<td>Nov. 25</td>
<td>123</td>
<td>3</td>
</tr>
</tbody>
</table>

* The experiment of guinea pig 110 was checked by subsequent inoculation to which the animal became infected.

The results of all the trials were negative, although it was ascertained that typical trypanosomes were present upon the labellum of one of the flies and in the stomach of another fly immediately after the experiment.

These experiments were supplemented by an experiment attempting to transfer infection to abrasions by the pulvilli of the contaminated fly. This also resulted negatively.

LENGTH OF TIME TABANUS STRIATUS HARBORS TRYpanosomes

An attempt was made in this series of experiments to determine the maximum length of time surra organisms remain alive in the gut of the fly. The fly was fed in each instance on a guinea pig showing numerous trypanosomes in its blood. As noted in Table IV trypanosomes indistinguishable from surra organisms were found in suspensions from flies up to thirty hours after biting a sick animal. Beyond ten hours, inoculations of infected flies into susceptible animals were negative.
TABLE IV.—Inoculations with suspensions of flies fed on infected animals.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Animal inoculated</th>
<th>Flies employed</th>
<th>Interval after feeding of fly on infected animal.</th>
<th>Presence of trypanosomes in the fly</th>
<th>Result.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guinea pig 86</td>
<td>1</td>
<td>6</td>
<td>Positive</td>
<td>Positive.</td>
</tr>
<tr>
<td>2</td>
<td>Guinea pig 89</td>
<td>1</td>
<td>10</td>
<td>...do</td>
<td>Do.</td>
</tr>
<tr>
<td>3</td>
<td>Guinea pig 131</td>
<td>1</td>
<td>24</td>
<td>...do</td>
<td>Negative.</td>
</tr>
<tr>
<td>4</td>
<td>Guinea pig 130</td>
<td>1</td>
<td>26</td>
<td>...do</td>
<td>Do.</td>
</tr>
<tr>
<td>5</td>
<td>Guinea pig 130</td>
<td>1</td>
<td>30</td>
<td>...do</td>
<td>Do.</td>
</tr>
<tr>
<td>6</td>
<td>Rabbit</td>
<td>1</td>
<td>30</td>
<td>...do</td>
<td>Do.</td>
</tr>
<tr>
<td>7</td>
<td>Guinea pig 97</td>
<td>1</td>
<td>48</td>
<td>Negative</td>
<td>Do.</td>
</tr>
<tr>
<td>8</td>
<td>Guinea pig 98</td>
<td>1</td>
<td>48</td>
<td>...do</td>
<td>Do.</td>
</tr>
<tr>
<td>9</td>
<td>Guinea pig 98</td>
<td>1</td>
<td>96</td>
<td>...do</td>
<td>Do.</td>
</tr>
</tbody>
</table>

* The dejecta from this fly were found swarming with trypanosomes two and one-half hours after the fly bit the sick animal.

CONCLUSIONS

1. *Tabanus striatus* Fabricus for the first time recorded has been found to play a rôle in the transmission of surra. Bred horseflies have been employed for the first time in such experiments. Errors resulting from naturally infected wild flies have thus been eliminated.

2. Three experiments were successful in the direct or mechanical transmission by "interrupted" feeding when only a short interval was allowed between the bites on infected and healthy animals. In 16 experiments the minimum number of flies with which the infection could be transmitted was 2.

3. Trypanosomes of surra were not found to be transmitted hereditarily in *Tabanus striatus* Fabricus.

4. The contaminated labellum of the fly does not appear to be a factor in the conveyance of infection.

5. The maximum length of time that *Trypanosoma evansi* has been demonstrated microscopically in the gut of this species of fly after feeding on infected blood is thirty hours; the organisms were found in the fly's dejecta two and one-half hours after biting the infected animal; and suspensions of flies, when injected subcutaneously, were found infective for animals for a period of ten hours after the flies had fed on infected blood.
AXILLARY TERATOMA

By P. K. Gilman

(From the Philippine General Hospital and the College of Medicine and Surgery, University of the Philippines)

Two plates

While numerous examples of embryomata occurring in the more frequently involved portions of the body are constantly being reported, a review of the available literature fails to reveal a similar case to the one here described.

Teratomata are tumors frequently containing a number of different forms of tissue, bone, teeth, hair, skin, muscle, and glands. They are more frequently found at the lower end of the spine where the division occurs between the neural and gut portions of the neuroenteric tube, about the head, and in the generative organs. Tissue derived from any or all of the three layers of the blastoderm occurs in these tumors, and a preponderance of any one type of tissue in the growth has led not only to a classification of these growths, but, previous to the work of Wilms, to some confusion as well.

The work of Wilms(1) in 1895 and in 1899 on dermoid cysts of the ovary did a great deal to clear up many questions concerning the anatomical peculiarities of these tumors and render their grouping possible. He showed these tumors to be complicated structures containing derivatives from all of the three blastodermic layers, applied the term embryomata, and believed them peculiar to the ovary, developing from the growth of an unimpregnated ovum.

It was shown later that embryomata of the ovary differ in no essential manner from embryomata occurring elsewhere in the body, thus doing away with the earlier idea of Wilms of ovum origin.

A few references from a large literature are cited to illustrate the more frequent situations in which these growths occur.

Weigert(2) studied a 3.5-centimeter teratoma of the pituitary body occurring in a boy of 14 years. The growth contained epidermis, hair follicles, hair, sebaceous glands, fat, cartilage, and smooth muscle.
Ford(3) reports the removal of a large teratoma from the right side of the face and neck of a mulatto woman aged 40 years. The tumor had been observed since the age of 18 years. It had increased rapidly in size for two years prior to its removal.

Musick(4) describes in detail a case of teratoma of the right lobe of the liver, causing death in an infant of 2 months. In this case structures were found having their origin in the mesoderm and entoderm, but not in the ectoderm.

Mandelbaum(5) reports a case of dermoid cyst of the medias-tinum in a Russian woman of 30 years, and has collected 36 other cases from the literature. He suggests as a result of study of the cases reported and the histological findings in his own case that these growths be classified with the teratomata and further suggests the following groupings:

I. True dermoids containing only ectodermal structures.
II. Teratomata, dermoids with the addition of structures from the en-toderm and mesoderm.
III. True dermoids or teratomata, with the addition of tumor formations.

Anspach(6) summarizes the opinion of Wilms and others regarding teratomata of the ovary, and reports a case of ovarian teratoma with a large proportion of thyroid gland tissue in the dermoid prominence removed from a woman of 36 years.

Ewing,(7) from a study of 2 cases of testicular tumors, concludes that teratomata of this region developing unequally or in a one-sided manner give rise to the myxomata, myomata, chon-dromata, carcinomata, and large round-celled sarcomata or car-cinomata of the testes.

Bergmann(8) reports 5 cases of congenital sacral teratomata operated upon in children. One of the cases possessed malignant characteristics with metastasis.

CASE REPORT

Mateo Serrer, Filipino, age 7 years. Operation, August 9, 1912.
Family history negative. Eleven brothers and sisters died, some of smallpox and some of beriberi.
Patient was born without medical attendance; delivery was normal. Child was breast fed at irregular hours, but with good success. Denti-tion was normal. There were no previous diseases with the exception of mild fevers.
Present illness is congenital, a swelling having been noted under the right arm shortly after birth. This swelling has increased slowly but steadily in each diameter until a few days ago when the patient had a slight fever and the tumor became red and increased noticeably in size.
The present condition of the child is, in general, good. He is fairly well nourished, although somewhat underdeveloped. The general examination is without especial interest.
Occupyng the right axilla and limiting considerably the movements of the arm in all directions is a tumor measuring about 12 centimeters in diameter, with a general spherical form. The growth as a whole is soft and elastic and is movable, although evidently attached to the apex of the axilla and upper portions of the chest wall; is not tender nor painful. The skin over the mass is more or less adherent in several linear areas, one of these on the external surface being reddened and inflamed. The vessels of the skin over the growth show great distention and considerable venous stasis. The tumor evidently contains fluid under considerable tension, and over the posterior portion of the wall of the cyst gives a sensation as though considerable fibrous thickening had taken place with probably a deposition of some mineral salts.

Under chloroform anaesthesia, an oblique incision was made over the tumor and the skin and subcutaneous tissues were stripped back from the cyst wall proper. The growth shelled out readily over nearly the entire surface, difficulty being experienced only in the apex of the axilla where the artery, several branches of the vein, and most of the nerve branches were enveloped in the fibrous capsule of the cyst and over the fifth rib, the periosteum of which was intimately connected with the wall.

The wound was closed with a cigarette drain and the child, although having taken the anaesthetic badly, made an uninterrupted recovery.

The tumor was sent to the pathological laboratory, and was examined by Dr. B. C. Crowell, whose report follows:

Specimen consists of a mass of tissue removed from the right axilla. This is irregularly globular in shape, and measures about 12 centimeters in diameter. There are some fibrous tags on the outer surface, and it is apparently cystic, being formed of larger and smaller cysts, some as large as 4 or 5 centimeters in diameter. A small portion of the fluid of the cyst was aspirated immediately after the specimen was received, and was seen to be clear and yellowish in color. The specimen was fixed intact, and after fixation was divided into two equal portions. The cysts of which it is made up are filled with a partially coagulated greenish fluid (after fixation). On removal of this fluid, one large cyst measuring 6 centimeters in diameter and other smaller cysts are seen; the walls of these are smooth and pale, and the larger cysts have thin walls. Across one of the larger cysts stretches a band of soft, gelatinous tissue about 2 centimeters in diameter. At the periphery of one-half of the cut section are seen numerous small cysts in the wall of the mass, these being separated by a pale tissue. Some of these cysts have a clear content, and in some it is yellowish and rather firm, and again in others of a deep red color.

Microscopically, sections taken from the walls of the cysts are seen to contain numerous fibrous connective tissue, muscular and adipose elements irregularly arranged, and in their midst are numerous, very thick-walled blood vessels. One small portion of a lymph node is also seen in one section imbedded in the wall. There are also numerous small cystic spaces, some of which are filled with blood and others filled with a pink, gelatinous material. The walls of these smaller cysts are formed by very low, flat, epithelial cells, the nuclei of which are rounded in many places. There is also considerable leucocytic infiltration of different parts
of the mass. The walls of the larger cysts are of the same character as
the walls of the small cysts.

Teratoma of mesodermal origin or autochthonous monophyllic terato-
blastoma of mesodermal origin (Adami).

REFERENCES

(2) Weigert, C. Zur Lehre von den Tumoren der Hirnanhänge. Vir-
chow's Arch. (1825), 65, 212.
(3) Ford, DeS., M. D. Extirpation of teratoma or teratoid tumor. Am.
Journ. Med. Sci. (1892), n. s. 77, 91-93.
(5) Mandelbaum, F. S. A case of dermoid cyst of the mediastinum, etc.
Am. Journ. Med. Sci. (1900), n. s. 120, 64.
(6) Anspach, B. M. Present conception of dermoid cysts of the ovary,
with the report of a case of teratoma strumosum thyreoidalii ovari.
ILLUSTRATIONS

PLATE I

Fig. 1. Anterior view of axillary teratoma.
2. Posterior view of same.

PLATE II

Fig. 1. Tumor after removal.
2. Same tumor after section.
Fig. 1. Anterior view of axillary teratoma.

Fig. 2. Posterior view of same.
A NOTE UPON STRANGLES IN THE PHILIPPINE ISLANDS

By WILLIAM HUTCHINS BOYNTON

(From the Veterinary Division, Bureau of Agriculture, Manila, P. I.)

It is believed by some members of the medical profession that streptococcic infection in man is of rare occurrence in the Philippine Islands, and, when present, is mild in effect.

Musgrave states that—

The most logical explanation of the ease with which infection may be avoided in both surgery and obstetrics is the rarity of virulent streptococci, such organisms seldom being encountered in laboratory work, in the morgues or in clinical work in the hospitals.

Streptococcic septicemia, streptococcic cellulitis, erysipelas, scarlet fever, and even streptococcic sore throat are extremely rare conditions in the Philippine Islands, and streptococcic metritis and peritonitis following child birth have not been encountered in autopsy work in Manila.

Since the diseases prevailing among the domesticated animals have not been worked out to any great extent here, it was not positively known whether or not they suffer from such infection.

The existence of strangles or distemper in horses appears to have attracted very little attention in the Philippines, and, although it has been recognized several times by veterinarians, the diagnosis in such instances was based entirely upon clinical symptoms, the causative agent not having been isolated and identified.

In searching for official reports on strangles in the Bureau of Agriculture files, none could be found, but by personal inquiry the writer has been able to gain some data as to its prevalence.

During the months of February, March, and April, 1909, shortly after the arrival of some old Army mares for breeding purposes at the Alabang stock farm, there was an outbreak of strangles which killed several colts, and ran a severe course in many others. By the strenuous work of the men in charge, the infection was cleaned up and has not appeared since.

1 Archibald R. Ward, chief.
There have been several outbreaks of this disease at the Trinidad stock farm during which several colts and older horses have died. Veterinarians who have worked in Manila say strangles is prevalent among the carromata horses, and also occurs frequently in the city stables. A few years ago this disease is said to have killed one of the finest race horses in the Philippine Islands.

Strangles appeared among the horses at the Singalong experiment station in Manila about March 10, 1911, and on March 26, horses, which had apparently not been affected by the disease during this outbreak, were shipped from Singalong to Trinidad. About April 15 the disease was observed among the animals at the Trinidad stock farm, but, as the disease first appeared in a pony belonging to an employee at the experiment station, it was thought probable that the infection was brought in by this pony rather than by the horses from Manila.

On April 3, 1911, the animal husbandry division of the Bureau of Agriculture shipped 11 horses suffering with osteoporosis from the Trinidad stock farm to the veterinary research laboratory at Alabang. The animals were transported in stock cars, arriving at Alabang in apparently good condition, except for the deformities caused by osteoporosis. They were placed in a shed previously occupied by cattle immunized to rinderpest, which were used in the production of antirinderpest serum. Each horse was tied in a separate stall, but could easily reach over into its neighbor's manger on either side.

Case 1.—April 10, 1911, the writer's attention was called to a horse, 1 year old, which had a slight purulent discharge from its left nostril, the left submaxillary gland being slightly enlarged. The animal was immediately separated from the rest, and its manger thoroughly disinfected. On April 16 this animal died. Post-mortem examination revealed typical osteoporosis lesions, the right femur being completely fractured about 7 centimeters from its head, and practically all the bones of the body were more or less affected, which was undoubtedly the cause of death. At the time of death, the discharge from the nostril had practically ceased, but the affected gland was somewhat enlarged. Neither culture or smear preparations were made from the discharge or gland of this animal.

Case 2.—April 17, a horse, 1 year old, which stood in the stall adjoining that of horse 1 had a purulent discharge from both nostrils. The animal was immediately isolated, and the stall and manger thoroughly disinfected. On the 19th, the submaxillary glands were enormously enlarged. Four days later an abscess broke, discharging a thick yellowish pus, and on the 26th two more abscesses broke. The horse was placed on the operating table, and two more abscesses were opened with aseptic precautions. From these, cultures were made on agar-agar and in plain bouillon. Several smear preparations were made and, after staining, showed upon micro-
scopie examination large numbers of cocci and a few short chains containing from 3 to 4 elements each. On the 28th, the agar culture showed several colonies of Micrococcus pyogenes aureus and albus. Besides these there were a few small grayish colonies which, upon microscopic examination of hanging-drop and stained smear preparations, proved to be streptococci. Long chains of streptococci were also found present in the bouillon culture but were mixed with numerous micrococci. Subcultures on agar and in bouillon were made from the small agar colonies. On April 29 the subcultures in both media revealed pure cultures of streptococci.

On May 1, 1 cubic centimeter of bouillon culture was injected subcutaneously into each of 2 rabbits and 2 guinea pigs, and 5 cubic centimeters subcutaneously into the neck of a horse 1 year of age. The rabbits and guinea pigs suffered no ill effects from the injection. The horse on the second day after injection showed a large swollen area around the point of inoculation which was very tender. On the fifth day the swelling was 14 centimeters in diameter. The lymphatic vessels extending from it were swollen, giving an identical picture of a typical, local mallein reaction in a glandered horse. On the eighth day the abscess thus formed broke and discharged a large amount of yellowish blood-stained pus, which upon microscopic examination of stained smear preparation revealed large numbers of streptococci in short chains.

At the time the abscess broke the horse ate very little and had a depressed appearance, lying down the greater part of the time. In a couple of days it regained its appetite, the swelling gradually subsided, and complete healing took place in fifteen days. The neighboring lymphatic glands were observed frequently, but no sign of enlargement or tenderness could be detected.

From all appearances, this inoculated horse had a mild attack of strangies, the infection remaining localized at the point of inoculation.

Case 3.—On April 25, a 2-year-old horse, which occupied the stall next to that of horse 2, was observed to have a slight discharge from both nostrils. It was immediately isolated, and the premises thoroughly disinfected. In three days it had a marked discharge from both nostrils, and both submaxillary glands were considerably enlarged. After ten days' duration the discharge ceased, and the glands gradually returned to normal size. A few short chains of streptococci were found on microscopic examination of the nasal discharge. In fourteen days this animal had apparently recovered from the attack.

Case 4.—On May 3, a small black mare, 1 year old, whose stall was next to that of horse 3, was observed to have a discharge from the right nostril. The animal was immediately isolated, and the premises thoroughly disinfected. The following day there was observed a discharge from both nostrils, and the submaxillary glands were slightly enlarged. At the end of ten days the discharge from both nostrils had ceased, and the glands were practically normal in size. Recovery was apparently complete in fourteen days.

CONCLUSIONS

1. From the results derived from the cultures and from microscopic examinations of the purulent discharges, it is evident that streptococcic infection exists in horses in the Philippine Islands.
2. Since bouillon cultures had no effect on rabbits and guinea pigs when inoculated subcutaneously, and did have decided effect upon a horse, it proves conclusively that the organism isolated was *Streptococcus equi*. No white mice were on hand, so the virulence of the culture could not be tested on them.

3. From the information gained through inquiry, it is very evident that strangles is a widespread disease among horses in the Islands, an interesting fact in view of the reputed rarity of streptococcic infections in man.
A FURTHER NOTE UPON THE INFLUENCE OF ATMOSPHERIC TEMPERATURE UPON THE SPREAD OF PNEUMONIC PLAGUE

By Oscar Teague

(From Cornell University Medical College, New York)

Plague has raged in India for almost two decades, and has carried off several millions of victims. The epidemic has remained essentially bubonic in type. Although 1 per cent or more of all the cases have been plague pneumonias, these numerous scattered cases have not led to epidemics of pneumonic plague. That plague pneumonitis may assume epidemic proportions under certain circumstances was demonstrated most conclusively in Manchuria during the winter of 1910–1911 by one of the most virulent epidemics of modern times. Teague and Barber recently offered the following explanation of the rapid spread of pneumonic plague in Manchuria and the failure to spread in India.

We believe we are justified in concluding from these experiments that were the plague organisms sprayed under similar conditions they would persist longer than cholera vibrios, but a shorter time than prodigious bacilli. Hence, it seems probable that the plague bacilli contained in fine droplets of pneumonic-plague sputum would suffer death from drying in a few minutes unless they were suspended in an atmosphere with an extremely small water deficit. Infection in pneumonic plague follows the inhalation of droplets of pneumonic sputum and obviously the longer these droplets remain suspended in the air, the greater is the danger of infection. As has just been stated, these fine droplets disappear very quickly except when they are suspended in an atmosphere with a very small water deficit. Such an atmosphere is, under ordinary circumstances, of common occurrence in very cold climates, whereas it is extremely rare in warm ones. Hence, since the droplets of sputum persist longer, the plague bacilli remain alive longer in the air, and there is a greater tendency for the disease to spread in cold climates than in warm ones.

During the Manchurian epidemic the temperature at Harbin (where the great majority of deaths occurred) ranged between

1 Assistant in experimental pathology, Cornell University Medical College, New York.

2 This Journal, Sec. B (1912), 7, 172.
—9° C. and —32° C. However, in considering the above explanation one must bear in mind that in most instances infection almost surely takes place indoors, whereas these low temperatures were measured in the open. Furthermore, a well-heated room with a low outside temperature affords a greater water deficit than a similar room with a high outside temperature. Hence, if the lodging houses in Harbin were well heated during the plague epidemic, there would remain but little basis for the above explanation.

As no records of indoor temperatures during the epidemic are available, I have endeavored to secure temperatures of inns and lodging houses in Harbin in which plague patients were found at the same season of the year that the epidemic occurred. For this purpose I forwarded blanks to the American minister at Peking with the request that he have them filled out by persons known to be reliable. As a result, the following observations were made at the request of the American consul at Harbin by Dr. T. N. Tang, thanks to the kindness of Dr. Wu Lien Teh, director of the North Manchurian Plague Prevention Service.

From Table I it is seen that 9 (Nos. 23, 24, 28, 29, 35, 36, 37, 38, and 39) of the houses investigated showed temperatures of 6° C. or less in at least one of the observations and 5 of these houses were known to have harbored plague cases during the epidemic. In 11 additional houses (Nos. 1, 2, 4, 9, 12, 17, 26, 31, 32, 33, and 41) temperatures of 10° C. or lower were recorded.

Therefore, it is clear that in Harbin there are native inns and lodging houses which during February are very inadequately heated and consequently must contain an atmosphere with a very low water deficit.

Since the publication of the article by Teague and Barber,

Dr. Wu Lien Teh has also furnished the following information, which has a definite bearing upon the subject under discussion:

"The native dwellings in Harbin are heated by brick stoves after the Russian style (very few); iron stoves in which coal is usually burnt; kangs (i.e., large rectangular brick structures 21 feet above the ground, in which the people sit and rest) heated usually by millet stalks; and open charcoal pans without chimneys.

"The windows usually consist of a wooden framework pasted with thin paper, thus letting in very little light. Beyond the doors and the cracks in the windows, walls, and roofs, there is seldom any ventilation inside the dwellings."

Rau⁴ has described a small epidemic of pneumonic plague at Santa Maria in the southern part of Brazil. For four years isolated cases of bubonic plague had occurred in Santa Maria at intervals of weeks or months. On July 21, 1912, there suddenly appeared a case of pneumonic plague which was followed in the course of the next three weeks by 17 other cases, all ending fatally.

It is worthy of note that these pneumonic cases occurred during the cool season and that the houses in Santa Maria are not heated artificially. (Personal communication from Doctor Rau.)

Next to Harbin, Changchun suffered most during the epidemic. After Doctor Tang had made the observations here recorded for Harbin, he was sent by Doctor Wu to Changchun, where he noted the temperatures recorded in Table II.

⁴ *Deutsche med. Wochenschr.* (1912), 38, 2314.
### Table I.—Temperature observations

<table>
<thead>
<tr>
<th>No. of observation</th>
<th>Variety of dwelling</th>
<th>Description of building</th>
<th>Approximate size of room.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Feet.</td>
</tr>
<tr>
<td>1[a] [b]</td>
<td>Native inn</td>
<td>Mud-walled room; old, no windows; native kangs on both sides; ground of room about 1 foot lower than that outside the door; earth floor.</td>
<td></td>
</tr>
<tr>
<td>2[a] [b]</td>
<td>do</td>
<td>Brick wall outside; mud inside; earth floor; 1 window; kangs on both sides leaving passage in the middle.</td>
<td>19</td>
</tr>
<tr>
<td>3[a] [b]</td>
<td>do</td>
<td>Earth floor; 6 paper windows; kangs on both sides.</td>
<td>24</td>
</tr>
<tr>
<td>4[a] [b]</td>
<td>do</td>
<td>Plank floor; native kangs on both sides; 2 paper windows.</td>
<td>23</td>
</tr>
<tr>
<td>5[a] [b]</td>
<td>do</td>
<td>Earth floor.</td>
<td>18.5</td>
</tr>
<tr>
<td>6[a] [b]</td>
<td>do</td>
<td>Plank floor; kangs on both sides of room; glass windows.</td>
<td>17</td>
</tr>
<tr>
<td>7[a] [b]</td>
<td>do</td>
<td>Plank floor.</td>
<td>16</td>
</tr>
<tr>
<td>8[a] [b]</td>
<td>do</td>
<td>Earth floor; kangs</td>
<td>19</td>
</tr>
<tr>
<td>9[a]</td>
<td>do</td>
<td>Earth floor; mud and straw wall; 4 paper windows.</td>
<td>19</td>
</tr>
<tr>
<td>10[a] [b]</td>
<td>do</td>
<td>Plank floor; 1 window.</td>
<td>9</td>
</tr>
<tr>
<td>11[a] [b]</td>
<td>do</td>
<td>Plank floor; kangs on both sides.</td>
<td>23</td>
</tr>
<tr>
<td>12[a] [b]</td>
<td>do</td>
<td>Earth floor; 4 windows.</td>
<td>19</td>
</tr>
<tr>
<td>13[a] [b]</td>
<td>do</td>
<td>Earth floor; 2 windows.</td>
<td>21</td>
</tr>
<tr>
<td>14[a] [b]</td>
<td>do</td>
<td>Plank floor; 1 window.</td>
<td>7</td>
</tr>
<tr>
<td>15[a] [b]</td>
<td>do</td>
<td>Brick floor; 1 paper window.</td>
<td>21</td>
</tr>
<tr>
<td>16[a] [b]</td>
<td>Private house</td>
<td>Earth floor; kangs; 2 paper windows.</td>
<td>19</td>
</tr>
<tr>
<td>17[a] [b]</td>
<td>Native Inn</td>
<td>Earth floor; 4 windows; mud wall.</td>
<td>23</td>
</tr>
<tr>
<td>18[a] [b]</td>
<td>Shop</td>
<td>Earth floor; 2 windows on either side; kangs on both sides.</td>
<td>10</td>
</tr>
<tr>
<td>19[a] [b]</td>
<td>Native Inn</td>
<td>Plank floor; kangs on both sides; 3 paper windows.</td>
<td>28</td>
</tr>
<tr>
<td>20[a] [b]</td>
<td>do</td>
<td>Earth floor; kangs on both sides; 8 windows.</td>
<td>28</td>
</tr>
<tr>
<td>21[a] [b]</td>
<td>do</td>
<td>Earth floor; kangs on both sides; 2 paper windows.</td>
<td>19</td>
</tr>
<tr>
<td>22[a] [b]</td>
<td>do</td>
<td>Plank floor; paper windows.</td>
<td>40</td>
</tr>
</tbody>
</table>
Teague: Spread of Pneumonic Plague in Fuchiatien (Harbin).

<table>
<thead>
<tr>
<th>Occupants at the time</th>
<th>How heated.</th>
<th>Occurrence or nonoccurrence of plague.</th>
<th>Temperature.</th>
<th>Date.</th>
<th>Time of day taken.</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Brick stove with chimney.</td>
<td>Not sure</td>
<td>°C.</td>
<td>°C.</td>
<td>1913.</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>10</td>
<td>3</td>
<td>Feb. 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>...do</td>
<td>do</td>
<td>7</td>
<td>2</td>
<td>do</td>
</tr>
<tr>
<td>12</td>
<td>...do</td>
<td>(?)</td>
<td>10</td>
<td>-4</td>
<td>do</td>
</tr>
<tr>
<td>14</td>
<td>Open charcoal pan</td>
<td>(?)</td>
<td>12</td>
<td>-2</td>
<td>do</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td>11</td>
<td>-5</td>
<td>do</td>
</tr>
<tr>
<td>12</td>
<td>Stove with chimney</td>
<td>(?)</td>
<td>10</td>
<td>-2</td>
<td>do</td>
</tr>
<tr>
<td>22</td>
<td>Open charcoal pan</td>
<td>(?)</td>
<td>14</td>
<td>-5</td>
<td>do</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>17</td>
<td>-2</td>
<td>do</td>
</tr>
<tr>
<td>8</td>
<td>Kangs</td>
<td>(?)</td>
<td>22</td>
<td>-10</td>
<td>do</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>16</td>
<td>-3</td>
<td>do</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>16</td>
<td>-3</td>
<td>do</td>
</tr>
<tr>
<td>14</td>
<td>Chimney stove</td>
<td>(?)</td>
<td>14</td>
<td>-3</td>
<td>do</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>15</td>
<td>-7</td>
<td>do</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>15</td>
<td>-4</td>
<td>do</td>
</tr>
<tr>
<td>6</td>
<td>Kangs</td>
<td>(?)</td>
<td>16</td>
<td>-8</td>
<td>do</td>
</tr>
<tr>
<td>14</td>
<td>Stove with chimney</td>
<td>(?)</td>
<td>19</td>
<td>-10</td>
<td>Feb. 4</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>19</td>
<td>-17</td>
<td>Feb. 5</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td>19</td>
<td>-17</td>
<td>Feb. 5</td>
</tr>
<tr>
<td>14</td>
<td>Stove with chimney</td>
<td>(?)</td>
<td>19</td>
<td>-17</td>
<td>Feb. 5</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>19</td>
<td>-17</td>
<td>Feb. 5</td>
</tr>
<tr>
<td>2</td>
<td>...do</td>
<td>(?)</td>
<td>16</td>
<td>-15</td>
<td>Feb. 6</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>17</td>
<td>-19</td>
<td>Feb. 6</td>
</tr>
<tr>
<td>12</td>
<td>...do</td>
<td>(?)</td>
<td>9</td>
<td>-19</td>
<td>Feb. 7</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>8</td>
<td>-24</td>
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<td>9</td>
<td>...do</td>
<td>(?)</td>
<td>11</td>
<td>-16</td>
<td>do</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>15</td>
<td>-24</td>
<td>do</td>
</tr>
<tr>
<td>11</td>
<td>...do</td>
<td>(?)</td>
<td>13</td>
<td>-17</td>
<td>do</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td>14</td>
<td>-23</td>
<td>do</td>
</tr>
<tr>
<td>11</td>
<td>Open charcoal pan</td>
<td>(?)</td>
<td>11</td>
<td>-18</td>
<td>do</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>14</td>
<td>-24</td>
<td>do</td>
</tr>
<tr>
<td>12</td>
<td>Charcoal pan</td>
<td>(?)</td>
<td>14</td>
<td>-18</td>
<td>Feb. 8</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td>16</td>
<td>-22</td>
<td>Feb. 8</td>
</tr>
<tr>
<td>9</td>
<td>Stove with chimney</td>
<td>(?)</td>
<td>12</td>
<td>-18</td>
<td>do</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td>19</td>
<td>-23</td>
<td>do</td>
</tr>
</tbody>
</table>

* Compiled by the North Manchurian Plague Prevention Service.
<table>
<thead>
<tr>
<th>No. of observation</th>
<th>Variety of dwelling</th>
<th>Description of building</th>
<th>Approximate size of room</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Length</td>
</tr>
<tr>
<td>25</td>
<td>Native inn</td>
<td>Earth floor; kangas on both sides of room, lighted once every evening.</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>do</td>
<td>Earth floor; kangas on both sides, burned once every evening; paper windows.</td>
<td>16</td>
</tr>
<tr>
<td>26</td>
<td>Native theater</td>
<td>Earth floor; kangas on both sides; paper window on one side.</td>
<td>21</td>
</tr>
<tr>
<td>27</td>
<td>Native theater</td>
<td>Earth floor; kangas on both sides; natural vent.</td>
<td>19</td>
</tr>
<tr>
<td>28</td>
<td>Native inn, room A</td>
<td>Earth floor; kangas on both sides, lighted once every evening; burn straw; 2 paper windows; dome-shaped roof.</td>
<td>19</td>
</tr>
<tr>
<td>29</td>
<td>Native inn, room A</td>
<td>Ear...</td>
<td>19</td>
</tr>
<tr>
<td>30</td>
<td>Native inn, room A</td>
<td>Ear...</td>
<td>19</td>
</tr>
<tr>
<td>31</td>
<td>Native inn, room B</td>
<td>Ear...</td>
<td>19</td>
</tr>
<tr>
<td>32</td>
<td>Native theater</td>
<td>Wooden floor; all windows on top; natural vent.</td>
<td>35</td>
</tr>
<tr>
<td>33</td>
<td>Private house, room A</td>
<td>Earth floor; kangas on both sides; 2 paper windows.</td>
<td>9</td>
</tr>
<tr>
<td>34</td>
<td>Private house, room B</td>
<td>Earth floor; kangas on both sides; 2 paper windows; dome-shaped roof.</td>
<td>19</td>
</tr>
<tr>
<td>35</td>
<td>Carpenter shop</td>
<td>Earth floor; kangas on both sides; 1 window, papered.</td>
<td>19</td>
</tr>
<tr>
<td>36</td>
<td>Cake shop</td>
<td>Earth floor; kangas on both sides; 2 paper windows; kangs on either side.</td>
<td>19</td>
</tr>
<tr>
<td>37</td>
<td>Private house</td>
<td>Earth floor; kangas on both sides; 2 paper windows.</td>
<td>23</td>
</tr>
<tr>
<td>38</td>
<td>Lodging house</td>
<td>Earth floor; 6 paper windows.</td>
<td>16</td>
</tr>
<tr>
<td>39</td>
<td>Eating house</td>
<td>Earth floor; 2 paper windows; kangs on either side.</td>
<td>19</td>
</tr>
<tr>
<td>40</td>
<td>Private house</td>
<td>Earth floor; kangas on both sides; 2 paper windows.</td>
<td>9</td>
</tr>
<tr>
<td>41</td>
<td>Earth floor; kangas on both sides; 2 paper windows.</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>42</td>
<td>Earth floor; kangas on both sides; 1 window.</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>43</td>
<td>Small inn</td>
<td>Earth floor; kangas on both sides; 1 window; brick wall.</td>
<td>9</td>
</tr>
<tr>
<td>44</td>
<td>Private house</td>
<td>Plank floor; kangas on one side; glass windows.</td>
<td>9</td>
</tr>
</tbody>
</table>
### Temperature.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td>1913.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inside of room.</td>
<td>Outside of room.</td>
<td>a. m.</td>
</tr>
<tr>
<td>12</td>
<td>Charcoal pan; no chimney.</td>
<td>(?)</td>
<td>4</td>
<td>-14</td>
<td>Feb. 9</td>
</tr>
<tr>
<td>11</td>
<td>Charcoal stove with chimney.</td>
<td>(?)</td>
<td>2</td>
<td>-23</td>
<td>Feb. 10</td>
</tr>
<tr>
<td>7</td>
<td>Coal stove with chimney.</td>
<td>(?)</td>
<td>8</td>
<td>-15</td>
<td>Feb. 9</td>
</tr>
<tr>
<td>16</td>
<td>Coal stove</td>
<td>(?)</td>
<td>5</td>
<td>-22</td>
<td>Feb. 10</td>
</tr>
<tr>
<td>18</td>
<td>2 big brick stoves, each 6 by 2 by 3 feet.</td>
<td>Yes, many</td>
<td>11</td>
<td>-22</td>
<td>Feb. 9</td>
</tr>
<tr>
<td>15</td>
<td>1 charcoal pan without light; 1 burning.</td>
<td>(?)</td>
<td>10</td>
<td>-10</td>
<td>Feb. 10</td>
</tr>
<tr>
<td>13</td>
<td>Only 1 charcoal pan, unlighted.</td>
<td>(?)</td>
<td>5</td>
<td>-20</td>
<td>Feb. 11</td>
</tr>
<tr>
<td>16</td>
<td>No stove or pan, but kangs on both sides.</td>
<td>(?)</td>
<td>12</td>
<td>-10</td>
<td>Feb. 10</td>
</tr>
<tr>
<td>20</td>
<td>1 charcoal pot, no chimney; burning.</td>
<td>(?)</td>
<td>11</td>
<td>-10</td>
<td>Feb. 10</td>
</tr>
<tr>
<td>11</td>
<td>2 big brick stoves, each 6 by 3 by 3 feet.</td>
<td>Yes</td>
<td>10</td>
<td>-20</td>
<td>Feb. 10</td>
</tr>
<tr>
<td>6</td>
<td>Charcoal pan, lighted</td>
<td>...do...</td>
<td>10</td>
<td>-7</td>
<td>Feb. 11</td>
</tr>
<tr>
<td>11</td>
<td>Charcoal pan, but without fire.</td>
<td>...do...</td>
<td>11</td>
<td>-7</td>
<td>...do...</td>
</tr>
<tr>
<td>5</td>
<td>No stove or charcoal pan whatever.</td>
<td>...do...</td>
<td>0</td>
<td>-9</td>
<td>...do...</td>
</tr>
<tr>
<td>2</td>
<td>Kangs only</td>
<td>...do...</td>
<td>6</td>
<td>-6</td>
<td>...do...</td>
</tr>
<tr>
<td>4</td>
<td>do</td>
<td>...do...</td>
<td>4</td>
<td>-6</td>
<td>...do...</td>
</tr>
<tr>
<td>8</td>
<td>Kangs; charcoal pan with fire.</td>
<td>Reported to have occurred.</td>
<td>10</td>
<td>-7</td>
<td>Feb. 12</td>
</tr>
<tr>
<td>12</td>
<td>Charcoal pan, burning</td>
<td>...do...</td>
<td>5</td>
<td>-19</td>
<td>Feb. 13</td>
</tr>
<tr>
<td>13</td>
<td>Stove with chimney; not lighted.</td>
<td>...do...</td>
<td>12</td>
<td>-7</td>
<td>Feb. 12</td>
</tr>
<tr>
<td>4</td>
<td>Charcoal pan, burning</td>
<td>...do...</td>
<td>8</td>
<td>-6</td>
<td>...do...</td>
</tr>
<tr>
<td>3</td>
<td>do</td>
<td>...do...</td>
<td>13</td>
<td>-7</td>
<td>Feb. 13</td>
</tr>
<tr>
<td>2</td>
<td>Stove with chimney; no fire.</td>
<td>...do...</td>
<td>14</td>
<td>-6</td>
<td>...do...</td>
</tr>
<tr>
<td>2</td>
<td>Stove with chimney; burning.</td>
<td>Yes; over 10 persons.</td>
<td>16</td>
<td>-6</td>
<td>...do...</td>
</tr>
<tr>
<td>6</td>
<td>Chimney stove with fire.</td>
<td>...do...</td>
<td>18</td>
<td>-7</td>
<td>...do...</td>
</tr>
</tbody>
</table>

* About.
* Downstairs, 9° C.; upstairs, 11° C.
<table>
<thead>
<tr>
<th>No. of observation</th>
<th>Variety of dwelling</th>
<th>Description of building</th>
<th>Approximate size of room.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Feet.</td>
</tr>
<tr>
<td>1</td>
<td>Chinese hotel</td>
<td>Plank floor; 1 glass window at the back wall; 1 entire glass window in front wall open to the yard; a kang inside room.</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>Chinese theater</td>
<td>Plank floor; a U-shaped amphitheater facing the stage; glass windows on all sides, but these always closed in cold weather; about 600 seats.</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>Small hawker’s dwelling</td>
<td>Earth floor; opposite kangs; dark; 2 paper windows, each about 2 feet square.</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>Hawker’s shop</td>
<td>Earth floor; 1 kang; 2 skylights; 2 paper windows; part of room used for cooking.</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>Coolie hut</td>
<td>Earth floor; one side of the room used; kaoliang (millet) stalks as a partition; 2 paper windows; 1 kang.</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>Public bath</td>
<td>Cement floor; glass windows in front wall; wooden benches along all sides of wall; bright but air-tight.</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>Chinese inn</td>
<td>Earth floor; 1 kang; wooden partition dividing room into 2 portions; 2 paper windows behind kang.</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>do</td>
<td>Plank floor; paper window in front; 2 kangs opposite each other, 1 was heated.</td>
<td>23</td>
</tr>
<tr>
<td>9</td>
<td>Chinese low-class inn</td>
<td>Earth floor; damp; dark; 3 kangs; paper window; dome-shaped roof.</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>Private residence</td>
<td>Earth floor; dark and damp; 2 kangs; millet-stalk roof, conical shape; paper window; half room used as kitchen.</td>
<td>17</td>
</tr>
<tr>
<td>11</td>
<td>Chinese Inn</td>
<td>Plank floor; paper window; walls lined with paper; have not been torn down since the epidemic, but have had a little alteration since then.</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>Police station</td>
<td>Earth floor; opposite kangs; paper windows; damp and dark; mud wall; millet-stalk roof.</td>
<td>37</td>
</tr>
<tr>
<td>13</td>
<td>Chinese low-class inn</td>
<td>Earth floor; opposite kangs; street wall has 2 paper windows about 3 feet square; 1 window at back wall; paper ceiling; mud wall.</td>
<td>16</td>
</tr>
</tbody>
</table>

\(a\) Compiled by the North Manchurian Plague Prevention Service.

\(b\) Medical officer himself.

\(c\) About.

\(d\) The houses of observations 3, 4, and 5 are situated 2 miles from Changchun, so a second observation was not made.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td>1913.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>a. m. p. m.</td>
</tr>
<tr>
<td>1</td>
<td>1 kang; charcoal pan burning; a stove with chimney extending from next room.</td>
<td>No; built after the epidemic.</td>
<td>7</td>
<td>-3</td>
<td>Feb. 21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>3 stoves with chimney at corners of ground floor.</td>
<td>do</td>
<td>5</td>
<td>-3</td>
<td>Feb. 21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.00</td>
</tr>
<tr>
<td>3</td>
<td>7 1 small charcoal pan kept burning.</td>
<td>Yes</td>
<td>-3</td>
<td>-7</td>
<td>Feb. 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.00</td>
</tr>
<tr>
<td>4</td>
<td>7 Earthen pot burning charcoal.</td>
<td>do</td>
<td>1</td>
<td>-7</td>
<td>Feb. 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.00</td>
</tr>
<tr>
<td>5</td>
<td>13 No stoves; kang lighted once or twice a day for cooking.</td>
<td>do</td>
<td>1</td>
<td>-8</td>
<td>Feb. 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.00</td>
</tr>
<tr>
<td>6</td>
<td>12 Chimney stove burning coal.</td>
<td>Yes; over 30</td>
<td>13</td>
<td>-8</td>
<td>Feb. 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes, but not this room; the infected room is now locked up.</td>
<td>14</td>
<td>-15</td>
<td>Feb. 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>-8</td>
<td>Feb. 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.15</td>
</tr>
<tr>
<td>7</td>
<td>10 Stove with chimney; burning coal.</td>
<td>No</td>
<td>17</td>
<td>-15</td>
<td>Feb. 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.30</td>
</tr>
<tr>
<td>8</td>
<td>11 Stove with chimney; burning.</td>
<td>Yes</td>
<td>16</td>
<td>-8</td>
<td>Feb. 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.00</td>
</tr>
<tr>
<td>9</td>
<td>20 No stove; but a square brick stove for cooking.</td>
<td>Yes; all in house were carried off by plague.</td>
<td>0</td>
<td>-8</td>
<td>Feb. 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>10</td>
<td>6 Stove with chimney extending into it from next room.</td>
<td>Yes</td>
<td>9</td>
<td>-8</td>
<td>Feb. 23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>11</td>
<td>18 No stove whatever; kangs burned twice a day.</td>
<td>Yes; 24 out of 26 persons.</td>
<td>9</td>
<td>-20</td>
<td>Feb. 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.00</td>
</tr>
<tr>
<td>12</td>
<td>29 Only 2 kangs that receive warmth from 2 cooking stoves outside.</td>
<td>Yes</td>
<td>11</td>
<td>-5</td>
<td>Feb. 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.00</td>
</tr>
<tr>
<td>13</td>
<td>31</td>
<td></td>
<td>3</td>
<td>-14</td>
<td>Feb. 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.00</td>
</tr>
<tr>
<td>14</td>
<td>16 Only 2 kangs that receive warmth from 2 cooking stoves outside.</td>
<td>Yes</td>
<td>5</td>
<td>-5</td>
<td>Feb. 24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.80</td>
</tr>
</tbody>
</table>

* All on the kang for warmth.

* Taken at corner of room.
TABLE II.—Temperature observations

<table>
<thead>
<tr>
<th>No. of observation</th>
<th>Variety of dwelling.</th>
<th>Description of building.</th>
<th>Approximate size of room.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Length</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Earth floor; 3 kangs like a □ in shape; opposite walls both were paper-lined; 3 square brick stoves attached to each of the kangs; old and damp.</td>
<td>Feet.</td>
</tr>
<tr>
<td>14</td>
<td>Chinese low-class inn.</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>15</td>
<td>do</td>
<td>Earth floor; opposite kangs; paper window on street side; old and wet.</td>
<td>20</td>
</tr>
<tr>
<td>16</td>
<td>Chinese inn</td>
<td>Earth floor; □-shaped kangs; 1 paper window; 1 brick stove for cooking; kaoliang-stalk roof.</td>
<td>34</td>
</tr>
<tr>
<td>17</td>
<td>Wagonmaker</td>
<td>Earth floor; 3 paper windows on one side; damp; 3 flat cooking stoves; mud wall.</td>
<td>24</td>
</tr>
</tbody>
</table>
### Teague: Spread of Pneumonic Plague

#### in Changchun—Continued.

<table>
<thead>
<tr>
<th>Occupants at the time</th>
<th>How heated</th>
<th>Occurrence or nonoccurrence of plague</th>
<th>Temperature</th>
<th>Date</th>
<th>Time of day taken</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Inside of room.</td>
<td>Outside of room.</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Only the 3 brick stoves for cooking.</td>
<td>Yes; about 3 or 4 persons lost lives here.</td>
<td>5°C.</td>
<td>−9°C.</td>
<td>1913</td>
</tr>
<tr>
<td>24</td>
<td>An open charcoal pan</td>
<td></td>
<td>3°C.</td>
<td>−14°C.</td>
<td>Feb. 24</td>
</tr>
<tr>
<td>5</td>
<td>1 charcoal pan burning</td>
<td></td>
<td>5°C.</td>
<td>−5°C.</td>
<td>Feb. 24</td>
</tr>
<tr>
<td>20</td>
<td>Only the 3 stoves attached to the kang</td>
<td></td>
<td>8°C.</td>
<td>−6°C.</td>
<td>Feb. 25</td>
</tr>
<tr>
<td>25</td>
<td>burned when cooking</td>
<td></td>
<td>6°C.</td>
<td>−13°C.</td>
<td>Feb. 26</td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td>9°C.</td>
<td>−13°C.</td>
<td>Feb. 26</td>
</tr>
</tbody>
</table>
It is seen from Table II that plague cases were known to have occurred in all but 3 of the houses investigated in Changchun and that temperatures of 6° C. or less were recorded in 11 of the 17 houses investigated.

Consequently, I am still of the opinion that atmospheric temperature is an important factor in determining the spread or failure to spread of pneumonic plague.
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EXPERIMENTAL ENTAMOEIC DYSENTERY

By Ernest Linwood Walker

with the cooperation of

Andrew Watson Sellards

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

One plate

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Part I. Introduction.
Part II. Feeding experiments with cultures of amóbæ.
Part III. Feeding experiments with Entamoeba coli.
Part IV. Feeding experiments with "Entamoeba tetrægæna" and Entamoeba histolytica.
Part V. Application of the results to the diagnosis, treatment, and prophylaxis of entamœbic dysentery.
Part VI. Summary and conclusions.

PART I. INTRODUCTION

By Ernest Linwood Walker

Entamœbic dysentery is, with the possible exception of malaria, the most widespread of the endemic tropical diseases, and it has been said to constitute one of the chief obstacles to the white man colonizing in tropical countries. Musgrave wrote in 1904 that amœbic dysentery caused more than 50 per cent of the invalidism of public servants in the Philippine Islands. Fortunately, owing to the great improvement in san-

2 Joint author on parts II and III.
itary conditions, the percentage of morbidity due to this cause is not so high at the present time, at least in the city of Manila. Gauducheau (1912), at the last biennial congress of the Far Eastern Association of Tropical Medicine, stated that amoebiasis, under the form of dysentery and abscess of the liver, causes nearly half of the deaths of Europeans at Tonkin, Indo China. While it is impossible to give accurate statistics, owing to the fact that the different types of dysentery are not usually separated in the reports of infectious diseases, it is probable that, except where modified by sanitation, this disease holds a more or less similar position in the morbidity and mortality statistics of other tropical countries.

Notwithstanding that amoebae have been associated with a certain type of dysentery since 1875, the etiologic relation of these organisms to this disease has been the most controverted question in tropical medicine. Between the extreme views that amoebae combat disease and are the true guardians of man’s health (Cassagrandi and Barbagallo, 1895), on the one hand; and, on the other hand, that all amoebae are or may become pathogenic (Musgrave and Clegg, 1904), every intermediate opinion of the etiologic relation of ameboid organisms to endemic tropical dysentery has been expressed. However, in recent years it is becoming more and more generally accepted that amoebae do play a rôle in the production of this type of dysentery; yet dissenters from this view still appear from time to time (Tanaka, 1910, and Duncan, 1912), and it has not been definitely proved whether this rôle is primary or secondary.

Our knowledge of the specific amoeba concerned in the production of this disease is equally uncertain. Eighteen species representing 4 genera of ameboid organisms have been described as parasites in the intestinal tract of man. Of these at least 5 have been definitely stated to be more or less pathogenic, and there exists no conclusive evidence to exclude the other 11 from the list of pathogenic species. Moreover, the observations and


experiments of several investigators have led them to believe that amoebae from water and other nonparasitic sources are capable, when taken into the intestine of man and other animals, of becoming facultative parasites, and in certain cases at least of causing dysenteric symptoms and ulcerative lesions in their host.

In a recent paper (Walker, 1911) I have attempted to establish the morphological distinction between the nonparasitic and the parasitic ameboid organisms and to differentiate the non-pathogenic from the pathogenic species. The conclusions reached in this morphological study include the following which bear directly upon this experimental investigation.

1. The ameboid organisms found in the Manila water supply and other nonparasitic sources belong to the genus *Amoeba* Ehrenberg.

2. The ameboid organisms cultivable from the intestinal tract, both of healthy persons and of cases of amœbic dysentery, also belong to the genus *Amoeba*.

3. The species of the genus *Amoeba* are not parasitic in the intestinal tract of man. When obtained in cultures of stools, they are probably derived from cysts of amœbæ that have been ingested with water or food and have passed unchanged through the intestine.

4. The amœboid organisms parasitic in the intestinal tract of man belong to a distinct genus, *Entamoeba* Casagrandi and Barbagallo.

5. The entamœbæ are strict or obligatory parasites, and are incapable of multiplying outside of the body of their host. They cannot be cultivated on Musgrave and Clegg's medium.

6. One nonpathogenic species, *Entamoeba coli* Schaudinn, parasitic in the intestinal tract of man, which includes *Entamoeba nipponica* Koidzumi, and which develops cysts containing 8 (or more) nuclei, is recognized.

7. One presumably pathogenic species, *Entamoeba histolytica* Schaudinn, which includes "*Entamoeba tetragena*" Viereck and "*Entamoeba minuta*" Elmassian and which develops cysts containing 4 nuclei, is recognized.

*Kartulis* (1891), Celli and Fiocca (1894), Musgrave and Clegg (1904), Noc (1909), Williams and Gurley (1909), Greig and Wells (1911), Gauducheau (1912), and Chatton and Lalung-Bonnaire (1912).

Hartmann in a recent paper (1912) has concluded that the "nipponica" type of entamœba represents degenerative changes in either *Entamoeba coli* or *Entamoeba histolytica* ("*E. tetragena*"). A more extended observation leads me to believe that this conclusion is correct.
A number of other authors, notably Schaudinn (1903), Craig (1905), Vedder (1906), Werner (1908), Hartmann (1908), Whitmore (1911), and Darling (1912), have each arrived at some of these conclusions.

The investigation which forms the subject of this paper has been undertaken to test experimentally the validity of these conclusions and to obtain such other information as may be possible concerning the etiology and epidemiology of entamoebic dysentery.

Two general methods of experimentation are available for determining the specific identity of microorganisms and their etiologic relation to a disease; namely, (a) the study of their immunity reactions and (b) the experimental infection of animals.

The application of immunity reactions to the determination of species of amœbæ and of their relations to entamoebic dysentery has been attempted by Sellards (1911). So far as this method was found applicable, it confirmed the conclusions of the morphological study. However, this method of experimentation was found to have certain serious limitations as applied to these organisms. The immunity reactions to protozoa have in general been found to be of a low grade, and those to amœboid organisms present no exception to the general rule. A second and more serious obstacle to the application of these reactions to the problem under consideration is the fact that it has been found impossible to cultivate the parasitic entamoebæ on artificial media or even to keep them alive outside of the body of their host long enough to test the immunity reactions against them. It is further to be noted that immunity reactions can at most only supply indirect evidence of the specific identity of a microorganism and its etiologic relation to a disease.

The experimental infection of animals with amœboid organisms has already been employed very extensively in attempts to prove their etiologic relation to dysentery. Dysentery has been produced by a number of investigators in a certain pro-

*Loesch (1875), Hlava (1877), Kartulis (1889), Kovacs (1892), Quincke and Roos (1893), Kreuse and Pasquale (1893), Zancoral (1893), Roos (1894), Gasser (1895), Fijardo (1896), Strong and Musgrave (1900), Harris (1901), Jaeger (1901), Ucke (1902), Huber (1903), Schaudinn (1903), Craig (1905), Hartmann (1908), Werner (1908), Darling (1912), Fantham (1912), Franchini (1912), Hartmann (1912), Wellman (1912), Wenyon (1912).
portion of the experimental animals (cat, dog, monkey) by feeding or injecting rectally dysenteric stools or so-called liver-abscess pus containing entamoebæ. In some cases these experiments have been controlled by feeding to another animal cultures of all of the bacteria that could be grown from the infectious material on ordinary culture media. Dysentery has also been produced experimentally in animals by rectal,7 and in one case by intravenous,8 injections of pus from liver abscesses containing entamoebæ, but free from bacteria cultivable on ordinary culture media. Finally, several investigators9 state that they have produced a disease in animals and, in one case, in man having the clinical symptoms of entamoebic dysentery, with entamoebæ in the stools, and exhibiting the characteristic lesions in the intestine at necropsy, by feeding or injecting rectally "pure mixed cultures" of amœbæ and nonpathogenic bacteria, which had been isolated not only from stools of dysenteric patients, but from water and other nonparasitic sources.

Certain of these experimental infections furnish considerable support to the belief in the etiologic relationship of amœboid organisms to endemic tropical dysentery, but most of the experiments are open to criticism. The infection experiments with dysenteric stools, uncontrolled, are of little more value than clinical observations; since not only the entamoebæ, but all of the other microœrganisms contained in the faeces were fed or injected in these experiments. The infections with dysenteric stools, controlled by feeding other animals all of the bacteria cultivable from the stools on ordinary media, and with liver-abscess pus free from bacteria cultivable on ordinary culture media, are more convincing; but they would not exclude bacteria cultivable on special media—or under anærobic conditions—or the filterable viruses. The work of those authors who obtained dysentery in experimental animals following the feeding of "pure mixed cultures" of amœbæ and nonpathogenic bacteria would appear to obviate all criticism. But these and many of the other experiments are open to a more serious criticism; namely, that the species of amœboid organism fed to, and recovered from, the experimental animal have not been accurately determined. The truth of this statement will be evident to

1 Kruse and Pasquale (1903), Strong and Musgrave (1900).
2 Gauducheau (1906).
3 Kartulis (1891), Musgrave and Clegg (1904), Williams and Gurley (1909).
anyone who takes the trouble to examine the indefinite descriptions of the amœboid organisms employed by these investigators in their experiments. In many instances the author has made no attempt to determine the species with which he has experimented, simply stating that it was an amœba found in a dysenteric stool or cultivated from such a source and that the amœba recovered from the experimental animal resembled, or was indistinguishable from, the amœboid organism fed to the animal. Such experiments supply no information as to the species of amœboid organism associated with the experimental dysentery; it does not prove the etiologic relationship of the associated amœboid organism to the experimental dysentery; and it vitiates every conclusion drawn by the investigator from his experiments.

The use of the lower animals for infection experiments is at best only a makeshift, and the application of the results to man is based on the assumption that the microœrganism in question behaves in the experimental animal as it would in the human body, an assumption that is not always borne out by the facts. There may exist a more specific objection to the employment of the lower animals for experiments on entamœbic dysentery. Although a number of authors claim to have been successful in infecting animals and in producing dysentery with different species of amœba and entamœba, I have not been able, in a limited number of experiments, to parasitize animals with the pathogenic entamœba. While these experiments have not been numerous enough to exclude the possibility of infecting animals, they at least indicate that the lower animals are less readily parasitized than man. Every species of animal appears to be parasitized with some species of entamœba, and dysentery is not uncommon in animals kept in captivity. It is probable that these facts, together with carelessness in identifying species of amœboid organisms, account for some of these apparently successful infection experiments on animals with amœbæ and entamœbæ.

The resort to human experimentation is usually not to be recommended, but in certain infectious diseases of wide geographical distribution and prevalence, or which give rise to devastating epidemics, and of which an accurate knowledge of the etiology or transmission cannot otherwise be obtained, experimental infections of man have been resorted to, and the knowledge thus obtained has enabled medical science to control
these diseases to a remarkable extent. The successful transmission of malaria by the anopheles mosquito from man to man (Sambon and Low, 1902) and the experimental infection of man with yellow fever by the stegomyia mosquito (Reed, Carroll, and Agramonte, 1901) are two notable examples of the solution of obscure etiologic and epidemiologic problems through human experimentation and of the vast benefit to mankind that has resulted from such experiments.

Entamöebic dysentery, while it does not cause the spectacular epidemics of some other infectious diseases, is of universal distribution in the Tropics and subtropics, and every year causes a large amount of sickness and death. The study of this disease by clinical and pathological methods and by experiments upon animals has led to no definite agreement upon the etiology or the epidemiology of this disease. Therefore, an attempt has been made to determine once for all the specific amoeboid organisms, if any, concerned in the production of endemic tropical dysentery by a series of carefully conducted experiments upon volunteers.

These experiments have been carried on within the endemic region at Manila, where material has been available and where the conditions for this investigation have been found to be as favorable as possible. Men at Bilibid Prison, who had long sentences to serve, who had been under observation for years in the prison, and who eat cooked food and drink distilled water exclusively, have been available for the experiments. Moreover, these men had been examined for intestinal parasites, including entamöebae, on admission to the prison, and those who were infected had received treatment. Consequently, the men have been under complete control, and the existence or possibility of natural infections with amoeboid organisms have been reduced to a minimum.

All of the men to whom pathogenic amoeboid organisms have been fed were volunteers. The nature of the experiment and the possibility of the development of dysentery as a result of the experiment were carefully explained to each of these men in his native dialect, and each man signed an agreement to the conditions of the experiment written in the native language. No promise of immunity to prison discipline, or commutation of sentence, and no financial inducements were employed to influence a man to volunteer, in accord with the authority under which this work was carried on.
These men were all Filipinos. There appears to exist no definite evidence of a racial immunity of the Filipino to entamœbic dysentery. The disease is endemic in the Philippine Islands, and many Filipinos suffer from it annually. Even if it be granted that a certain degree of immunity does exist, it is believed that the essential results of these experimental infections of Filipinos are applicable to all races of man.

The condition of the men selected for the experiments, with reference to previous attacks of dysentery and to present infections with amœboid organisms, was determined by the clinical history and physical examination of the men and by cultural and microscopic examination of their stools. It was found necessary to include some men who gave a definite history of a mucous and bloody dysentery at some earlier period of their lives. While there is no evidence of the existence of an appreciable immunity following an attack of entamœbic dysentery, the possibility of such a condition was controlled by feeding the same material to other men who had negative dysenteric histories. It is interesting to note in this connection that none of the men who had a positive dysenteric history failed to become parasitized with the pathogenic entamœba. The clinical histories were supplemented in most cases by physical examinations. In some of the later experiments, in which men were used who had negative dysenteric histories, physical examinations were not made. In the majority of the cases, records of one or more examinations of the stools of the men for entamœbæ were available in the hospital records. Cultures on Musgrave and Clegg's medium and microscopic examinations of the stools of all of the men, usually before and after a purgative, were made for amœboid organisms before using them in these feeding experiments. Those men who showed amœbæ after either test, with certain intentional exceptions, were excluded.

In order to cover all of the genera and species of amœboid organisms which were established by the morphological study (Walker, 1911) and which might be concerned in the etiology of entamœbic dysentery, a considerable variety of material has been fed in these experiments. This included all of the species of Amœba that could be cultivated from the Manila water supply, from a variety of other nonparasitic sources both within and outside of the Tropics, from the stools of healthy persons, and from the stools of cases of entamœbic dysentery; Entamœba coli from healthy persons and of persons suffering from diseases other than
dysentery; cysts of "Entamoeba tetragena" from "convalescent" and "contract" carriers;\(^9\) and motile Entamoeba histolytica from acute cases of entamoebic dysentery and from an entamoebic liver abscess.

Each culture of amöba consisted of a single species of amöba, but associated with it was the mixed bacterial growth from the source from which it was cultivated. No attempt was made to isolate the amöbæ in "pure mixed cultures" with one species of bacterium; first, on account of the difficulty of obtaining really "pure mixed cultures"; secondly, since it was impossible to obtain the entamöbæ in "pure mixed cultures," it was considered advisable for the sake of uniformity to feed all of the amœboid organisms with mixed bacterial cultures; and, thirdly, because it was found to be unnecessary.

In the feeding experiments, the growth of amöbæ scraped from the surface of the culture medium and the material containing the entamöbæ, either alone or mixed with powdered starch or magnesium oxide, was inclosed in gelatine capsules and ingested by the men. The powdered starch served to absorb the excess of moisture that would tend to dissolve the gelatine capsule and to facilitate the ingestion of the material. The magnesium oxide served the same purposes as the starch and, in addition, to neutralize the acidity of the contents of the stomach of the experimental man. In every case the capsules of infective material were personally administered.

Following the ingestion of the infectious material, the stools of the men were saved daily until parasitization, or the failure to parasitize, with the specific amœboid organisms was definitely determined, and thereafter at frequent intervals. These stools were examined culturally and microscopically for amœboid organisms, and the species of such organisms, when found, was carefully determined. It has not been considered sufficient to determine the species of amœboid organism fed to, and recovered from, the experimental man, but necessary to follow each case carefully to guard against the possibility of double infection, resulting from a previous latent infection of the man, from impure material used in the feeding experiments or from a sub-

\(^9\) By "convalescent" carrier is meant a person who has suffered from an attack of entamoebic dysentery and has recovered but who is still carrying the specific entamoeba; in contrast, the "contact" carrier is a person who, without having had entamoebic dysentery, is carrying the pathogenic entamoeba.
sequent natural infection. The clinical symptoms of the parasitized men have been carefully noted, and whenever conditions appeared to warrant a physical examination of them has been made. The men who developed dysentery have been promptly treated after the manifestation of typical clinical symptoms, and all have been cured.

Experimental infections with material such as has been described are subject to certain limitations and sources of error, as are all experiments made with other than pure cultures. Just what these limitations and sources of error are in the present case and how far they can be avoided or controlled is worth considering at this point. In the first place it is to be noted that the presence of other microorganisms in the material fed can in no way interfere with the determination of the parasitism for man of the different species of Amoeba and Entamoeba, since they can be identified by their morphological characters in the microscopic examination of the stools. Secondly, it is evident that in feeding experiments with ameboid organisms which are not followed by the development of dysentery, the presence of other microorganisms in the infectious material will not complicate the results. Therefore, it will be possible to eliminate the nonpathogenic species with certainty. Finally, only in infection experiments followed by the development of dysentery will the presence of other organisms in the infectious material be a source of error. In such cases the experiments uncontrolled would not prove the specific ameboid organism to be the primary etiologic agent in the production of the dysentery.

Feeding experiments that were followed by dysenteric symptoms might be controlled by feeding other men all of the bacteria that could be cultivated from the infectious material on ordinary and special media and under aerobic and anaerobic conditions. This would eliminate everything but noncultivable organisms, such as the filterable viruses. However, controls of these experiments were available which made it unnecessary to undertake the work involved in the bacterial cultures and which were considered to be more efficient. It was found that in feeding material containing the presumably pathogenic entamoebae not all of the men become parasitized with the entamoebae. Such individuals were equivalent to controls that had been fed not only all of the cultivable but also any noncultivable microorganisms that the infectious material might contain, and they have been reserved as controls of the men fed at the same time with
the same material, but who did become parasitized with the entamaebæ. Furthermore, it has been found that not all of the individuals parasitized with the presumably pathogenic entamaebæ developed dysentery; that is, some of them become "contact carriers." A number of feeding experiments have been made with entamaebæ from such "carriers" who had not, and have not subsequently, developed dysentery. In several cases the entamaebæ have been passed successively through two such "carriers" to a third man, in some of whom dysentery was produced. By these controls the attempt has been made to eliminate the possible etiologic action of the bacteria or other microorganisms associated with the pathogenic entamaebæ.

This large series of experimental infections has been conducted to a successful finish with a minimum of discomfort and without danger to the men. By these experiments it is believed that the specific entamaeba concerned in the etiology of endemic tropical dysentery has been definitely determined, the endemiology of this disease elucidated, and information obtained of the greatest value for the diagnosis, treatment, and prophylaxis of this important tropical disease.

PART II. FEEDING EXPERIMENTS WITH CULTURES OF AMOEBAE

By Ernest Linwood Walker and Andrew Watson Sellards

This series of experiments was undertaken to obtain cumulative evidence refuting the conclusions of several authors that amoebe cultivated from water and other nonparasitic sources and from dysenteric stools are capable of living parasitically and, in certain cases, of producing dysenteric symptoms and ulcerative lesions in the intestine of man and other animals.

Kartulis (1891) reports the production of dysentery in 1 cat by rectal injections of pure cultures of amoebe, isolated from a liver abscess, and in 2 cats with impure cultures of amoebe, isolated from a dysenteric stool, grown on a straw-infusion medium. These experiments were controlled by feeding and injection experiments with the bacteria isolated from dysenteric stools, which were followed by negative results.

Celli and Fiocca (1894) cultivated 6 species of amoebe from the intestine of man, which they identified with species which they had cultivated from water and soil. No experiments were made to test the pathogenicity of the species.

Musgrave and Clegg (1904) state that they produced dysentery in monkeys, and in one case in man, having the symptoms and lesions of entamaebic dysentery, with amoebe in the stools, by feeding, or injecting subcutaneously, "pure mixed cultures" of amoebe and harmless bacteria, which had been cultivated not only from dysenteric stools but also from
washings from lettuce and from the Manila water supply. These authors maintained that all amœbæ are, or may become, pathogenic.

One of us (Walker, 1908) obtained in culture on Musgrave and Clegg's medium an amœba, "Amœba hominis," from the intestinal tract of a woman at necropsy which was at that time believed to be a parasitic species. No tests were made of the pathogenicity of this amœba.

Noc (1909) considers the amœbæ found in dysenteric stools, in the sections of dysenteric intestines, and in liver abscesses to be identical with an amœba common in the drinking water in Indo China and which he has cultivated on artificial media. Animal experiments with cultures from both the water and the dysenteric stools gave negative results.

Williams and Gurley (1910) produced attacks of typical bloody dysentery in a kitten by feeding Amœba limax cultivated from potato parings, while a kitten fed the bacteria associated with the amœbæ in culture showed no symptoms.

Greig and Wells (1911) believe that the amœba found in the stools of dysenteric patients and in the pus from liver abscesses in Bombay is not Entamœba histolytica Schaudinn, but the same species that is found in Cochin China. This same amœba, these authors state, is found in the conduit water of Bombay.

Gauducheau (1912) is of the opinion that the so-called limax amœbæ are in size and structure like Amœba phagocytoïdes, cultivated by him in 1907, and found in the intestine of dysenteric cases and in water. These amœbæ, he says, are capable of multiplying in the intestine of animals, and there can be no doubt of their parasitic nature.

Chatton and Lalung-Bonnaire (1913) describe an amœba (Amœba limax) which they cultivated from a case of chronic intermittent diarrhœa and which they believe to be the same as the amœboid organism which they found microscopically in the stools of the patient.

One of us (Walker, 1911) has already determined that morphologically the cultivable amœbæ belong to the genus Amœba Ehrenberg. Species of this genus are characterized morphologically by the more or less central position of the nucleus in the resting organism, by the arrangement of the greater part of the chromatin of the nucleus in a central karyosome, by the presence (with rare exceptions) of a contractile vacuole, by the development of mononuclear cysts; and, biologically, by the absence of schizogony in the encysted stage, by their ability to live non-parasitically, and to multiply on artificial culture media (Plate I, figs. 1 and 2).

Twenty feeding experiments have been made with cultures from 11 different sources, representing 13 strains and 8 species of Amœba, as detailed in Table I.
Table I.—Strains of amoebae used in feeding experiments.

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<td>1</td>
<td>Water supply</td>
<td>Manila, P. I</td>
<td>A</td>
<td>2</td>
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<td>2</td>
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<td>3</td>
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<td>B</td>
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<td>4</td>
<td>Clover</td>
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<td>5</td>
<td>Hay</td>
<td>Illinois, U. S. A</td>
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<td>6</td>
<td>Algae</td>
<td>Kansas, U. S. A</td>
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<td>1</td>
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<td>7</td>
<td>Normal stool</td>
<td>Manila, P. I</td>
<td>F</td>
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<td>8</td>
<td>do</td>
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<td>9</td>
<td>do</td>
<td>do</td>
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<td>1</td>
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<td>10</td>
<td>Diarrhoeal stool</td>
<td>Kansas, U. S. A</td>
<td>G</td>
<td>3</td>
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<tr>
<td>11</td>
<td>Dysenteric stool</td>
<td>Manila, P. I</td>
<td>G</td>
<td>1</td>
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<tr>
<td>12</td>
<td>do</td>
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To these 8 distinct species of *Amoeba* no names have been given, since to do so in the present chaotic state of the nomenclature of the free-living amoebae would only add to the confusion. Species *A, B, F, G, and H* are illustrated in the plates of an earlier paper by one of us (Walker, 1911). Among these 13 strains of amoebae are represented all of the different species that could be cultivated from the Manila water supply, from normal stools, and from dysenteric stools in Manila. A larger series of experiments with cultures from dysenteric stools was not considered necessary, in view of the behavior of all of the amoebae in the intestinal tract of man.

The amoebae fed in these experiments were cultivated on a medium which had the following composition:

- Agar-agar: 2.5 grams
- Sodium chloride: 0.05 gram
- Liebig’s beef extract: 0.05 gram
- Normal sodium hydroxide: 2 cc.
- Distilled water: 100 cc.

Without clarifying, it was sterilized at 7 kilograms’ pressure per square centimeter for about three-quarters of an hour. After the sterilization, its reaction was neutral to phenolphthalein. This medium, which is essentially that of Musgrave and Clegg (1904), has proved satisfactory, all of the amoebae growing well upon it.

The cultures of amoebae used in the feeding experiments were pure with reference to the protozoön; that is, they consisted of a single species of *Amoeba*, but they were cultivated with the
mixed growth of bacteria with which they had been isolated. The presence of bacteria growing with the amœbebæ in mixed cultures would be objectionable only if the experiments were followed by the development of dysentery, and since all of the cultures of amœbebæ were confirmed to be neither pathogenic nor parasitic the objection has no foundation.

The 20 experiments were made on 10 different men, some of them being used for several successive experiments. When the ingested organism failed to parasitize the man, he was used, after the lapse of a sufficient interval and after repeated negative cultural and microscopic examinations of his stools, to repeat the experiment with the same or a different amœboid organism. Since the species of amœba ingested could in every case be identified microscopically, the use of the man subsequently for feeding another species of amœboid organism in no way interfered with the continued observation of him with reference to the former experiment. Such men were in certain respects more desirable for subsequent experiments than new men, for they had been under more immediate observation and the possibility of previous amœbic infection had been more certainly excluded by the large number of cultural and microscopic examinations that had been made of their stools.

Some of the men used in these experiments gave a history of one or more attacks of dysentery at some earlier periods of their lives. Their present condition with reference to dysentery or amœbic infection was determined by physical examination and by cultural and microscopic examination of their stools. The microscopic examinations were made both before and after the administration of a saline purgative. With one exception, men showing any evidence of amœbic infection by either method were excluded from the experiments. One man was already infected with Entamoeba coli when employed for a feeding experiment with a culture of amœbe. Use was made of him to control our differentiation of the amœbebæ from the entamoebæ by cultural and microscopic examinations.

The amœbebæ were fed for the most part in the encysted condition, since this is the resistant stage and seemed most likely to be capable of infecting the men. In a few cases they were fed in the amœboid stage. Some precautions were necessary in the latter case to eliminate the presence of the encysted amœbebæ. This was accomplished by making several successive transplants of the cultures to fresh medium at from fifteen to eighteen hours' interval; for it is only in old cultures that the amœbebæ become encysted. In feeding experiments with encysted amœbebæ, a trans-
plant to fresh culture medium was made in every case to test the viability of the cysts.

The growth scraped from several agar-slant cultures or Petri-plate cultures, sometimes alone, but more often mixed with magnesium oxide to absorb the excess of moisture that would dissolve the gelatine capsule and to neutralize the acidity of the contents of the stomach that might prevent infection, was inclosed in a gelatine capsule and ingested by a man. The motile amœbæ were ingested as an emulsion in water with magnesium oxide. It might be argued that the action of the gastric juices was necessary to dissolve the cysts of the amœbæ, and, consequently, the use of magnesium oxide to neutralize the gastric acidity in feeding the encysted amœbæ would tend to prevent infection. This argument would not be applicable, however, to the cultivable amœbæ. The cysts of these amœbæ are dissolved or ruptured from within whenever they are placed on fresh culture medium or in any medium suitable for growth, and, moreover, acids are extremely antagonistic to the growth of these amœbæ. For these reasons there is no objection to the neutralization of the acidity of the stomach contents of the men used in these experiments.

Following the ingestion of cultures of amœbæ, the stools of the men were examined daily, both culturally and microscopically, for amœboid organisms until the parasitization or non-parasitization with the specific amœba was determined, and thereafter at frequent intervals.

A complete protocol is given of each man in order to put on record the details of these experiments.

Experiment I.—Man 3, aged 31 years, had been under observation in the prison for six years. He gave a history of one attack of dysentery of one month's duration sixteen years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 2 Petri-plate cultures of Amœba IA, mixed with magnesium oxide. This amœba was one of the 2 species isolated in culture from the Manila water supply. The culture fed was an old one containing only encysted amœbæ. Transplant cultures made on fresh culture media to test the viability of the cysts gave a luxuriant growth of Amœba A. This man has been under observation two years and seven months since the experiment began. Cultures of his stools on Musgrave and Clegg's medium and microscopic examinations of his stools for amœboid organisms have been constantly negative. No dysenteric symptoms have developed.

Experiment II.—Man 4, aged 34 years, had been under observation in the prison one year and six months. He had a negative dysenteric history,
and had not been used previously for feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amoeboid organisms were negative. He ingested the growth on 2 Petri-plate cultures of Amoeba 2A, isolated in culture from the Manila water supply, mixed with magnesium oxide. These cultures contained both motile and encysted amœbæ. Transplant cultures made to test the viability of the organisms showed a luxuriant growth of Amoeba A. This man was under observation eleven and one-fourth months after the experiment began. Following the feeding, cultures of this man's stools on Musgrave and Clegg's medium and microscopic examination of his stools for amoeboid organisms were constantly negative. No symptoms of dysentery have developed.

Experiment III.—Man 3, aged 31 years, had been under observation in the prison for six years. He gave a history of one attack of dysentery of one month's duration sixteen years ago. He had been previously used for another feeding experiment with negative results (see experiment I). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœbæ were negative. He ingested the growth on 4 Petri-plate cultures of Amoeba 1A, isolated from the Manila water supply, mixed with magnesium oxide. The cultures ingested by this man were old ones containing only encysted amœbæ. Transplant cultures to test the viability of the cysts gave a luxuriant growth of Amoeba A. This man has been under observation two years and six months since the experiment began. Cultures of his stools on Musgrave and Clegg's medium and repeated microscopic examinations of his stools for amoeboid organisms have been negative. No symptoms of dysentery have developed.

Experiment IV.—Man 4, aged 34 years, had been under observation in the prison one year and six months. He had a negative dysenteric history. He had been used previously for another feeding experiment with negative results (see experiment II). Physical examination of the abdomen and cultural and microscopic examinations of his stools for amoeboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of Amoeba 2A, isolated from the Manila water supply, mixed with magnesium oxide. The cultures ingested contained both motile and encysted amœbæ. This man has been under observation eleven months and seven days since the experiment began, during which time Amoeba A has never been found in his stools nor have dysenteric symptoms developed.

Experiment V.—Man 2, aged 40 years, had been under observation in the prison five years and one month. He had a negative dysenteric history. He had been used for another experiment sixty-nine days previously with negative results (see experiment VII). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amoeboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of Amoeba 2A, isolated from the Manila water supply, mixed with magnesium oxide. The cultures fed to this man contained encysted amœbæ only. Transplant cultures to test the viability of the cysts showed an abundant growth of Amoeba A. This man has been under observation two years and four and one-half months since this experiment began. Cultures and microscopic examinations of his stools for amoeboid organisms have been constantly negative, and no symptoms of dysentery have developed.
Experiment VI.—Man 7, aged 30 years, had been under observation in the prison for five years and nine months. He gave a history of 3 attacks of dysentery, each of one week’s duration, six years ago. He had been used for 3 previous feeding experiments (experiments XII, XIII, and XVI), the last of which was ninety-two days previously, and all of which were followed by negative results. Physical examination of his abdomen and microscopic and cultural examinations of his stools for ameboid organisms were negative. He ingested the growth on 7 Petri-plate cultures of Amoeba 5B, mixed with magnesium oxide. Amoeba B was the second of 2 species which had been isolated in culture from the Manila water supply. The cultures ingested by this man consisted exclusively of encysted amœbæ. Transplant cultures made to test the viability of the cysts showed an abundant growth of amœbæ. Amoeba B was recovered in cultures from the stools of this man from the first to the fifth day after ingestion but never subsequently. Microscopic examination of his stools for amœboid organisms have been constantly negative. This man has been under observation two years and one and one-half months since this experiment began. No symptoms of dysentery have developed.

Experiment VII.—Man 2, aged 40 years, had been under observation in the prison four years and ten months. He had a negative dysenteric history, and had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 1 agar-slain culture of Amoeba 4C, mixed with magnesium oxide. Amoeba C was isolated from clover grown in the United States. The culture ingested was an old one containing only encysted amœbæ. A transplant culture made from this culture to test the viability of the cysts gave an abundant growth of Amoeba C. Following the feeding, cultures and microscopic examinations of his stools for amœboid organisms were constantly negative, and no dysenteric symptoms developed. He was under observation one hundred twenty-six days when he was used for a feeding experiment with Entamoeba histolytica (part IV) and developed dysentery on the twentieth day with Entamoeba histolytica in his stools. Altogether, this man has been under observation two years and seven months, during which time Amoeba C has never been found culturally or microscopically in his stools.

Experiment VIII.—Man 8, aged 57 years, had been under observation in the prison for seven years and eight months. He had a history of 1 attack of dysentery of one month’s duration eight years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organism were negative. He ingested the growth on 2 Petri-plate cultures of Amoeba 5D, mixed with magnesium oxide. Amoeba D had been isolated in culture from an infusion of hay coming from Illinois, United States. The cultures fed to this man contained encysted amœbe only. Transplant cultures made to test the viability of the cysts showed an abundant growth of Amoeba D. Following the ingestion, cultures of the stools of this man showed a growth of Amoeba D on the first day after feeding, but never subsequently. Microscopic examinations of his stools have been constantly negative. This man has been under observation two years and five and one-half months since this experiment began, but has never shown any symptoms of dysentery.
Experiment IX.—Man 3, aged 31 years, had been under observation in the prison for six years and two months. He gave a history of dysentery of one month's duration sixteen years ago. He had been used for 3 previous feeding experiments (experiments I, III, and XV) the last of which was thirty-four days previously, and all of which were followed by negative results. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 4 agar-slant cultures of Amœba 5D, isolated in culture from hay, mixed with magnesium oxide. The cultures ingested by this man contained motile forms of the amoeba exclusively. Amœba D was recovered in cultures from the stools of this man on the second and third days after feeding, but never subsequently. Microscopic examinations of his stools for amœboid organisms have been constantly negative. This man has been under observation two years and four and one-half months since the experiment began. No symptoms of dysentery have developed.

Experiment X.—Man 9, aged 27 years, has been under observation in the prison for five years and three months. He had a negative dysenteric history, and had not been used for previous feeding experiments. Physical examination of his abdomen was negative. Microscopic examination of his stools showed a few Entamoeba coli. Cultural examinations of his stools for amoebae were negative. He ingested the growth on 2 agar-slant cultures of Amœba 6E, mixed with magnesium oxide. Amœba E had been isolated in culture from fresh-water algae obtained from Kansas, United States. The cultures fed to this man contained encysted amoebae only. Transplant cultures made to test the viability of the cysts showed a good growth of Amœba E. Following the feeding, cultures of this man's stools showed Amœba E on the first and second days after feeding, but never subsequently. Microscopic examinations of his stools have constantly showed a few Entamoeba coli. This man was under observation three months. No symptoms of dysentery developed.

Experiment X demonstrates the morphological and biological differences between the cultivable amœbæ and the parasitic entamœbæ. Amœba E was recovered in culture, but could not be found microscopically in the stools of this man; on the other hand, Entamoeba coli was identified microscopically, both before and after feeding Amœba E, but could not be cultivated.

Experiment XI.—Man 7, aged 30 years, had been under observation in the prison for four years and eight months. He gave a history of 3 attacks of dysentery, each of one week's duration, six years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amoeboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of Amœba 8A, mixed with magnesium oxide. This strain of Amœba A, which is the same species as the amoeba common in the Manila water supply, had been isolated in culture from a stool of a healthy man. The cultures fed in this experiment contained only encysted amoebæ. Transplant cultures to test the viability of these cysts showed a luxuriant growth of Amœba A. Following the feeding, cultures and microscopic examinations of the stools of this man for amœboid organisms have been
constantly negative. This man was under observation for forty-three days after the experiment began, when he was used for another feeding experiment (experiment XVI). Altogether, he has been under observation for two years and six months. During this time Amoeba A has never been found microscopically or culturally in his stools, nor has he ever shown any symptoms of dysentery.

Experiment XII.—Man 4, aged 34 years, had been under observation in the prison one year and seven months. He had a negative dysenteric history. He had been used for 2 experiments forty-one and twenty-nine days previously, respectively, with negative results (experiments II and IV). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 2 Petri-plate cultures of Amoeba 7F, mixed with magnesium oxide. Amoeba 7F had been cultivated from a stool of a healthy man. The cultures fed in this experiment contained only encysted amoebæ. Transplant cultures to test the viability of the cysts gave an abundant growth of Amoeba F. Following the ingestion, cultures of this man's stools showed a growth of Amoeba F on the second day only after feeding. Microscopic examination of his stools for amœboid organisms were constantly negative. This man was under observation ten months after the experiment began. No symptoms of dysentery developed.

Experiment XIII.—Man 7, aged 30 years, had been under observation in the prison for four years and five months. He had a history of 3 attacks of dysentery, each of one week's duration, six years ago. He had been used for a previous feeding experiment with negative results (experiment XI). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 2 Petri-plate cultures of Amoeba 9F cultivated from the stool of a healthy man, mixed with magnesium oxide. The cultures ingested by this man contained encysted amoebæ only. Transplant cultures made to test the viability of the cysts showed an abundant growth of Amoeba F. Following the feeding, cultures of this man's stools on Musgrave and Clegg's medium showed a growth of Amoeba F on the first day, but never subsequently. Microscopic examinations of his stools for amœboid organisms have been constantly negative. This man has been under observation two years and six and one-half months since the experiment began, but has never shown any dysenteric symptoms.

Experiment XIV.—Man 1, aged 29 years, had been under observation in the prison three years and ten months. He had a negative dysenteric history, and had not been used for previous feeding experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested the growth on 3 agar-slabt cultures of Amoeba 10G, mixed with magnesium oxide. Amoeba G had been cultivated from a diarrheal stool in Kansas, United States. The cultures ingested by this man were old, and contained encysted forms exclusively. Transplants from each of the cultures fed, made on fresh culture media to test the viability of the cysts, showed an abundant growth of Amoeba G. Following the feeding, Amoeba G was recovered in cultures of this man's stools on Musgrave and Clegg's medium on the first and second days after feeding, but never subsequently. Microscopic examinations of this man's stools were made daily, Sundays excepted, for thirty-five days with negative results. On the thirty-fifth day his stool
was still formed, but was surrounded by considerable mucus streaked with blood. Microscopic examination and cultures were negative for ameboid organisms. On the thirty-sixth day his stool was partly formed and partly fluid, the fluid portion consisting of mucus and blood. Three cultures of the material were negative. Microscopic examination showed not *Amoeba G*, which had been ingested by the man, but a distinct genus and species of ameboid organism, *Entamoeba histolytica* (part IV). The dysenteric condition persisted for only two days, and he recovered without treatment. *Entamoeba histolytica* has persisted in this man's stools up to the present time, two hundred forty-one days after the beginning of the experiment. During the period of observation no relapse of the dysenteric symptoms has occurred. At no time has *Amoeba G* been found microscopically in this man's stools, nor was it ever recovered in cultures after the first two days subsequent to feeding. This man either had a previous latent infection with *Entamoeba histolytica*, or became infected with the pathogenic *entamoeba* subsequent to ingesting *Amoeba G*.

Experiment XIV shows several things besides that which it was planned to demonstrate. First, it shows the possibility of latent or secondary infections with other ameboid organisms in such experiments; secondly, it emphasizes the care necessary to exclude such secondary infections in experimental work; thirdly, it illustrates the chief source of error in the conclusions of previous experimenters; and, fourthly, it has demonstrated our ability to exclude such sources of error from our experiments.

Experiment XV.—Man 3, aged 31 years, had been under observation in the prison for six years and one month. He gave a history of dysentery of one month's duration sixteen years ago. He had been used for 2 feeding experiments with cultures of amebæ, thirty-six and twenty-four days previously, respectively, with negative results (experiments I and III). Physical examination of his abdomen and microscopic and cultural examinations of his stools for ameboid organisms were negative. He ingested the growth on 4 Petri-plate cultures of *Amoeba 10G*, isolated from a diarrheal stool in Kansas, mixed with magnesium oxide. The cultures ingested by this man contained only encysted amebæ. Transplant cultures made to test the viability of the cysts showed an abundant growth of *Amoeba G*. Following the feeding, cultures of this man's stools showed a growth of *Amoeba G* on the second day after feeding, but never subsequently. Microscopic examinations of his stools for ameboid organisms have been constantly negative. This man has been under observation two years and five and one-half months since the experiment began. During this time *Amoeba G* has never been found microscopically in his stools, and he has never shown any symptoms of dysentery.

Experiment XVI.—Man 7, aged 30 years, had been under observation in the prison for four years and six months. He gave a history of 4 attacks of dysentery, each of one week's duration, six years ago. He had been used for 2 previous feeding experiments (experiments XI and XIII), the latter of which was thirty-four days previously, and both of which were followed by negative results. Physical examination of his abdomen and microscopic and cultural examinations of his stools for
ameoboid organisms were negative. He ingested the growth on 4 agar-
slant cultures of *Amoeba 10G*, isolated from a dysenteric stool in Kansas,
mixed with magnesium oxide. The cultures ingested by this man con-
tained motile forms of the amoeba exclusively. *Amoeba G* was recovered
in cultures of this man's stools on the sixth day only after feeding. Micro-
scopic examinations of his stools for ameoboid organisms have been con-
stantly negative. He has been under observation two years and four and
one-half months since this experiment began. No symptoms of dysentery
have developed.

*Experiment XVII.*—Man 5, aged 30 years, had been under observation
in the prison for six years and nine months. He gave a history of mucous
dysentery seven years ago. He had not been used for previous feeding
experiments. Physical examination of his abdomen and microscopic and
cultural examinations of his stools for ameoboid organisms were negative.
He ingested the growth on 3 Petri-plate cultures of *Amoeba 11G*, mixed
with magnesium oxide. This amoeba was isolated in culture from a man
suffering from an acute attack of entamebic dysentery. The culture fed
contained only encysted forms. Transplant cultures made to test the
viability of the cysts all showed a growth of *Amoeba G*. Cultures of this
man's stools showed a growth of *Amoeba G* on the first, second, and third
days after feeding, but never subsequently. Microscopic examinations of
his stools have been constantly negative. This man has been under observation
two years and six and one-half months since this experiment began.
During this time ameoboid organisms were never found microscopically or
culturally in his stools, and he has never exhibited any symptoms of
dysentery.

*Experiment XVIII.*—Man 6, aged 27 years, had been under observation
in the prison for five years and six months. He gave a history of bloody
mucous stools for four months, two years ago. He had not been used for
previous feeding experiments. Physical examination of his abdomen and
microscopic and cultural examinations of his stools for ameoboid organisms
were negative. He ingested the growth on 4 Petri-plate cultures of
*Amoeba 12H*, unmixed with other substance. This amoeba was isolated in
culture from a dysenteric stool. The cultures ingested contained both mo-
tile and encysted forms of the amoeba. Transplant cultures made to test
their viability showed a luxuriant growth of *Amoeba H*. Cultures of this
man's stools on Musgrave and Clegg's medium showed a growth of *Amoeba
H* on the first day after feeding, but never subsequently. Microscopic
examinations of his stools have been constantly negative. This man was
under observation one year and five months following the beginning of this
experiment. During this time *Amoeba H* has never been found micro-
scopically in his stools, and he has never shown any symptoms of dysentery.

*Experiment XIX.*—Man 6, aged 27 years, had been under observation
in the prison for five years and seven months. He had a history of a
bloody mucous dysentery for four months, two years ago. He had been
used sixteen days previously for another feeding experiment with the
same strain and species of amoeba, with negative result (experiment
XVIII). Physical examination of his abdomen and microscopic and cul-
tural examinations of his stools for ameoboid organisms were negative.
This man ingested the growth on 4 Petri-plate cultures of *Amoeba 12H*,
cultivated from a dysenteric stool, mixed with magnesium oxide. The cul-
tures ingested by this man contained encysted amoebae only. Transplant
cultures made to test the viability of the cysts showed a good growth of *Amoeba H*. Cultures of this man's stools showed a growth of *Amoeba H* on the first and second days after feeding, but never subsequently. Microscopic examinations of his stools were constantly negative. This man was under observation one year four months and eighteen days after this experiment began. No symptoms of dysentery developed.

*Experiment XX.*—Man 10, aged 45 years, had been under observation in the prison four years and eleven months. He gave a history of dysentery three years ago. He had not been used for previous feeding experiments. Physical examination of his abdomen disclosed thickened bands along the sigmoid. Microscopic and cultural examinations of his stools for amoeboid organisms were negative. He ingested the growth on 3 Petriplate cultures of *Amoeba 13F*, mixed with magnesium oxide. This strain of *Amoeba F* had been isolated in culture from a stool of an acute case of entamoebic dysentery in Manila. The cultures of this amoeba ingested by this man contained encysted forms only. Transplant cultures made to test the viability of the cysts showed a luxuriant growth of the amoeba. *Amoeba F* was recovered in cultures from this man's stool from the first to the third day after feeding, but never subsequently. Microscopic examinations of his stools for amoeboid organisms were constantly negative. This man was under observation five months after this experiment began. No symptoms of dysentery developed.

The protocols are summarized in Table I.

From the protocols and Table I it will be seen that, with the exception of species A and C, the specific amoeba ingested in these experiments was in every case recovered in cultures on Musgrave and Clegg's medium from the stools of the man to whom they were fed on the first to the sixth day after ingestion, but never subsequently. Species C was ingested only once, and was not recovered in cultures. Three strains of species A, fed five times, were never recovered in cultures of the stools of the experimental men.

On the other hand, microscopic examination of the stools of these men were, with one apparent exception, always negative, although in many of these experiments the men have been under observation for over two years. The one exception to this result was in experiment IX, in which the man was already parasitized with *Entamoeba coli* before ingesting a culture of *Amoeba E*. *Amoeba E* was recovered in cultures on the first and second days after feeding and not subsequently, but it was never found microscopically in the stools of this man. *Entamoeba coli*, on the other hand, was never obtained in cultures, but it was constantly found microscopically in the stools of this man. Thus *Amoeba E* behaved like the other amoebae in the intestinal tract of man, and it was distinguished morphologically and biologically from *Entamoeba coli* in this experiment.
### Table 1—Feeding experiments with cultures of Amoeba

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*Note: Amoeba infected with Entamoeba histolytica or the type of feeding Amoeba.*

*This note is essential for the correct interpretation of the results.*
In consequence of the failure in every case to find the amœbæ microscopically in the stools of the men who had ingested cultures of amœbæ and the ability to recover them in cultures only during the first few days after the ingestion, it is probable, as Werner (1908) and later one of us, Walker (1911), have concluded, that the cysts of the ingested amœbæ pass unchanged through the intestinal tract and find conditions suitable for development when the faeces are placed on the culture medium. In the case of experiments X and XVI, in which only motile amœbæ were ingested, the use of magnesium oxide to neutralize the acidity of the contents of the stomachs of the men may have favored the existence of the amœbæ in their passage through the intestinal tract of these men. It is also possible that the amœbæ became encysted (a protective reaction that takes place under any unfavorable conditions) in the intestines of these men. The failure to recover in cultures of the stools 3 strains of species A, fed five times, indicates that the cysts of this species are ordinarily incapable of withstanding passage through the human stomach.

Therefore, in consequence of our failure to parasitize men in 20 ingestion experiments with 13 strains of 8 species of amœbæ, we believe that the conclusion reached in the morphological study (Walker, 1911), that the cultivable amœbæ are not capable of living as parasites in the human intestine, is experimentally proved.

Following the feedings with the cultivable amœbæ, one man (experiment XIV) who had ingested a culture of Amœba G, isolated from a diarrhœa stool in Kansas, United States, outside of the endemic region, developed a slight attack of dysentery of two days' duration, thirty-five days after feeding. Amœba G ingested by this man was recovered in cultures on the first and second days after feeding and never afterward. It could never be found microscopically in the stools of this man. Two other men (experiments XV and XVI), who ingested the same strain of amœba, showed a similar behavior of the amœba, but did not develop dysentery. On the other hand, the amœboid organism found in the stools of the man in experiment XIV during the attack of dysentery belonged to a species and genus (Entamœba histolytica) distinct from the organism ingested by this man. It could not be cultivated on Musgrave and Clegg's medium, but was demonstrable microscopically in the stools during, and subsequent to, the attack of dysentery. Moreover, Entamœba histolytica from the stools of this man has been used to infect
other men in whom this entamoeba maintained its characters of noncultivability and persistence microscopically in the stools and in some of whom dysentery has developed.

This case well illustrates the erroneous conclusions that have been drawn from experiments by investigators who have neglected to determine the species of amœboid organism fed to, and recovered from, the experimental animal. This man apparently either had a latent infection with *Entamoeba histolytica* or had become infected with this entamoeba after ingesting the culture of *Amœba G*. If the species of the amœboid organisms fed to this man, and that recovered in his stools during the attack of dysentery, had not been determined, and if the case had not been carefully followed with daily microscopic and cultural examinations, the conclusions would have been inevitable that *Amœba G*, cultivated from a diarrheal stool in Kansas, was capable of producing entamoebic dysentery in man. As it is, we are in position to make the unqualified statement that *Amœba G* had nothing to do with the development of dysentery in this man; and, moreover, that *Amœba G* is not only not pathogenic, but that it is incapable of living as a parasite in the intestine of man.

In view of the fact that it has not been found possible to produce dysentery in man by 20 ingestion experiments made with 13 strains of 8 species of amœbæ cultivated from a variety of nonparasitic sources and from normal and dysenteric stools and that it has been demonstrated experimentally that none of these amœbæ are capable of living parasitically in the intestinal tract of man, the conclusion appears warranted that the *Amœbæ* play no part in the etiology of endemic tropical dysentery. The sound basis of this conclusion will be more evident when we consider the behavior of the *Entamoeba* in the human intestine.

**PART III. FEEDING EXPERIMENTS WITH ENTAMŒBA COLI**

By ERNEST LINWOOD WALKER and ANDREW WATSON SELLARDS

It has been shown in a previous paper by one of us (Walker, 1911) that the amœboid organisms, living parasitically in the intestinal tract of man and other animals, differ morphologically and biologically from those found in water and soil, and occasionally cultivable from feces, sufficiently to justify the establishment of the genus *Entamoeba* by Cassagrandi and Barbagallo (1897) for the former species. Morphologically the parasitic *Entamoeba* are differentiated from the nonparasitic *Amœba* by the absence of a contractile vacuole, by the eccentric instead of
central position occupied by the nucleus in the resting organism, by the peripheral instead of central arrangement of the chromatin in the nucleus, and by the presence of 4 or 8 nuclei, instead of a single nucleus, in the encysted stage; and biologically they are distinguished by their parasitic instead of saprozoic mode of life, by the occurrence of a reproductive process (schizogony) in the encysted stage, by their inability to propagate outside of the body of their host, and by not being cultivable on Musgrave and Clegg's medium (compare figs. 1 and 2 with figs. 3 to 8, Plate I).

*Entamoeba coli* was first distinguished from another species, *Entamoeba histolytica*, found in the intestinal tract of man by Schaudinn in 1903. This species was described by Schaudinn as follows. The entamoeba shows no separation of the ectoplasm from the entoplasm in the resting stage. In the motile entamoeba the ectoplasm is apparent in the hyaline pseudopodes, which are always less strongly refractive than the entoplasm. The nucleus is vesicular, spherical in the resting entamoeba, and has a thick nuclear membrane. In the center of the nucleus of the vegetative entamoeba are one or more small granules of plastin and chromatin. The chromatin is distributed as fine granules through the achromatic network of the nucleus, and appears to be collected particularly about the nuclear membrane. Multiplication takes place in the vegetative stage by simple division and by schizogony into 8 daughter entamoebæ. Cysts are developed, within which an autogamous sexual process takes place, followed by the development of 8 nuclei which give rise to 8 daughter entamoebæ when the cyst is ingested by a new host.

Schaudinn (1903) found *Entamoeba coli* in 50 per cent of healthy persons in West Prussia, in 20 per cent at Berlin, and in 66 per cent of the population on the shores of the Adriatic Sea. Craig (1905) found 65 per cent of 200 American soldiers recruited from various parts of the United States parasitized with *Entamoeba coli*. The occurrence of this species in the United States has been confirmed by Sistrunk (1911) who found it in the stools of 11 out of 145 patients suffering from diseases other than dysentery at Rochester, Minnesota; by Stiles (1911) who has observed it in North Carolina; and by Rosenberger and Terrell (1913) who found entamoebæ in 112 out of 137 males and in 81 out of 141 females examined at Philadelphia. In none of these cases was there a history of diarrhœa or dysentery. Vedder (1906) found *Entamoeba coli* in 50 per cent of healthy
American soldiers and in 72 per cent of Filipino scouts in the Philippine Islands. These figures for the Philippines have been confirmed by Craig and Ashburn who found 71 per cent of healthy American soldiers parasitized. Evidence of the wide distribution of *Entamoeba coli* is further substantiated by McCarrison in India, by Wenyon (1908) in Khartum, by Elmasian (1909) in South America, by Whitmore (1911) in Manila and Saigon, by Prowazek (1911) in Samoa, and by Darling (1912) upon the Isthmus of Panama.

Recently several other entamoebae of the *coli* type have been described as distinct species. Prowazek (1911) found associated with *Entamoeba coli* in human faeces in Suwaii and Sai-pipi an entamoeba which he called *Entamoeba williamsii*. This species is said to differ from *Entamoeba coli* in the presence of “excretion crystals” in its cytoplasm, in its movements and feeding habits, in its peculiar chromidia formation, and in that it develops cysts containing 10 instead of 8 nuclei.

Beaurepaire Aragao (1912) describes an entamoeba from the stools of a child in Brazil which is said to differ from *Entamoeba coli* by the presence of a bundle of “siderophile substance,” sometimes double, which divides the cyst into two approximately equal parts. The author designates this entamoeba by the name *Entamoeba brasiliensis*.

Prowazek (1912) found another entamoeba associated with *Entamoeba coli* in the stools of a woman in Sawai, which he considers a distinct species, and which he named *Entamoeba hartmanni*. This entamoeba is said to differ from *Entamoeba coli* in its small size, in the variable size of its nuclei, and by the very characteristic minute chromidia in the cytoplasm.

It is noteworthy that 2 out of these 3 so-called species have been found but once, that 2 out of the 3 have been found associated in the same patient with typical *Entamoeba coli*, and that all 3 were found in regions where infections with *Entamoeba coli* are common. The differential characters of these so-called new species consist chiefly in differences in size and cytoplasmic contents, variable size of the nuclei, and the number of nuclei in the cyst. Such differences are not uncommon mingled with typical *coli* forms. Abnormally large or small *coli* are frequently met with, and within certain limits the size of the nuclei and the cytoplasmic contents of entamoebae are exceedingly variable. These variations represent chiefly metabolic and reproductive, but sometimes degenerative, changes in the entamoeba. The number of nuclei in the cysts of *Entamoeba coli* is also subject
to variation. While 8 is the usual number, individual cysts containing from 9 to 16, usually mingled with the 8 nuclear cysts, are not uncommon. Therefore, it is believed that the enteramœba found so commonly in the stools of healthy persons in tropical and subtropical countries is of one species, *Entamœba coli* Schaudinn. It is unquestionably the common species in the Philippine Islands.

This enteramœba is distinguished from *Entamœba histolytica*, to be considered in part IV, by its porcelaneous appearance; greater refractiveness; more sluggish motility; the possession of a nucleus that is distinctly visible in the living enteramœba and contains a relatively large amount of chromatin; by the development of cysts that are larger, more refractive, and contain 8 or more, instead of 4, nuclei; and by their frequent occurrence in the stools of healthy persons (compare figs. 3 and 4 with figs. 5 to 8, Plate I).

Among the numerous experimental infections with enteramœbæ that have been attempted by various authors, the following were made with enteramœbæ identified as *Entamœba coli*.

Schaudinn (1903) experimented with kittens and also parasitized himself on two occasions by swallowing cysts of *Entamœba coli*. In both of his own infections the enteramœbæ were said to have persisted in his stools two months. None of these experimental infections of himself or of kittens were followed by the development of dysenteric symptoms.

Craig (1905) made a large number of experiments on the pathogenicity of *Entamœba coli*, using kittens to which the faeces containing the enteramœbæ were fed in milk or injected rectally. In some cases the injections were repeated from five to ten times in the same cat. In none of these experimental animals did symptoms of diarrhoea or dysentery develop.

Wenyon (1912) attempted to infect cats with faeces containing cysts of *Entamœba coli* which were administered per asophagus and per rectum in large doses. In no case were these experimental infections followed by dysenteric symptoms. These same animals were subsequently infected with "*Entamœba tetragna*" and developed enteramœbic dysentery.

Craig (1913) quotes a personal communication from Creighton Wellman. He attempted to infect 5 kittens by injecting rectally 4 to 5 cubic centimeters of faeces containing *Entamœba coli*. None of the animals developed symptoms of dysentery, and no dysenteric lesions were found in their intestines at necropsy about one month after injection.

In our series of experiments 20 men have ingested *Entamœba coli*. The men employed were carefully examined before use with reference to previous attacks of dysentery and to present parasitization with ameboid organisms. A few of the men gave a history of dysentery at some earlier period of their lives. Cultures on Musgrave and Clegg's medium and microscopic
examinations, both before and after a purgative, were made of their stools, and all men showing ameboid organisms after either method of examination were excluded.

Five distinct strains of *Entamœba coli* were employed as follows:

Strain A, from a healthy Filipino.
Strain B, from a Filipino suffering from an epithelioma of the jaw.
Strain C, from a healthy Filipino.
Strain D, from a Filipina, suffering from lobar pneumonia.
Strain E, from a healthy American.

*Entamœba coli* was fed for the most part in the encysted stage, since there are reasons for believing it to be the stage naturally infective and it was the purpose of these experiments to secure as high a percentage of parasitization as possible in order to determine the pathogenesis of this species.

The entamœbæ were mixed with powdered starch or magnesium oxide, inclosed in gelatine capsules, and ingested by the men. The starch or magnesium oxide was used, as in the experiments with the cultures of amœbæ, to absorb the excess of moisture that might dissolve the gelatine capsule and to facilitate the ingestion of the material. The magnesium oxide, when used, served also to neutralize the acidity of the contents of the stomach of the man. It is doubtful if this be necessary to secure parasitization with the entamœbæ, since the action of the gastric juices probably plays an important part in the dissolution of the cyst wall of the parasite. However, it was employed in certain of these experiments to correspond with the feeding experiments with the cultures of amœbæ. The percentage of parasitizations with *Entamœba coli* did not appear to be materially affected by its use.

Following the ingestion of material containing *Entamœba coli*, the stools of the men were examined daily, culturally and microscopically, for ameboid organism until it was determined that the ingested entamœbæ had parasitized or failed to parasitize the man, and thereafter at frequent intervals. The men were examined clinically and physically whenever conditions seemed to warrant.

A complete protocol is given of each man in order to put on record the details of these experiments.

*Experiment XXI.*—Man 11, aged 26 years, had been under observation in the prison for six years and two months. He had not been used for previous experiments. He gave a history of one attack of dysentery eight years ago. Physical examination of his abdomen and microscopic and cultural examinations of his stools for ameboid organisms were
negative. He ingested cysts of *Entamoeba coli*, strain *A*, mixed with magnesium oxide. This man has been under observation two years since the experiment began. Following the feeding, cultures of this man's stools were constantly negative. Microscopic examinations showed *Entamoeba coli* in his stools on the fourth day after feeding and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXII.**—Man 6, aged 27 years, had been under observation in the prison for five years and seven months. He gave a history of a bloody mucous dysentery of four months' duration two years ago. He had been used previously for 1 feeding experiment with a culture of amœbe, which was followed by negative results (experiment XVIII, part II). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested encysted and motile *Entamoeba coli*, strain *A*, mixed with magnesium oxide. This man has been under observation one year and four months since this experiment began. Following the ingestion, cultures of this man's stools were constantly negative for amœboid organisms. Microscopic examinations showed *Entamoeba coli* in his stools on the seventh day and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXIII.**—Man 8, aged 57 years, had been under observation in the prison seven years and nine months. He gave a history of 1 attack of dysentery of one month's duration eight years ago. He had been used for 1 feeding experiment with a culture of amœbe with negative results (experiment VIII, part II). Physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain *A*, which had been kept at room temperature for ten days, mixed with powdered starch. This man has been under observation two years and five months since this experiment began. Cultures of his stools were constantly negative. Microscopic examination showed *Entamoeba coli* in his stools on the seventh day and more or less constantly ever since. No symptoms of dysentery have developed.

**Experiment XXIV.**—Man 12, aged 44 years, had been under observation in the prison for seven years and eight months. He had not been used for previous experiments. He had a negative dysenteric history. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain *A*, which had been kept at room temperature ten days before use in this experiment, mixed with powdered starch. This man was under observation one year and ten months after this experiment began. Cultures of his stools on Musgrave and Clegg's medium were constantly negative for amœbe. Microscopic examinations showed *Entamoeba coli* in his stools on the second day and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXV.**—Man 13, aged 48 years, had been under observation in the prison seven years and four months. He had not been used previously for experiments. He gave a history of 1 attack of dysentery of one week's duration twenty years ago. Physical examination of his abdomen showed a sigmoid that was palpable, firm, and smooth. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain *A*, which had been kept at room temperature for ten days, mixed with powdered starch. This
man has been under observation two years and five months since this experiment began. Cultures of his stools on Musgrave and Clegg's medium for amœbæ have been constantly negative. Microscopic examination showed *Entamoeba coli* in his stools on the seventh day and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXVI.**—Man 14, aged 24 years, had been under observation in the prison seven years and seven months. He had not been used previously for experiments. History and physical examination of his abdomen and microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain B, which had been kept at room temperature two days, mixed with powdered starch. This man was under observation one year and three months after this experiment began. Cultures of his stools on Musgrave and Clegg's medium were constantly negative. Microscopic examination showed *Entamoeba coli* in his stools on the first day after ingestion and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXVII.**—Man 15, aged 25 years, had been under observation in the prison seven years and four months. He had not been used previously for experiments. His history with reference to dysentery was negative. Physical examination of his abdomen was not made. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain B, which had been kept at room temperature for two days, mixed with powdered starch. This man has been under observation one year and eight months since this experiment began. Cultures of his stools on Musgrave and Clegg's medium were uniformly negative. Microscopic examinations showed *Entamoeba coli* in his stools on the eleventh day and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXVIII.**—Man 16, aged 26 years, had been under observation in the prison six years and five months. He had not been used previously for experiments. His history with reference to dysentery was negative. No physical examination was made of his abdomen. Microscopic and cultural examinations of his stools for amœboid organisms were negative. He ingested encysted *Entamoeba coli*, strain C, mixed with magnesium oxide. Since this experiment began this man has been under observation one year and six months. Cultures of his stools on Musgrave and Clegg's medium have been constantly negative. Microscopic examinations showed *Entamoeba coli* in his stools on the first day and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXIX.**—Man 17, aged 30 years, had been under observation in the prison five years and five months. He had suffered from 2 attacks of entamobic dysentery four years and seven months and one year and five months previously, respectively. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain C, mixed with magnesium oxide. This man was under observation four months after this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examinations of his stools were also negative; that is, this man failed to become parasitized with *Entamoeba coli*. No symptoms of dysentery developed.

**Experiment XXX.**—Man 18, aged 32 years, had been under observation in the prison five years and two months. He had not been used for previous
experiments. His history with reference to dysentery was negative. No physical examination of his abdomen was made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain *C*, mixed with magnesium oxide. This man was under observation four months after this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examination showed *Entamoeba coli* in his stools on the second day and more or less constantly thereafter. No symptoms of dysentery developed.

**Experiment XXXI.**—Man 19, aged 30 years, had been under observation in the prison six years and four months. He had not been used for previous experiments. His history with reference to dysentery was negative. No physical examination of his abdomen was made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain *C*, mixed with magnesium oxide. This man has been under observation one year and six months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examination of his stools for entamoebæ have also been negative; that is, this man failed to become parasitized with *Entamoeba coli*. No symptoms of dysentery have developed.

**Experiment XXXII.**—Man 20, aged 47 years, had been under observation in the prison for four years and one month. He had not been used previously for experiments. He had a negative dysentery history. No physical examination of his abdomen was made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain *D*, mixed with magnesium oxide. This man has been under observation one year and three months since this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examination showed *Entamoeba coli* in his stools on the sixth day and thereafter. No symptoms of dysentery have developed.

**Experiment XXXIII.**—Man 21, aged 30 years, had been under observation in the prison five years and one month. He had not been used previously for experiments. His history with reference to dysentery was negative. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested encysted *Entamoeba coli*, strain *D*, mixed with magnesium oxide. This man has been under observation one year and three and one-half months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed *Entamoeba coli* in his stools on the eighth day and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXXIV.**—Man 22, aged 31 years, had been under observation in the prison five years and eleven months. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination of his abdomen was made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba coli*, strain *D*, mixed with magnesium oxide. This man was under observation ten months after this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examinations showed *Entamoeba coli* in his stools on the eighth day after ingestion and more or less constantly thereafter. No symptoms of dysentery have developed.

**Experiment XXXV.**—Man 23, aged 38 years, had been under observation
in the prison five years and five months. He had not been used previously for experiments. He had a negative dysenteric history. Physical examination of his abdomen was not made. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of \textit{Entamoeba coli}, strain \textit{E}, mixed with powdered starch. This man was under observation eight months after this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed \textit{Entamoeba coli} in his stools on the second day and more or less constantly thereafter. No symptoms of dysentery have developed.

\textit{Experiment XXXVI.}—Man 24, aged 25 years, had been under observation in the prison three years and nine months. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of \textit{Entamoeba coli}, strain \textit{E}, mixed with powdered starch. This man has been under observation eight months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed \textit{Entamoeba coli} in his stools on the seventh day and more or less constantly thereafter. No symptoms of dysentery have developed.

\textit{Experiment XXXVII.}—Man 25, aged 26 years, had been under observation in the prison four years and one month. He had not been used previously for experiments. His history with reference to dysentery was negative. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of \textit{Entamoeba coli}, strain \textit{E}, mixed with powdered starch. This man was under observation eight months after this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed \textit{Entamoeba coli} in his stools on the second day and more or less constantly thereafter. No symptoms of dysentery have developed.

\textit{Experiment XXXVIII.}—Man 26, aged 19 years, had been under observation in the prison two years and eleven months. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of \textit{Entamoeba coli}, strain \textit{E}, mixed with powdered starch. This man has been under observation eight months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations of his stools were also negative; that is, this man failed to become parasitized with \textit{Entamoeba coli}. No symptoms of dysentery have developed.

\textit{Experiment XXXIX.}—Man 27, aged 40 years, has been under observation in the prison seven years and one month. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of \textit{Entamoeba coli}, strain \textit{E}, mixed with powdered starch. This man has been under observation eight months since this experiment began. Cultures of his stools for amœbæ were constantly negative. Microscopic examination showed \textit{Entamoeba coli} in his stools on the second
Table II.—Feeding experiments with Entama:.-

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Note: Observations on stools continued until recovery or death.

Results: Positive

Negative

Reference: Clegg's results.
day after ingestion and more or less constantly thereafter. No symptoms of dysentery have developed.

Experiment XL.—Man 28, aged 30 years, had been under observation in the prison six years and eleven months. He had not been used previously for experiments. He had a negative dysenteric history. No physical examination was made of his abdomen. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of Entamoeba coli, strain E, mixed with powdered starch. This man has been under observation eight months since this experiment began. Cultures of his stools for amœbæ have been constantly negative. Microscopic examinations showed Entamoeba coli in his stools on the second day after ingestion and more or less constantly thereafter. No symptoms of dysentery have developed.

The protocols of these experiments with Entamoeba coli are summarized in Table II.

The results of the experiments with Entamoeba coli present a striking contrast to those obtained after feeding cultures of amœbæ (part II). The Amœbæ were, with the exception of 2 species, always recovered in cultures on Musgrave and Clegg's medium from the stools of the men the first few days after feeding; while similar cultures of the stools of men who had ingested Entamoeba coli have been invariably negative. Furthermore, the Amœbæ could never be found microscopically in the stools of the men who had ingested them; on the other hand, Entamoeba coli has been found microscopically, after a short incubation period, in the stools of every man who became parasitized (88 per cent of the men), and the entamœbæ have persisted in the stools for an indefinite time.

Of the 20 men who ingested Entamoeba coli, 17 became parasitized after the first feeding and 3, who did not become parasitized, were reserved as controls. Of the 12 men who ingested the entamœbæ mixed with powdered starch and of the 8 men who ingested the entamœbæ mixed with magnesium oxide, 11 and 6, respectively, became parasitized.

The incubation period of Entamoeba coli, that is, the time elapsing from the day of ingestion to the appearance of the entamœbæ in the stools of the men, as determined by the 20 experiments, varies from one to eleven days, with an average of 4.7 days.

None of the 17 men experimentally parasitized with Entamoeba coli nor the 3 nonparasitized controls have developed any symptoms of dysentery, although some of these have been under observation for two years and five months.

From the uniform results obtained in these experiments with
Entamoeba coli, we believe that we are justified in the conclusions that Entamoeba coli, unlike the Amoebœ, is an obligatory parasite and cannot be cultivated on Musgrave and Clegg’s medium, and that it is nonpathogenic and consequently plays no rôle in the etiology of entamoebic dysentery.

PART IV. FEEDING EXPERIMENT WITH “ENTAMŒBA TETRAGENA” AND ENTAMŒBA HISTOLYTICA

By Ernest Linwood Walker

Of the identified species of Entamoeba, 3, Entamoeba histolytica Schaudinn, “Entamoeba tetragena” Viereck, and “Entamoeba minuta” Elmassian, have been found associated with endemic tropical dysentery and have been definitely implicated in the etiology of this disease.

Entamoeba histolytica was first described by Schaudinn in cases of dysentery from Egypt, China, and Siam in 1903. It is distinguished, according to this author, by its morphology and its developmental cycle. This entamoeba possesses a distinct, refractive ectoplasm and a granular, vacuolated entoplasm. The nucleus is scarcely visible in the living entamoeba, is eccentric in position, is frequently deformed by the movements of the entamoeba, possesses no limiting membrane, and is poor in chromatin, which is arranged chiefly about the periphery. Entamoeba histolytica, according to Schaudinn, does not become encysted in toto and undergo schizogony within the cyst, as does Entamoeba coli; instead, there are developed small peripheral buds, containing chromidia derived from the nucleus, which are constricted off from the parent entamoeba and become surrounded by a resistant capsule.

Of the numerous feeding experiments that have been made upon animals, in the following only has Entamoeba histolytica been specifically identified:

Schaudinn (1903) produced a typical dysentery in 3 cats with characteristic lesions and entamoeba in the bloody mucous stool by feeding a dysenteric stool containing Entamoeba histolytica.

Craig (1905) produced dysentery in 50 per cent of the kittens injected rectally and in 66 per cent of 8 kittens fed dysenteric stools containing Entamoeba histolytica. At necropsy, typical lesions were observed, and on section Entamoeba histolytica was found in the tissues.

Shirota (1912) was able to produce a dysentery, having the same lesions as in man and with Entamoeba histolytica in the stools and lesions, by introducing the stools of a human dysenteric patient into the rectum of young cats. The bacteria isolated in cultures from the same dysenteric faeces, when introduced into the rectum of other kittens, produced no clinical symptoms or pathological changes.
Walker and Sellards: Entamæbic Dysentery

Wenyon (1912) has recently conducted the most extensive and successful experimental infections of animals with dysenteric faeces containing *Entamoeba histolytica* yet attempted. He introduced the material containing the *entamæbæ* *per æsophagus* and *per rectum* into cats. Of 14 experiments on 12 cats, 8 were followed by the development of acute dysentery with typical ulcerations and *entamæbæ* in their stools, and one cat developed, in addition to the dysentery, 4 abscesses of the liver. He was able to pass the infection successively through 4 cats when it was lost. This author was further able to study the invasion of the tissues of infected cats. "The amœbæ make their way to the bottom of the tubular glands in the large intestine. Then they multiply and by pressure of their numbers or by the exertion of their pseudopodia, and probably through some toxic substance excreted by them, the lining cells are weakened and separated and the amœbæ pass into the connective tissue beneath."

Craig (1913) quotes a personal communication from Creighton Welman giving the data of 5 infection experiments of kittens with *Entamoeba histolytica* performed in 1910. Two of the kittens received rectal injections of faeces containing the *entamæbæ* and 3 were fed the same material. Four out of the 5 kittens developed dysenteric symptoms or showed the characteristic lesions at necropsy.

"*Entamoeba tetragena*" was first described by Viereck in 1907 in 2 cases of dysentery from India. Shortly afterward it was described under the name of "*Entamoeba africana*" by Hartmann (1908), in cases of dysentery from Southwest Africa and South America. Subsequently it has been observed by several investigators in dysenteries in different parts of the Tropics, and it is stated by Whitmore (1911) and Hartmann (1912) to be the most common pathogenic species in the Philippine Islands. This amœba is distinguished from *Entamoeba histolytica*, according to Hartmann (1908 and 1912), by the nuclear structure of the vegetative stage, by its reproduction, and by the structure of its cysts. The nucleus of the vegetative stage, unlike that of *Entamoeba histolytica*, is distinctly visible in the living amœba, has a double-contoured membrane, and is rich in chromatin which has a characteristic arrangement. There is a peripheral layer of chromatin and a central karyosome. This karyosome undergoes cyclical changes, but in its most characteristic stage consists of a central granule, the "centriol," which is surrounded by a clear halo bounded by a layer of chromatin granules. "*Entamoeba tetragena*," unlike *Entamoeba histolytica*, becomes encysted *in toto* and undergoes schizogony within the cyst, but differs from *Entamoeba coli* in that only 4 merozoites are formed. The unincubated cyst, therefore, contains 4 instead of 8 nuclei.

The following experimental infection of animals have been attempted with *entamæbæ* identified as "*Entamoeba tetragena*":

...
Hartmann (1908) states that this species is as a rule less pathogenic for cats than *Entamoeba histolytica*. Of 3 cats used in his experiments, 1 did not develop dysentery, 1 showed after from eight to ten days slightly bloody stools for a few days only, and the third developed a more severe dysentery and died three weeks after infection.

Werner (1909) experimented with 5 strains of "*Entamoeba tetragena*." Two of these strains when injected into the rectum of cats gave rise to no infection, the other 3 strains produced a dysentery in the experimental animals. One of these latter strains was passed through 5, another through 3 cats, and a third through 1 cat, after which their virulence was lost. The period of incubation is given as from five to twelve days with an average of seven and one-half days. The duration of the disease in cats was from eight to thirty-two days. Of the successfully infected cats, 6 died. These showed typical ulcerations of the large intestine, and one had an abscess of the liver.

Franchini (1911) introduced into the rectum of a healthy monkey, which had been under observation in the laboratory more than one year, feces from a case of tropical dysentery containing blood, mucus, and numerous "*Entamoeba tetragena*." Three injections of this material were made, on February 10, 18, and 20, respectively. On May 10 the monkey developed dysentery with numerous entamoebae in his stools. At necropsy the cecum was found to contain one large and numerous small ulcers, and the rest of the intestine showed more or less colitis. "*Entamoeba tetragena*" was found in the intestinal contents and in sections of the large intestine.

Darling (1912) fed 2 kittens with the cysts of "*Entamoeba tetragena*" from a case of entamoebic dysentery. On the twelfth day both kittens had prolapse of the rectum following intussusception and entamoebic enteritis. He was unable, in numerous feeding experiments with monkeys, dogs, and cats, to infect with the motile or trophozoite stage of this entamoeba.

Craig (1913) quotes a personal communication from Dr. H. B. Pantham who had succeeded in producing dysentery in 1 of 2 kittens fed faces containing "*Entamoeba tetragena*" from an infection contracted in Algeria. The kitten died in three weeks, and ulcerations containing "*Entamoeba tetragena*" were found in the intestine. All of his experiments by rectal injections of the material into kittens were negative.

"*Entamoeba minuta*" was found by Elmassian (1909) in the stools of a case of recurrent dysentery in a European who had resided in Paraguay, South America. This *Entamoeba* had, in the living organism, an indistinct nucleus like *Entamoeba histolytica*, but in stained preparations the nucleus showed a heavy peripheral ring of chromatin like *Entamoeba coli*. No distinction existed between ectoplasm and entoplasm, and its movements were sluggish. Small cysts, 12 to 14 microns in diameter, were developed which contained 4 nuclei. The author considers this species to be pathogenic, but no experiments were undertaken to prove its pathogenicity.

In a previous paper (Walker, 1911) I have expressed the opinion, based upon morphological evidence, that "*Entamoeba tetragena*" Viereck is identical with *Entamoeba histolytica* Schau-
dinn and that the life cycle of Entamoeba histolytica includes the development of "tetragena" cysts.

Wenyon (1912), Darling (1912), and Hartmann (1912) have subsequently come to the same conclusion, although Darling and Hartmann persist in calling the species "Entamoeba tetragena." However, on the basis of priority, Entamoeba histolytica Schaudinn must remain the valid name of this species. Craig (1913) still maintains that Entamoeba histolytica and "Entamoeba tetragena" are distinct species.  

The view that we are here dealing with but one species has received further support from observation of the morphological changes that take place in Entamoeba histolytica during the course of the disease in experimentally infected men. There has been found to exist a more or less definite series of morphological changes in the entamoebae that are found in the stools which appear to be correlated with the clinical symptoms in the host. Men fed Entamoeba histolytica show "tetragena" cysts in their stools, after a short incubation period, and these cysts persist so long as the stools of the parasitized individual remain formed. When the stools become soft or diarrhoeal, the "tetragena" cysts are replaced by postencysted or preencysted entamoeba which are small and inactive and have a nucleus more or less rich in chromatin. These forms correspond to Elmasian's "Entamoeba minuta." If chronic dysentery with faecal stools mixed with mucus and blood develops, larger and more active forms appear which still contain nuclei rich in chromatin and many of which show the karyosome structure characteristic of "Entamoeba tetragena." In acute attacks of dysentery, in which mucus and blood practically free from faeces are passed, these forms are largely replaced by entamoebae having an indistinct nucleus that contains a minimum amount of chromatin, which is characteristic of Entamoeba histolytica Schaudinn. In untreated cases that recover spontaneously from the attack of dysentery, this series of morphological changes is repeated in the inverse order, ending with the reappearance of "tetragena" cysts in the formed stools of the convalescent individual. These changes in the morphology of Entamoeba histolytica, which are connected with the developmental cycle of the organism, probably

Since this paper was written, Craig (1913a and 1913b) has changed his opinion and now agrees that Entamoeba tetragena Viereck is identical with Entamoeba histolytica Schaudinn. He gives me full credit for being the first definitely to state the identity of these two species.
account for the several species of Entamoeba that have been described by different observers in dysenteric stools.

An attempt has been made to obtain experimental evidence of the truth of these conclusions by the use in feeding experiments of either motile entamoebae of the histolytica type or“tetraxen” cysts only. The material for these feeding experiments was selected after a careful microscopic examination of both fresh and stained preparations.

Experiment 1.—Men 5 and 34 ingested motile and resting entamoebae of the histolytica type only from a case of acute entamoebic dysentery. Both men became parasitized, and “tetraxen” cysts appeared in the formed stools of these men on the fourth day after ingestion and have persisted ever since.

Experiment 2.—Man 2 ingested “tetraxen” cysts from a convalescent case of entamoebic dysentery. This man became parasitized, and “tetraxen” cysts appeared in his stools on the second day after the ingestion of the entamoebae. These cysts persisted in the stools of this man until the twentieth day, when acute dysentery developed with typical motile histolytica only in his bloody mucous stools. The patient was given treatment, and recovered from the attack of dysentery on the thirtieth day, when “tetraxen” cysts reappeared in his normal stools. On the sixtieth day he suffered a relapse with motile histolytica only in his bloody mucous stools. Treatment again relieved the dysenteric symptoms, but “tetraxen” cysts soon reappeared and have been found more or less constantly ever since in his stools.

Therefore, in these experiments it has been possible to obtain (a) “tetraxen” cysts in the stools of men fed motile histolytica only, (b) motile histolytica in the stools of a man fed exclusively with “tetraxen” cysts, and (c) an alternation of motile histolytica and “tetraxen” cysts in the stools of a man having recurrent attack of entamoebic dysentery. The conclusions, therefore, appear warranted that Entamoeba tetraxen Viereck is identical with Entamoeba histolytica Schaudinn, that “tetraxen” cysts are produced in the life cycle of Entamoeba histolytica, and that Entamoeba minuta Elmassian is the preœncysted stage of Entamoeba histolytica.

Entamoeba histolytica Schaudinn (which includes “Entamoeba tetraxen” Viereck and “Entamoeba minuta” Elmassian) is distinguished from Entamoeba coli, previously considered, by a less refractive and more hyaline appearance; by a more active motility; by an indistinct nucleus that is relatively poor in chromatin; by cysts that are smaller and less refractive, which usually contain one or more refractive bodies that stain with chromatin stains and are designated by Hartmann (1912) as “Chromidialkörper,” and 4 instead of 8 nuclei; and by their
less frequent occurrence in the stools of healthy persons and their constant presence in the stools of cases of endemic tropical dysentery (compare figs. 5 to 8 with figs. 3 and 4, Plate I).

In the following series of experiments 20 men have ingested material containing Entamoeba histolytica. Four of these men had a history of attacks of dysentery from six to sixteen years previously; the other 16 had negative dysenteric histories. Four of the men had been used previously for ingestion experiments with cultures of amœbæ with negative results. All of the men were free from dysenteric symptoms, and their stools were proved to be free from amœboid organisms by cultures on Musgrave and Clegg's medium and by microscopic examinations before being used for these experiments.

The entamœbæ ingested by these men were from 7 different sources and represented 4 distinct strains of Entamoeba histolytica. The history of these strains will be given in connection with the protocols of the experiments.

The material containing Entamoeba histolytica, as in the case of the Amœbae and Entamoeba coli, was mixed with powdered starch or magnesium oxide, inclosed in a gelatine capsule, and ingested by the experimental men. In cases where motile entamœbæ were ingested and it was consequently undesirable to absorb the moisture of the material, the infectious material was inclosed in a small gelatine capsule and this inclosed in a larger gelatine capsule containing magnesium oxide. The use of magnesium oxide to neutralize the acidity of the contents of the stomach in the experiments with Entamoeba histolytica was in order to secure parasitization with the motile entamœbæ and to insure infection with any possibly pathogenic microorganisms that might be associated with the entamœbæ and be the primary etiologic agent in the production of dysentery, especially in the control cases that did not become parasitized with Entamoeba histolytica.

Cultures and microscopic examinations were made daily of the stools of the men after ingesting the infectious material until parasitization or nonparasitization with Entamoeba histolytica was determined, and thereafter at frequent intervals. In every case the species of amœboid organism found by either method of examination was carefully determined. Clinical symptoms of dysentery were carefully watched for, and men who developed dysentery received ipecac treatment as soon as the diagnosis of entamœbic dysentery was clinically and microscopically established. Treatment of the cases of dysentery was
controlled by microscopic examination of the stools until each man was cured.

Complete protocols of each man are given in order to put on record the details of these experiments.

**ENTAMOEBA HISTOLYTICA, STRAIN A, FIRST PASSAGE**

Strain A of *Entamoeba histolytica* was from a man convalescent from a slight attack of entamebic dysentery of two days' duration. This man had been convalescent from fifty-nine to one hundred sixty-one days when the entamebæ from his stools were used for these feeding experiments, and he has not subsequently suffered a relapse of the dysentery. His formed stools contained many cysts of *Entamoeba histolytica*. This strain was ingested by 3 men and carried by subsequent passages through 9 other men.

**Experiment XLII.**—Man 2, aged 40 years, had been under observation in the prison five years and one month. He had a negative dysenteric history. He had been used previously for 2 feeding experiments with cultures of amoebæ, ninety-six and seventeen days previously, both of which were negative (part II, experiments VII and V). Physical examination of his abdomen and cultural and microscopic examinations of his stools for amoeboid organisms were negative. He ingested cysts of *Entamoeba histolytica*, strain A, mixed with magnesium oxide. This man received a saline purgative by mistake in the evening of the day that he ingested the infectious material. He became parasitized with *Entamoeba histolytica*, the encysted entamebæ appearing in his stools on the eleventh day. Cultures of his stools on Musgrave and Clegg's medium were negative. On the twentieth day he developed an attack of dysentery with bloody mucous stools containing motile *Entamoeba histolytica*, many of which were filled with red blood corpuscles. Physical examination disclosed pain over the abdomen, no tenderness except over the sigmoid and caecum, sigmoid not palpable, and liver dulness normal. He was put on ipecac treatment, and on the thirtieth day all symptoms of dysentery had disappeared. Encysted *Entamoeba histolytica*, however, continued to be present in his stools. On the seventieth day there was a relapse of the dysentery with motile entamebæ in the bloody mucous stools. Cultures of his stools on Musgrave and Clegg's medium were again negative. The patient was again put on ipecac treatment, the symptoms soon abated, and, up to the present time, he has suffered no further relapse, but encysted entamebæ soon reappeared in his stools and have persisted ever since. He has been under observation for one year since the beginning of this experiment.

**Experiment XLII.**—Man 29, aged 35 years, had been under observation in the prison for seven years and ten months. He had not been used previously for experiments, and had no history of dysentery. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amoeboid organisms were negative. He ingested cysts of *Entamoeba histolytica*, strain A, mixed with magnesium oxide. Following the ingestion, both cultures and microscopic examination of the stools of man 2 were negative. Therefore, this man failed to
become parasitized with *Entamoeba histolytica*. In order to determine whether this man possessed a relative or an absolute immunity, he ingested cysts of *Entamoeba histolytica* a second time on the seventy-sixth day after the first. The infectious material was from the same "carrier" as the first ingestion, now convalescent one hundred thirty-six days, but still showing encysted *Entamoeba histolytica* in his stools, and was mixed with magnesium oxide. Following this ingestion experiment, cultures of his stools were negative. Microscopic examination showed many small *Entamoeba histolytica* in his stools on the seventy-ninth day, or three days after this second feeding, but subsequent examinations were negative. The entamoebae evidently had failed again to establish themselves permanently as parasites in the intestine of this man. On the one hundred second day he ingested for the third time entamoebae from the same "carrier," now convalescent one hundred sixty-two days. Following this ingestion experiment, cultures of the stools of this man were negative for amoebe. Microscopic examination showed encysted *Entamoeba histolytica* on the one hundred seventh day, or the fifth day after this last ingestion, and the entamoebae have persisted in his stools ever since. This man has been under observation nine months since the last ingestion experiment, and he has not shown any dysenteric symptoms.

*Experiment XLIII.*—Man 30, aged 25 years, had been under observation in the prison seven years and five months. He had a negative dysenteric history, and had not been used for previous experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amoeboid organisms were negative. He ingested cysts of *Entamoeba histolytica*, strain *A*, mixed with magnesium oxide. Following the ingestion, cultures of the stools of man 30 were negative for amoebe. Microscopic examination of his stools showed encysted *Entamoeba histolytica* on the day following the ingestion, and the entamoebae have persisted in his stools ever since. On the ninety-fifth day a stool from this man was semifluid and greenish, contained mucus, a little blood, and many motile and resting *Entamoeba histolytica*, some of which contained red blood corpuscles. No examination of his stools had been made for six days previously, and another stool was not obtained until the second day after this examination. At the former examination the stool was soft and no entamoebae were found; at the latter examination the stool was formed and contained a few encysted entamoebae. *Entamoeba histolytica* had been found in his stools eight days previous to this slight attack of dysentery. Physical examination of this man was negative. He has been under observation one year since the beginning of the experiment. No relapse of the dysentery has occurred, but the entamoebae have persisted in his stools.

**ENTAMOEBA HISTOLYTICA, STRAIN A, SECOND PASSAGE, SERIES 1**

In this series of experiments strain *A* of *Entamoeba histolytica* had been passed from the original case, convalescent sixty days from an attack of spontaneous entamoebic dysentery, through man 2. Man 2 had been infected one hundred forty-four days previously with strain *A*, had developed an attack of entamoebic dysentery one hundred twenty-five days, with a relapse seventy-four days, previously (experiment XLI), and was now a "con-
valvescent carrier,” passing cysts of *Entamoeba histolytica* in his formed stools.

Experiment XLIV.—Man 31, aged 36 years, had been under observation in the prison six years and nine months. He had a negative history for dysentery, and had not been used previously for experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba histolytica*, strain A, second passage, series 1, mixed with magnesium oxide. Cultures of the stool of man 31 following the feeding were negative for amœbæ. Microscopic examination of his stool showed *Entamoeba histolytica* on the fifth day. This man has been under observation one year since the experiment began. The entamœbæ have been present in the stools of this man up to the present time, but no symptoms of dysentery have developed.

Experiment XLV.—Man 32, aged 39 years, had been under observation in the prison four years and eight months. He had a negative history for dysentery, and had not been used for previous experiments. Physical examination of his abdomen and cultural and microscopic examination of his stools for amœboid organisms were negative. He ingested cysts of *Entamoeba histolytica*, strain A, second passage, series 1, mixed with magnesium oxide. Cultures of the stools of man 32 after the ingestion were negative. Microscopic examination of his stools showed *Entamoeba histolytica* on the third day after ingestion and thereafter. This man was under observation five and one-third months after the experiment began. No dysenteric symptoms developed.

Experiment XLVI.—Man 33, aged 30 years, had been under observation in the prison two years and seven months. He had a negative dysenteric history, and had not been used for experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amœboid organisms were negative. This man ingested cysts of *Entamoeba histolytica*, strain A, second passage, series 1, mixed with magnesium oxide. Following the ingestion, cultures of the stools of man 33 were negative. *Entamoeba histolytica* was found microscopically in his stools on the fifth day and thereafter. He has been under observation one year since the experiment began. No symptoms of dysentery have developed.

**ENTAMOEBA HISTOLYTICA, STRAIN A, SECOND PASSAGE, SERIES 2**

This series of experiments was conducted with strain A which had been passed through man 29 (experiment XLII), who had been parasitized with this strain of *Entamoeba histolytica* one hundred twenty-one days previously, but who had not, and has not subsequently, developed dysentery. This series of experiments, therefore, was made with *Entamoeba histolytica* from a “contact carrier.”

Experiment XLVII.—Man 36, aged 45 years, had been under observation in the prison eight years and five months. He had a negative
dysenteric history, and had not been used previously for experiments. Physical examination of his abdomen and cultural and microscopic examination of his stools for ameboid organisms were negative. This man ingested encysted *Entamoeba histolytica*, strain A, second passage, series 2, mixed with powdered starch. Cultures of the stool of man 36 after the ingestion were negative for amebae. Microscopic examination of his stools showed *Entamoeba histolytica* on the fourth day after the ingestion. This man has been under observation one year since the experiment began. *Entamoeba histolytica* has been found constantly in his stools, but no dysentery has developed.

*Experiment XLVIII.*—Man 7, aged 30 years, had been under observation in the prison five years and two months. This man gave a history of 3 attacks of dysentery, each of one week’s duration, six years ago. He had been used previously for the following experiments. Two hundred forty-six days previously he had ingested a culture of *Amoeba 9F*. Result negative (part II, experiment XIII). Two hundred twenty-five days previously he had ingested a culture of *Amoeba 8A*. Result negative (part II, experiment XI). Two hundred fifteen days previously he had ingested a culture of *Amoeba 10G*. Result negative (part I, experiment XVI). One hundred fifty-seven days previously he had ingested a culture of *Amoeba 8B*. Result negative (part II, experiment VI). Physical examination of his abdomen and cultural and microscopic examinations of his stools for ameboid organisms were negative. He ingested cysts of *Entamoeba histolytica*, strain A, second passage, series 2, mixed with powdered starch. Cultures of the stools of man 7 after the ingestion were negative for amebae. Microscopic examination of his stools showed *Entamoeba histolytica* on the fourth day after the ingestion. This man has been under observation one year since the experiment began. The entamebæ have persisted in his stools, but no dysentery has developed.

**Entamoeba histolytica, strain A, third passage**

The history of strain A of *Entamoeba histolytica* employed in this series of experiments is as follows. Coming originally from a man convalescent sixty days from a slight attack of entamebic dysentery, it has been passed through men 29 and 36. Man 29 had been parasitized with this strain for one hundred sixty-one days, without the development of dysentery, when it was passed through man 36. Man 36 had been parasitized one hundred twenty-one days with the strain, without the development of dysentery, when it was used for the present series of experiments. Neither man 29 nor 36 has subsequently developed symptoms of dysentery although both have shown the cysts of *Entamoeba histolytica* more or less constantly in their stools up to the present time. Therefore, this strain of entamebæ had been passed from a “convalescent carrier” through 2 “contact carriers” before being employed in the present series of experiments.
Experiment XLIX.—Man 41, aged 50 years, had been under observation in the prison three years and seven months. He had a negative dysenteric history. No physical examination was made of his abdomen. He had not been used for previous experiments. His stools were negative, both culturally and microscopically, for amœboid organisms. He ingested cysts of Entamoeba histolytica, strain A, third passage, mixed with magnesium oxide. Cultures of the stools of man 41 after the ingestion were negative for amœbæ. Microscopic examination of his stools showed Entamoeba histolytica on the first day after feeding and thereafter. This man developed dysentery on the fifty-seventh day with abdominal pain and bloody mucous stools containing motile Entamoeba histolytica. The dysentery lasted two weeks. He was treated with ipecac, has recovered, and has had no relapses. Entamoeba histolytica disappeared temporarily from this man’s stools following the ipecac treatment, but reappeared shortly and has persisted ever since.

Experiment L.—Man 42, aged 30 years, had been under observation in the prison four years and eleven months. His history was negative for dysentery. No physical examination was made of his abdomen. He had not been used previously for experiments. Cultural and microscopic examinations of his stools for amœboid organisms were negative. He ingested encysted Entamoeba histolytica, strain A, third passage, mixed with magnesium oxide. Cultures of the stools of man 42 after the ingestion were negative for amœbæ. Microscopic examination of his stools showed Entamoeba histolytica on the fourth day and constantly thereafter. He has been under observation one year since the experiment began. No dysenteric symptoms have appeared up to the present time.

Experiment LI.—Man 43, aged 38 years, had been under observation in the prison three years and two months. His history was negative for dysentery. No physical examination was made of his abdomen. He had not previously been used for experiments. His stools were negative, culturally and microscopically, for amœboid organisms. This man ingested encysted Entamoeba histolytica, strain A, third passage, mixed with magnesium oxide. Cultures of the stools of man 43 after the ingestion were negative for entamebæ. Microscopic examination of his stools have been constantly negative for entamebæ, and he has been reserved as a non-parasitized control. He has been under observation one year since the experiment began. No dysentery has developed up to the present time.

Experiment LII.—Man 44, aged 27 years, had been under observation in the prison one year and three months. His history was negative for dysentery. No physical examination was made of his abdomen. He had not been used for previous experiments. Cultures and microscopic examinations of his stools for amœboid organisms were negative. This man ingested encysted Entamoeba histolytica, strain A, third passage, mixed with magnesium oxide. Cultures of the stools of this man after the ingestion were negative for amœbæ. Microscopic examinations of his stools up to the sixth day were negative for entamebæ. By an oversight his stools were not examined again until the thirty-third day, when Entamoeba histolytica was found, and has been found more or less constantly ever since, in his stools. This man has been under observation one year since the experiment began. He has shown no symptoms of dysentery up to the present time.
This strain of *Entamoeba histolytica* was from a man suffering from an acute attack of entamoebic dysentery, whose dysenteric stools contained many motile entamoebae. The strain was used in the following two experiments, and there were no subsequent passages of it.

**Experiment LIII.**—Man 5, aged 30 years, had been under observation in the prison for seven years and six months. He gave a history of a mucous dysentery of one month's duration seven years ago. He had been used for a feeding experiment with a culture of *Amoeba* 11G, two hundred fifty-seven days previously (part II, experiment XVII). The result of this experiment was negative. At the time of the present experiment, physical examination of his abdomen and cultural and microscopic examinations of his stools for ameboid organisms were negative. He ingested motile *Entamoeba histolytica*, strain B. After the ingestion, cultures of the stools of man 5 were negative for amebae. Microscopic examinations of his stools were negative for several weeks, and he was considered as a nonparasitized control. However, on the forty-fourth day *Entamoeba histolytica* was found in his stool and has persisted ever since. He has been under observation one year since the beginning of the experiment. No dysenteric symptoms have appeared.

**Experiment LIV.**—Man 34, aged 30 years, had been under observation in the prison six years. He had a negative dysenteric history, and had not been used previously for experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for ameboid organisms were negative. He ingested motile *Entamoeba histolytica*, strain B. Following the ingestion, cultures of the stools of man 34 were negative for amebae. *Entamoeba histolytica* was found microscopically in the stools of this man on the fourth day after the ingestion and has persisted ever since. This man was under observation nine months after the beginning of this experiment. No symptoms of dysentery developed.

**ENTAMOEBA HISTOLYTICA, STRAIN C**

Strain C of *Entamoeba histolytica* was obtained post mortem from a fatal case of entamoebic liver abscess. The patient from whom these entamebæ were obtained died at 11 o'clock in the morning, a necropsy was performed at 1.30, and the entamebæ were ingested by men 3 and 35 at 2.30 in the afternoon. There were no subsequent passages of this strain.

**Experiment LV.**—Man 3, aged 31 years, had been under observation in the prison six years. He had a history of dysentery of one month's duration sixteen years ago. He had been previously used for the following experiments. Two hundred seventy-nine days previously he ingested a culture of *Amoeba* 1A. Result negative (part II, experiment I). Two hundred sixty-seven days previously he had ingested a culture of *Amoeba* 1A. Result negative (part II, experiment III). Two hundred forty-three days previously he had ingested a culture of *Amoeba* 10G. Result negative (part II, experiment XV). Two hundred eight days previously
he had ingested a culture of *Amoeba 5D*. Result negative (part II, experiment IX). Physical examination of his abdomen and cultural and microscopic examinations of the stools for amoeboid organisms were negative at the time of the present experiment. This man ingested motile *Entamoeba histolytica*, strain C, mixed with magnesium oxide. Following the ingestion, cultures of the stools of this man were negative for amœbæ. *Entamoeba histolytica* was found microscopically in his stools on the day following the ingestion and has persisted in his stools ever since. This man was under observation two months after the experiment began. No symptoms of dysentery developed.

*Experiment LVI.*—Man 35, aged 30 years, had been under observation in the prison six years and nine months. He had a negative history for dysentery, and had not been used for previous feeding experiments. Physical examination of his abdomen and cultural and microscopic examinations of his stools for amoeboid organisms were negative. He ingested motile *Entamoeba histolytica*, strain C, mixed with magnesium oxide. This man has been under observation one year since the experiment began. Cultures and microscopic examination of his stools have been constantly negative for amoeboid organisms. He has consequently been reserved as a nonparasitized control. This man has shown no dysenteric symptoms.

**ENTAMŒBA HISTOLYTICA, STRAIN D**

Strain D consisted of encysted entamoebae from a naturally parasitized "contact carrier" of *Entamoeba histolytica*. This woman had no history of entamoebic dysentery, but her stools contained a moderate number of encysted *Entamoeba histolytica*. This strain was used in 4 experiments, and there were no subsequent passages of it.

*Experiment LVII.*—Man 37, aged 32 years, had been under observation in the prison seven years and eight months. He had a history of an acute dysentery four years previously. He had not been used for previous experiments. Physical examination of his abdomen and microscopic and cultural examinations of his stools for amoeboid organisms were negative. He ingested cysts of *Entamoeba histolytica*, strain D, mixed with powdered starch. Following the ingestion, cultures of the stools of man 37 were negative. Microscopic examination of his stools showed *Entamoeba histolytica* on the eighth day after feeding. This man was under observation eight months and seventeen days after the experiment began. The entamoebæ have been found constantly in his stools, but no symptoms of dysentery have developed.

*Experiment LVIII.*—Man 38, aged 37 years, had been under observation in the prison six years and eight months. Dysenteric history was negative, and he had not been used for previous experiments. No physical examination of his abdomen was made. Microscopic and cultural examinations of his stools for amoeboid organisms were negative. He ingested cysts of *Entamoeba histolytica*, strain D, mixed with powdered starch. Cultures of the stools of man 38 following the ingestion were negative. Microscopic examination of his stools showed *Entamoeba histolytica* on the twenty-first day after the ingestion and more or less constantly ever since. This man has been under observation one year since the beginning of the experiment. No dysenteric symptoms have developed.
Experiment LIX.—Man 40, aged 25 years, had been under observation in the prison eight years and eight months. He had a negative dysenteric history. No physical examination of his abdomen was made. He had not been used for previous experiments. Microscopic and cultural examinations of his stools were negative for amœboid organisms. He ingested cysts of Entamoeba histolytica, strain D, mixed with powdered starch. Cultures of the stools of man 40 after the ingestion were negative. Microscopic examination of his stools showed Entamoeba histolytica on the eleventh day after the experiment began and more or less constantly thereafter. Since then he has been under observation one year, but has had no symptoms of dysentery.

Experiment LX.—Man 39, aged 32 years, had been under observation in the prison six years and two months. He had a negative dysenteric history. No physical examination of his abdomen was made. He had not been used previously for experiments. Microscopic and cultural examinations of his stools were negative for amœboid organisms. He ingested cysts of Entamoeba histolytica, strain D, mixed with powdered starch. Cultures of the stools of man 39 following the ingestion were negative. Microscopic examination of his stools showed Entamoeba histolytica on the eleventh day after feeding and more or less constantly ever since. On the eighty-seventh day this man had a slight dysentery with abdominal pain and motile Entamoeba histolytica in his bloody mucous stools, which lasted only one day and from which he recovered without treatment. He has been under observation one year since the beginning of the experiment, but has had no relapse of the dysentery.

These protocols are summarized in Table III.

As the protocols and Table III show, amœboid organisms could not be recovered in cultures on Musgrave and Clegg's medium from the stools of any of the men who had ingested Entamoeba histolytica. On the other hand, Entamoeba histolytica has been found microscopically in the stools of every man who became parasitized, and the entamebæ have persisted in the stools of these men for an indefinite time. Therefore, it is demonstrated experimentally that Entamoeba histolytica, like Entamoeba coli, and in contrast to the Amœbe, is an obligatory parasite which cannot be cultivated on Musgrave and Clegg's medium.

Of the 20 men who ingested Entamoeba histolytica, 17 became parasitized at the first feeding, 1 required 3 successive feedings before becoming permanently parasitized, and 2, who did not become parasitized at the first feeding, were reserved as controls. Of the 16 men who ingested encysted Entamoeba histolytica, 14, or 85.5 per cent, and of the 4 men who ingested motile Entamoeba histolytica, 3, or 75 per cent, became parasitized. However, all of the men who ingested motile entamebæ had the acidity of the contents of their stomachs neutralized with magnesium oxide. It is doubtful whether so large a percentage of them would become parasitized under natural con-
ditions. All 6 of the men who ingested encysted *Entamoeba histolytica* without neutralizing the acidity of the gastric juices became parasitized.

The incubation period of the parasite, that is, the period of time elapsing between the ingestion of the infectious material and the appearance of the entamœbæ in the stools, is, as shown in these experimentally parasitized men, from one to forty-four days, with an average of nine days. In one case, man 44, in which microscopic examination of the stools was by an oversight not made between the sixth and thirty-third day, *Entamoeba histolytica* was not found on the sixth and was found on the thirty-third day. If we exclude this case and that of man 5, who ingested motile entamœbæ and who had an abnormally long incubation period, forty-four days, the average incubation period of the parasite would be 5.7 days, which is approximately the same as in the case of *Entamoeba coli*. It is probable that the number of entamœbæ ingested would in part account for the considerable variation in the incubation period in the different men.

Of the 18 men experimentally parasitized with *Entamoeba histolytica*, 4, or 22 per cent, have developed entamœbic dysentery. Two of these cases, men 30 and 32, had only slight attacks of dysentery, lasting one or two days, and recovered without treatment. The other 2 cases, men 2 and 41, had more severe attacks of dysentery lasting one or more weeks, and recovered only after receiving ipecac treatment. One of the latter, man 2, had a relapse thirty days after recovery which persisted until treated.

The 4 cases of experimental dysentery were obtained with material from 3 different sources and representing 2 distinct strains of *Entamoeba histolytica*. Men 2 and 30 were infected with entamœbæ from the same individual, man A, who was convalescent eighty-four days from a slight attack of entamœbic dysentery of two days' duration. Man 41 ingested a strain of *Entamoeba histolytica* coming originally from the same source as in cases 2 and 30, but which had been passed through two other men, 29 and 36, who did not develop and have not subsequently developed dysentery, before being ingested by man 42. Case 39 was obtained after the ingestion of a distinct strain of *Entamoeba histolytica*, strain D, which came from a "contact carrier" of *Entamoeba histolytica* without symptoms or previous history of dysentery.

The passages of the 4 strains of *Entamoeba histolytica* in these
experiments and the cases of experimental dysentery are shown in the following diagram:

Diagram.

**Strain A.**
"Convalescent carrier."

2  
Dysentery.  

29  
"Contact carrier."

30  
Dysentery.

31 32 33  

7  
"Contact carrier."

36  

41 42 43 44  
Dysentery.  Did not become parasitized.

**Strain B.**
Acute entamoebic dysentery.

5 34 3

**Strain C.**
Liver abscess.

3  
Did not become parasitized.

**Strain D.**
"Contact carrier."

35 37 38 39 40  
Dysentery.

The incubation periods of these cases of experimental dysentery were twenty, ninety-five, eighty-seven, and fifty-seven days, respectively, with an average of 64.8 days. It is possible that the incubation period might in certain cases be shorter than
twenty days; on the other hand, on account of the latency characteristic of this disease, it is probable that the incubation period may often be much longer than ninety-five days.

The large percentage of latent infections (78 per cent) among these experimentally parasitized men was somewhat unexpected. However, the frequent occurrence of latent infections in entamoebic dysentery has been noted by a number of authors, and is well known to every clinician and pathologist working in the Tropics.

Vincent (1909) has reported cases of entamoebic dysentery in soldiers returned from French Indo China, of whom some had never suffered from dysentery and others had recovered from attacks while in the Tropics. After their return to France, where infection was considered impossible, these men showed not the slightest intestinal symptoms for three, six, and, in 1 case, eleven months, and then developed attacks of entamoebic dysentery. In such cases Vincent observed that the attacks followed certain adjuvant causes, such as indigestion, alcoholic excess, chill, or excessive and prolonged fatigue.

Musgrave published in 1910 a study of 50 cases of intestinal entamoebiasis without diarrhœa which came to necropsy. Two of these cases showed a perforation of the appendix from entamoebic ulcerations, 5 had perforations of entamoebic ulcers of the large intestine, and 4 had entamoebic liver absceses. The necropsies of the other 39 cases showed more or less extensive entamoebic ulcerations of the large intestine. None of these cases had bloody mucous stools or even diarrhœa; indeed, constipation was a characteristic feature of several of them up to the time of death.

Moreover, individuals who are infected with the pathogenic microorganism, but who show no clinical manifestations of the specific disease, are well known in most, if not all, infectious diseases. As an example of the prevalence of such "contact carriers," which is comparable with the condition found in entamoebic dysentery, the results of the investigation carried out under the auspices of the Medical Commission for the Investigation of Acute Respiratory Diseases of the Department of Health of the City of New York may be cited. This investigation disclosed the fact that pneumococci could be isolated from the

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a Dock (1891), Councilman and Lafleur (1892), Musgrave (1905), Martini (1908), Vincent (1909), Musgrave (1910).

b Park and Williams (1905), Longcope and Fox (1905), Duval and Lewis (1905), Buerger (1905).
mouts and throats of from 53 to 100 per cent of healthy persons, and that from 69 to 83 per cent of these organisms proved to be virulent on inoculating them into animals.

Therefore, it appears that the relatively small number of cases of dysentery obtained up to the present time in the men experimentally parasitized with *Entamoeba histolytica* is thoroughly consistent with our knowledge of the prevalence of latent infections in this disease. It should also be borne in mind that not *Entamoeba histolytica* alone, but all of the microorganisms contained in the dysenteric stool, were fed in each case and, consequently, the small percentage of dysenteries resulting from these feedings has no bearing upon the etiology, but is evidence only of the frequent latency, of this disease.

To what extent this latency, which is characteristic of entamoebic dysentery, is due to the chronicity of the ulcerative process, and whether or not the inability of *Entamoeba histolytica* to penetrate the healthy intestinal epithelium plays a part in it, cannot at present be definitely answered. In the latter case the entamebæ might be conceived to live as commensals in the intestine of their host, and only when there occurred some depression of the natural resistance of the host or of its tissues or some inflammation or actual lesions in the intestine, were the entamebæ able to penetrate the intestinal epithelium, become tissue parasites, and produce the characteristic lesions of entamoebic dysentery.

It was hoped that information on this aspect of the subject might be obtained by post-mortem examination of men who had been parasitized for a considerable time with *Entamoeba histolytica*, but who had never shown any dysenteric symptoms. However, only one necropsy has been obtained. This man, who had been parasitized with *Entamoeba histolytica* for one hundred sixty-five days without showing any dysenteric symptoms, died of pulmonary and intestinal tuberculosis. Necropsy showed an extensive ulceration of the small intestine and cæcum which was clearly tubercular. In the lower large intestine there were a few small ulcers of doubtful anatomical character. Sections of these ulcers, however, showed no entamebæ, but only large numbers of tubercle bacilli. The tubercular lesions in this case would seem to have afforded openings in the intestinal epithelium for the entrance of the entamebæ, but the products of the tubercular ulcerations may have been inimical to the entamebæ, and it is consequently unsafe to draw any conclusions from this case.
A further attempt was made to solve the question of latent infections by the experimental infection of animals, which could be killed at different times after infection and in which the behavior of the entamœbæ in the intestine could be studied post mortem. In these experiments 2 monkeys were fed repeatedly with fæces containing encysted *Entamœba histolytica*; 1 monkey was fed a dysenteric stool; 1 monkey received, *per œsophagus* after washing out the stomach with a suspension of magnesium oxide, a bloody mucous stool by stomach tube; 1 monkey received an injection of dysenteric stool into the rectum; 1 monkey received an injection of dysenteric stool into the cæcum; and 1 monkey received an inoculation of entamœbic liver-abscess pus into the liver. Two cats were fed encysted *Entamœba histolytica*, and 6 kittens received rectal injections of dysenteric stools containing motile *Entamœba histolytica*. One young pig was twice fed large numbers of encysted entamœbæ. All of the animals not only did not develop dysentery, but in no case have they become parasitized with *Entamœba histolytica*. These results are surprising in view of the experience of other authors who have found animals, especially cats and monkeys, readily infected with entamœbæ by feeding or rectal injections of dysenteric fæces. My results, while not numerous enough absolutely to exclude the possibility of parasitizing animals with *Entamœba histolytica*, at least indicate that they are less readily parasitized than men, 90 per cent of whom became parasitized in my experiments. In consequence of these negative results from attempts to infect animals with *Entamœba histolytica*, no further information was obtained in these experiments on the nature of latency in entamœbic dysentery.

Some light by analogy may be thrown on this subject by the results of experimental infections of monkeys with *Balantidium coli* which are now in progress. *Balantidium coli* produces a serious and often fatal dysentery in man, but latent infections which give rise to no clinical symptoms are as prevalent as in infections with *Entamœba histolytica*. In the experimental infections of monkeys, which almost invariably become parasitized after feeding encysted *Balantidium coli*, there is the same latency of clinical symptoms as in the human infections. One parasitized monkey killed one month after infection showed a slight colitis with the balantidia penetrating the sound tissues of the mucosa. Another monkey killed two months after infection showed no colitis or ulcerations. And a third monkey, which was dying five months after infection, was chloroformed, and at necropsy the large intestine was found to have an extensive
ulcerative colitis, although no dysentery had manifested itself during the five months the monkey had been infected. Therefore, so far as these experiments have gone, it appears that latent balantidiasis in the monkey may be due to several different causes, namely: (1) In certain cases to the failure of the balantidia to penetrate the tissues of the intestine, (2) in other cases to the chronicity of the ulcerative process, and (3) to the fact that more or less extensive colitis and ulceration may exist in the intestine of the infected animal without any dysenteric symptoms. It is conceivable, indeed probable, that the latency of entamoebic dysentery is due to similar causes.

Notwithstanding that the prevalence of latent infections has caused the results of the experimental infections with Entamoeba histolytica to appear less clean-cut than the results with the Amœbe and with Entameba coli and has rendered the nonparasitized controls of these experiments inconclusive, nevertheless, I am of the opinion that these experiments supply evidence which, when considered with the morphological, clinical, and pathological data, is sufficient to establish beyond a reasonable doubt the etiologic relationship of Entamoeba histolytica to endemic tropical dysentery.

In the first place, it is to be noted that the uniformly negative results of the feeding experiments with the Amœbe and Entamoeba coli suffice to exclude them once for all from the etiology of this disease. Therefore, the question of the etiologic factor must lie between Entamoeba histolytica and some unidentified microorganism present in dysenteric stools.

If the entamoebæ are only commensals, the multiplication of which is favored by the dysenteric condition and which invade the dysenteric lesions as a secondary infection, it would be expected that the common Entamoeba coli would be most frequently associated with the disease; on the contrary, it is the relatively rare Entamoeba histolytica only that is constantly found in the stools and lesions of entamoebic dysentery.

Post mortem, the entamoebæ are found not only in the lesions, but in the sound tissues underlying the lesions, where they are usually the only microorganisms demonstrable. They penetrate between the cells of the sound mucosa, the submucosa, and even of the muscularis, where by their migrations and multiplication, aided probably by the secretion of a ferment, they produce histolysis. They are found in the lymph spaces and in the blood vessels in which they are carried by the way of the portal circulation to the liver and give rise to abscesses as sequelæ to the intestinal infection. In these liver abscesses, which are usually
bacteriologically sterile, *Entamoeba histolytica* is found not so much in the necrotic material as in the sound tissues at the borders of the abscess.

Anatomically and histologically both the intestinal and liver lesions are characteristic and do not correspond to lesions due to other microorganism. The typical ulcerations are of the so-called undetermined type, which undoubtedly result from the widespread wandering of *Entamoeba histolytica* in, and the consequent histolysis of, the tissues underlying the intestinal epithelium. The cellular reaction about those lesions, when uncomplicated by secondary bacterial invasion, is not inflammatory but regenerative in character and consists not of polymorphonuclear leucocytes but of formative cells and lymphocytes. In liver abscesses, which are less frequently complicated by secondary bacterial infection than are the intestinal lesions, the nature of the morbid process is most clearly perceived. The so-called pus of these abscesses is not pus at all, but chiefly cellular detritus resulting from the histolysis of the liver tissue by *Entamoeba histolytica*.

In the experimental infections with *Entamoeba histolytica* no dysenteries have developed in the cases parasitized from a case of acute entamebic dysentery, in those from an entamebic liver abscess, nor in those who did not become parasitized with *Entamoeba histolytica*, although the acidity of the stomach contents of all of these men was neutralized with magnesium oxide at the time of ingestion of the infectious material. If a bacterium or other unidentified microorganism should be concerned in the etiology of this disease, it would seem that these feeding experiments were made under conditions most favorable to secure infection.

On the other hand, all of the experimental dysenteries were obtained after ingesting *Entamoeba histolytica* from healthy persons who were "carriers" of this parasite. Case 2 developed dysentery after ingesting *Entamoeba histolytica* from a man convalescent fifty-nine days from a slight spontaneous attack of entamebic dysentery, who has not subsequently shown any symptoms of dysentery, and who was, therefore, a "convalescent carrier" of *Entamoeba histolytica*. Case 30 resulted from the ingestion of material from the same carrier convalescent one hundred sixty-one days. Case 39 suffered an attack of entamebic dysentery after ingesting *Entamoeba histolytica* obtained from a person who had not, and has not subsequently, developed
dysentery; that is, a "contact carrier." And case 41 developed entamoebic dysentery after ingesting *Entamoeba histolytica* that had been passed through 2 "contact carriers." The history of this strain of *Entamoeba histolytica* is as follows. It was originally from the same convalescent carrier (man A) from whom cases 2 and 30 were infected. *Entamoeba histolytica*, from this "carrier" convalescent fifty-nine days, was ingested by man 29, who became parasitized, but did not develop dysentery. *Entamoeba histolytica* from this "contact carrier," one hundred twenty-one days after infection, was next ingested by man 36, who likewise became parasitized, but did not develop dysentery. Finally, *Entamoeba histolytica* from this second "contact carrier," one hundred thirty-four days after infection, was ingested by man 41, who became parasitized with *Entamoeba histolytica* and developed entamoebic dysentery after fifty-seven days. Therefore, three hundred seventy-one days and the passage through 2 "contact carriers"—who had not, and have not subsequently, developed dysentery—separated this experimental case from the spontaneous case of entamoebic dysentery, a gap bridged by the innumerable and continuous generations of *Entamoeba histolytica* in 4 different men.

No case of spontaneous entamoebic dysentery has occurred in this ward during the period of these experiments with *Entamoeba histolytica*.

Therefore, it is believed that the results of these experiments warrant the conclusions that *Entamoeba histolytica* is a strict or obligatory parasite, that it cannot be cultivated on Musgrave and Clegg's or other ordinary culture media, and that this entamoeba is the essential etiologic factor in endemic tropical dysentery.

PART V. APPLICATION OF THE RESULTS TO THE DIAGNOSIS, TREATMENT, AND PROPHYLAXIS OF ENTAMOEIC DYSENTERY

By Ernest Linwood Walker

The information concerning the etiology and endemiology of entamoebic dysentery and the morphology, biology, and parasitism of *Entamoeba histolytica*, which has been obtained in the course of this experimental investigation, is believed to be of the greatest value for the diagnosis, treatment, and prophylaxis of this important tropical disease.

The identification of the specific microorganism in the laboratory constitutes the final word in diagnosis, upon which must
be based the treatment and prophylaxis of an infectious disease. On account of the relatively long incubation period usually existing in entamebic dysentery, the prevalence of chronic and latent cases of the disease, and the frequent inefficiency of treatment to kill all of the entamebæ in the intestine, the microscopic identification of the pathogenic Entamoeba histolytica is of particular importance in the diagnosis, the control of treatment, and the prophylaxis of entamebic dysentery. Hitherto, on account of the uncertainty existing as to the specific entameba concerned in the production of this disease and the supposed difficulty of identifying the organism, especially in the resting and encysted stage, the microscopic diagnosis of infections with Entamoeba histolytica has been subject to many errors; indeed, in many laboratories, no attempt is made to distinguish between the pathogenic species and the common, harmless Entamoeba coli, or to diagnose latent and chronic infections. An extensive practical experience gained in the course of this investigation has demonstrated that the microscopic diagnosis of infections with Entamoeba histolytica can be made with certainty, and has disclosed many practical points in the technique and the application of this diagnosis. Therefore, it is believed that a somewhat extended treatment of this subject is warranted.

The material for the microscopic examination for Entamoeba histolytica should be a stool obtained, contrary to the prevailing practice, without the previous administration of a purgative. In stools obtained after a purgative, Entamoeba histolytica, if present in the fluid stool, is in a preëncysted stage at which it most closely resembles the nonpathogenic species, Entamoeba coli; consequently, a differential diagnosis between the two species is difficult and often impossible.

It may be objected that without a purgative infections with Entamoeba histolytica will frequently be overlooked. However, such is not the case. It has been my experience in following many cases of entamebic infection with daily stool examinations, including cases doubly infected with Entamoeba histolytica and Entamoeba coli, that the entamebæ are rarely absent from the normal stools several successive days and that Entamoeba histolytica is more constantly present, and usually present in larger numbers, in the stools of infected persons than is Entamoeba coli. In 930 microscopic examinations made of stools, without the previous administration of a purgative, from men known to be parasitized with Entamoeba histolytica, and who were not undergoing treatment, the entamebæ were found 664 times, or 71.39 per cent; that is, in nearly 3 out of every 4 of such exami-
infections. Moreover, the negative results were based on the examination of a single coverslip which was often hurriedly made. The examination under similar conditions of 303 stools of men known to be parasitized with *Entamoeba coli* showed the entamoebae in 171, or 56.44 per cent of the examinations; in other words, in about 1 out of every 2 of such examinations.

A further objection, that may be raised to the examination of formed stools, is the fact that in such stools usually only encysted entamoebae are to be found. It is an opinion generally held, and which is supported by the statement in all textbooks, that a positive diagnosis of entamoebic infection should never be made unless motile entamoebae are observed. It is of the greatest importance, however, for the diagnosis of chronic and latent infections that one should be able to distinguish resting and encysted entamoebae from other bodies found in faeces and to differentiate the cysts of *Entamoeba histolytica* from those of *Entamoeba coli*. This can be done with certainty by the experienced protozoologist. The majority of the 1,233 examinations mentioned in the preceding paragraph were made of formed stools containing nonmotile and encysted, chiefly encysted, entamoebae. Moreover, it is the encysted stage of the entamoeba that furnishes the most unequivocal characters for the differentiation of the pathogenic *Entamoeba histolytica* from the harmless *Entamoeba coli*.

In the examination of liver-abscess pus for *Entamoeba histolytica*, the pus first obtained after the operation usually does not contain entamoebae; frequently they appear in the pus from the drainage tube only after several days. The explanation of this is to be found in the fact that the entamoebae are not found in the pus of the abscess, but only in the tissues at the borders of the abscess. Consequently, it is only when the borders of the abscess begin to slough off that the entamoebae appear in the drainage from the abscess. Therefore, a negative diagnosis of entamoebic liver abscess should never be made except after negative examinations obtained for several successive days after operation.

Dysenteric or diarrheal stools should be examined as soon as possible after they are passed, since the motile entamoebae present in such stools quickly die and disintegrate. On the other hand, in the formed stools of chronic and latent infections, the encysted entamoebae persist unchanged for days, and consequently the examination can be made at one's leisure.

In making the examination, a small platinum loopful of the fluid or semifluid material should be placed on a microscope
slide and the cover-glass dropped upon it. Slight pressure may be exerted, if necessary, upon the cover-glass with the forceps to cause the material to flow as a thin film between the cover-glass and slide. If the stools be more or less formed, a small drop of water should be placed upon the slide and a minute portion of the stool rubbed up in it, forming a fairly thick suspension of the feces in the water, upon which the cover-glass should be placed. A satisfactory preparation must be thin, but there should not be an excess of fluid which will permit the cover-glass to float about when the oil-immersion objective is applied to it. A warm stage is not necessary for making the examination.

The advantage of a preliminary examination of the preparation with low magnification (Leitz 3 or Zeiss AA objective) cannot be overestimated. It enables the examiner to make a rapid survey of the whole preparation and to pick out the individual entamoebae for examination with the oil-immersion objective. When the entamoebae are few in the preparation, they can be found with difficulty, if at all, with higher magnification. With a Leitz 3 or Zeiss AA objective and a 3 ocular, the entamoebae appear as round, oval, or irregular, colorless, and refractive dots which with proper focusing stand out distinctly in the background of the preparation. Practical experience will enable the microscopist to distinguish them from certain other bodies that are met with in stools. By applying the oil-immersion objective—most conveniently used with the dry objective on a revolving nose-piece—to every body in the preparation which looks suspicious under low magnification, this experience will soon be attained. Indeed, it is not only possible for the experienced microscopist to identify an entameba with the low magnification, but to distinguish a cyst of Entamoeba histolytica from one of Entamoeba coli with a considerable degree of certainty by the difference in its size and refractiveness.

A suspected entameba, having been located in the preparation with the low-power objective, should then be examined with the 1/12 oil-immersion objective. With this magnification the entamoebae present certain morphological characters that enable the experienced investigator to identify them whether they be in the motile, resting, or encysted stage.

The motile forms will give little difficulty, even to the novice, since their movements are characteristic.

The resting entamoebae is distinguished from other bodies found in the stool by its size, distinctness, regularity of contour, degree of refractiveness, and especially by its nuclear structure.
The entamæbæ vary in size within considerable limits, but are usually from 20 to 30 microns in diameter. They are, therefore, larger than pus cells, or other protozoa, with the exception of Balantidium coli, that are found in the stools of man. They are also more refractive than pus, epithelial, or other cells found in the stools. The nuclear structure of the entamæba is particularly characteristic. The unencysted entamæba possesses, unless in the process of division, only a single nucleus. This nucleus is round, or occasionally slightly oval or irregular, small with reference to the size of the cell, and appears not solid but as a refractive ring (Plate I, figs. 3, 5, 6, 7). This relatively small, ring-shaped nucleus appears to be absolutely diagnostic of an entamæba. Only one other kind of cell observed in stools possesses a nucleus in any way resembling that of an entamæba. This is an epitheloid cell, sometimes found in mucous stools, which has a ring-form nucleus relatively much larger than that of an entamæba, occupying one-fourth to one-half of the cell. While an entamæba may occasionally be observed with an abnormally large nucleus, probably preparatory to division, the nucleus never approaches the size of the nucleus of this epitheloid cell. The latter cells are also less refractive and granular than entamæbæ.

The encysted entamæba is round or slightly oval, more refractive than the resting or motile stage, and is surrounded by a more or less distinct cyst wall. The nuclear structure here also is characteristic. The cyst contains several (from 2 to 8, depending upon the species of entamæba and the stage of development of the cyst) ring-form nuclei usually smaller than, but of the same structure as, the nucleus of the motile entamæba (Plate I, figs. 4 and 8).

The technique and descriptions so far given refer to the examination of living entamæbæ in fresh stools. This method of stool examination for entamæbæ is the quickest and for general purposes of diagnosis the most satisfactory. The preparation of stained specimens takes more time and a more extensive technique, and certain distinctive characters of the entamæba are lost in the fixed and stained preparation. On the other hand, staining sometimes assists in bringing out the details of nuclear structure, and is necessary for making permanent preparations of entamæbæ.

The technique of fixing and staining entamæbæ which has given the most uniformly satisfactory results is as follows. A thin smear of the fresh faeces or liver-abscess pus is made on a cover-glass, fixed in sublimate-alcohol mixture or Zenker's
fluid for from five to fifteen minutes, thoroughly washed in distilled water, stained in aqueous alum haematoxylin from three to five minutes, washed in distilled water, passed through successive grades to absolute alcohol, cleared in xylol or oil origanum, and mounted in xylol-balsam. All of the stages of this process are most conveniently carried out by floating the cover-glass, preparation downward, upon the surface of the different liquids contained in watch glasses. The preparations should be fixed moist and should not be allowed to become dry throughout the process of staining and mounting.

The sublimate-alcohol mixture consists of 2 parts of a saturated aqueous solution of mercuric chloride and 1 part of absolute alcohol. The sublimate solution should be saturated warm and should be kept in stock. The absolute alcohol should be added in proper proportion only at the time of using, because alcohol evaporates and the solution changes in standing.

The aqueous alum haematoxylin has the following composition:

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematoxylin crystals</td>
<td>1</td>
</tr>
<tr>
<td>Saturated aqueous solution of ammonia alum</td>
<td>100</td>
</tr>
<tr>
<td>Distilled water</td>
<td>300</td>
</tr>
<tr>
<td>Thymol</td>
<td>a crystal</td>
</tr>
</tbody>
</table>

The haematoxylin crystals are dissolved in the water by the aid of heat, and the other substances added to the solution. The stain should be ripened for from a week to ten days in a flask or bottle loosely plugged with cotton. It is then ready for use and should be kept in a tightly stoppered bottle away from the light. It will keep in good condition for several months.

Bodies that are liable to be mistaken for entamœbæ in the stools include air bubbles, fat globules, starch or proteid grains, pus and epithelial cells of the host, and certain unicellular vegetable organisms. Of these air bubbles, fat globules, and starch or proteid granules of undigested food, while possibly deceptive with low magnification, should from their structure cause no difficulty when examined with high magnification. Stools containing mucus or pus often contain many cells which are confusing to the inexperienced microscopist. It will assist the observer if he remembers that these pus and epithelial cells with few exceptions are distinctly smaller than entamœbæ. It will, therefore, be necessary only to take into consideration cells which, when viewed with the low magnification, are distinctly larger than the average.

In faeces containing pus there are sometimes present large round cells of uncertain identity which in size and general appearance
closely resemble resting or encysted entamaebae. The cells contain from one to several small, round or irregular, refractive, nucleus-like bodies that stain like chromatin. It is possible that they are cells showing degenerative changes with fragmentation of the nucleus. These cells are, however, readily distinguished with high magnification from entamaebae by the structure of the nucleus-like bodies, which are not ring-form, but solid chromatin masses. Motile forms of entameba also will be frequently found in such stools, which will aid in the diagnosis.

Certain unicellular vegetable organisms known as Blastocytis hominis Brumpt, which are believed to be allied to the yeasts, are found rather frequently in the stools of man in the Tropics. Smaller forms of these cells have been mistaken for the cysts of Trichomonas intestinalis, and the larger forms simulate the encysted entamaebae very closely in size and general appearance. They are, however, slightly less refractive than the cysts of Entamæba, and can, therefore, be distinguished by the experienced observer, even with low magnification. Under high magnification they are seen to have a wholly different structure from the cysts of Entamæba. They are round, oval, or slightly irregular, and possess a distinct wall. The protoplasm is restricted to several narrow segments of the cell, and contains from one to several granules staining like chromatin. The main body of the cell is filled with a homogeneous, hyaline, slightly refractive, and often faintly yellow mass, the nature of which is doubtful, but it probably represents reserve food substance.

An examination of figs. 3 to 8 on Plate I will give a good idea of the general morphology of the entamaebae. Figs. 1 and 2 show the motile encysted stages of a typical nonparasitic amoeba for comparison with the entamaebae.

The differentiation of Entamæba histolytica from Entamæba coli depends upon certain morphological characters of the two species which are very distinctive at certain stages, but less distinctive at other stages, of the development of the two species. These stages of the development of the parasites are correlated with the clinical manifestations of the infection and especially with the consistence of the stools of the host. Therefore, the comparative morphology of Entamæba histolytica and Entamæba coli are most conveniently discussed in relation to the nature of the stools in which they are found; namely, (1) in dysenteric stools, (2) in diarrheal stools and stools after a purgative, and (3) in formed stools.
1. IN DYSENTERIC STOOLS

Both *Entamoeba histolytica* and *Entamoeba coli* occur only in the motile stage in dysenteric stools; and, when double parasitization exists, *Entamoeba histolytica* is usually the more numerous in such stools.

*Size.*—The size of both *histolytica* and *coli* are subject to wide variations, and little dependence can be placed on this character for diagnostic purposes. In dysenteric stools *histolytica* often appears larger than *coli* (Plate I, figs. 3 and 5). That this larger size of *histolytica* is only apparent, and not real, is probable from the fact that in the encysted stage (the only stage in which reliable measurements can be made) *histolytica* is almost invariably smaller than *coli* (Plate I, figs. 4 and 8). This apparently larger size of motile *histolytica* is probably connected with the more active movements of this species; while *coli* is sluggishly motile and tends to retain a more or less spherical shape, *histolytica* is actively motile and is extended flat over the surface of the substratum.

*Shape.*—*Entamoeba histolytica*, being more actively motile than *Entamoeba coli*, presents a more varied form to the observer. While *coli* is usually round, oval, or slightly irregular, *histolytica* is more often long oval, ligulate, or irregular in fresh dysenteric stools.

*Appearance.*—*Entamoeba histolytica* is hyaline and feebly refractive while *Entamoeba coli* is more porcelaneous and refractive in appearance.

*Motility.*—The amœboid movements of *Entamoeba histolytica* are very active in fresh dysenteric stools, and the motility of this species often persists for some hours after the stool has become cold. On the other hand, the movements of *Entamoeba coli* are always sluggish, and all motility is usually soon lost in cold stools.

*Cytoplasm.*—The cytoplasm of *Entamoeba histolytica* is homogeneous, and in the stained entameba is seen to have a coarsely reticulated structure (Plate I, fig. 5). In cold stools it frequently appears much vacuolated. Contrary to the description given by some authors, there is no true distinction to be seen between ectoplasm and entoplasm in the resting entameba. Individual entamebea, which contain granular material from partly digested food, sometimes present the appearance of a granular entoplasm. In motile *histolytica* the extended pseudopods may present a more dense, hyaline appearance than the reticulated body of the cytoplasm. The cytoplasm of *histolytica* may contain
various cells, including red blood corpuscles, of its host. On the other hand, the cytoplasm of *Entamœba coli* is more granular in appearance (Plate I, fig. 3). A hyaline ectoplasm is apparent only in the pseudopods of the motile entamœba. The cytoplasm of *coli* more often contains bacteria, starch and proteid grains, and other débris from the faeces than cells from the body of its host. The absence from the cytoplasm of red blood corpuscles and other cells of its host may result rather from the fact that *coli* is a strict commensal and is more often found in non-dysenteric stools than from its incapacity to ingest red blood corpuscles.

**Nucleus.**—The nucleus of *Entamœba histolytica* is usually indistinct in the motile organism, especially if it be actively motile or much vacuolated. In the latter case it is sometimes impossible to distinguish the nucleus from the vacuoles. In stained preparation the nucleus of *histolytica* is seen to possess a thin membrane and to be relatively poor in chromatin. This chromatin is distributed as a thin peripheral layer or as scattered granules about the inner surface of the nuclear membrane, with a few granules scattered in the network of the nonrefractive, nonstainable nucleoplasm (Plate I, fig. 5). This type of nucleus is characteristic of *histolytica* found in stools in acute dysentery that consist exclusively of mucus and blood. In stools of chronic cases of dysentery that consist of more or less mucus and blood mixed with faecal matter, the so-called "*tetragena*" type of nucleus is commonly met with. This type of nuclear structure contains more chromatin than the preceding type, and the chromatin has a characteristic arrangement. It is distributed partly as a more or less extensive but loose layer, which frequently shows radial projections, about the inner surface of the nuclear membrane, and partly as a loose central karyosome of varying structure. This karyosome consists typically of a central granule, the "centriol," surrounded by a clear halo that is bounded by a layer of chromatin granules (Plate I, fig. 6). All intermediate stages between the typical *histolytica* and the "*tetragena*" types of nuclei are to be observed in dysenteric stools. On the other hand, the nucleus of *Entamœba coli* is distinctly visible in the living and motile entamœba as a heavy refractive ring. It consists of a nuclear membrane and a relatively large amount of chromatin which is arranged as a heavy, dense, continuous or broken layer about the inner surface of the nuclear membrane and sometimes also in a small, central, dense karyosome. The interior of the nucleus consists of a nonrefractive, nonstainable
nucleoplasm (Plate I, fig. 3). Therefore, the nucleus of Entamoeba histolytica differs from that of Entamoeba coli in being less distinct, often invisible, in the living entamoeba, and in being poorer in chromatin. The "tetragena" type of Entamoeba histolytica found in stools of chronic cases of dysentery has a nucleus more closely resembling that of Entamoeba coli; but the peripheral layer of chromatin is less dense, often shows radial projections, and the karyosome is loose instead of dense in structure.

2. IN DIARRHEAL STOOLS

In diarrheal stools and stools after a purgative, Entamoeba histolytica is usually small, sluggishly motile or immobile, and possesses a nucleus that is distinctly visible in the living entamoeba as a more or less heavy peripheral ring of chromatin (Plate I, fig. 7). Therefore, it more or less closely resembles Entamoeba coli. These forms appear to represent changes in Entamoeba histolytica preparatory to encystment. They are spoken of by Darling (1913) as the "small generation" of Entamoeba histolytica, and were mistaken by Elmassian (1909) for a distinct species of Entamoeba. Although the small size in part may be due to less volume, it is probable that it results in part from the contraction and rounding up of the much extended motile entamoeba. The increase of chromatin content of the nucleus may be considered as a preparation for the multiple nuclear division that is to take place in the cyst. While all stages from the typical histolytica through the "tetragena" to this pre-encysted stage of Entamoeba histolytica may be found in diarrheal stools or stools after a purgative, the predominance of the preencysted stage and the more or less resemblance of it to Entamoeba coli make the differentiation of the two species difficult and sometimes impossible in such stools.

3. IN FORMED STOOLS

In formed stools both Entamoeba histolytica and Entamoeba coli are present in the encysted stage, and it is this stage of the entamoeba that presents the most distinctive character for making a differential diagnosis. Furthermore, the identification of Entamoeba histolytica in the encysted stage in formed stools is extremely important for the diagnosis of chronic and latent infections and for the control of treatment of entamoebic dysentery, and constitutes one of the most efficient factors in the prophylaxis of this disease.
The cysts of *Entamoeba histolytica* (Plate I, fig. 8) are relatively small, from 10 to 15 microns in diameter. They are round, or occasionally oval, moderately refractive, and have a thin cyst wall. The completely encysted entamoeba contains 4 ring-form nuclei and, usually, one or more elongated refractive bodies, that stain with chromatin stains and which have been designated by Hartmann as chromidial bodies. On the other hand, the cysts of *Entamoeba coli* (Plate I, fig. 4) are larger (from 15 to 20 microns in diameter), more refractive, and usually possess a thicker cyst wall. The completely encysted entamoeba contains 8 (occasionally more) nuclei and does not include “chromidial bodies.” The encystment of *Entamoeba coli* appears to proceed more rapidly than that of *Entamoeba histolytica*, so that from 2 to 6 nuclear stages are infrequently met with. In the case of *Entamoeba histolytica*, nuclear multiplication appears to take place early, so that from 2 to 4 nuclear stages are frequently seen before encystment is complete; indeed, occasionally in the motile entamoeba.

For convenience of reference, the more distinctive and constant characters of *Entamoeba histolytica* and *Entamoeba coli* are tabulated.

**Motile stage.**

<table>
<thead>
<tr>
<th>A. Entamoeba histolytica</th>
<th>B. Entamoeba coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Appearance hyaline.</td>
<td>1. Appearance porcelaneous.</td>
</tr>
<tr>
<td>2. Refractiveness more feeble.</td>
<td>2. Refractiveness more pronounced.</td>
</tr>
<tr>
<td>4. Nucleus more or less indistinct.</td>
<td>4. Nucleus distinct.</td>
</tr>
</tbody>
</table>

**Encysted stage.**

<table>
<thead>
<tr>
<th>A. Entamoeba histolytica</th>
<th>B. Entamoeba coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Cyst less refractive.</td>
<td>2. Cyst more refractive.</td>
</tr>
<tr>
<td>3. Cyst usually contains elongated refractive bodies known as “chromidial bodies.”</td>
<td>3. Cysts do not contain “chromidial bodies.”</td>
</tr>
<tr>
<td>4. Nuclei never more than 4.</td>
<td>4. Nuclei 8, occasionally more.</td>
</tr>
</tbody>
</table>

Therefore, in dysenteric stools and sometimes in diarrheal stools, the characters of the motile *Entamoeba histolytica* are fairly distinctive, and the experienced observer will have little difficulty in identifying the species. Unusually, however, in diarrheal stools and in stools after a purgative *Entamoeba histolytica* is in a pre-encysted stage in which it closely resembles *Entamoeba coli*, especially in its sluggish motility and its distinct
nucleus containing much chromatin. It is for this reason that I have insisted upon stool examinations without the administration of a purgative. In the case of natural diarrhoeal stools, diagnosis can usually be made by an experienced protozoologist by a careful study of the stools on successive days; but it is always advisable to endeavor to obtain a formed stool. Formed stools, when they can be obtained, are always to be preferred for making a laboratory diagnosis of entamoebic infection, because the encysted entamoebae in such stools present the most distinctive morphological characters for the differential diagnosis between Entamoeba histolytica and Entamoeba coli. Finally, it is to be insisted upon that a negative diagnosis should never be made on a single stool examination, since the entamoebae may occasionally be absent from the stools of an infected person; nor upon the identification of Entamoeba coli in a stool, since there may exist a double parasitization with this species and Entamoeba histolytica. In all such cases a diagnosis should be based on several examinations made on different days.

The treatment of entamoebic dysentery in the Philippines has been based hitherto upon the presence of entamoebae in the stools without regard to the species. With the establishment of a morphological and pathogenic distinction between Entamoeba histolytica and Entamoeba coli, and the consequent ability to make a differential diagnosis between the two species, there no longer exists a justification for the indiscriminate treatment of every person showing entamoebae in his stool. Entamoeba coli is a very common commensal of man in the Tropics, but it is usually present in small numbers in the intestine and is harmless. Consequently, there is no reason why a patient parasitized with this species should, unless he desired, be subjected to the more or less disagreeable course of treatment. The indiscriminate treatment of all persons showing entamoebae in their stools is as indefensible as would be the treatment with diphtheria antitoxin of every person showing a culture of any bacillus whatsoever from his throat.

The evidence so far secured in this investigation points to the conclusion that the ordinary routine treatment with ipecac, while efficient in relieving attacks of dysentery and in causing the entamoebae to disappear temporarily from the stools, frequently does not kill all of the entamoebae in the intestine; consequently, the patient is liable to a relapse of the dysentery. This tendency to relapse after chemotherapeutic or drug treatment is, as is well known, characteristic of other protozoan and of spirochaete infections. Two acute attacks and 1 relapse of dysentery and 4
latent infections with *Entamoeba histolytica* have been treated during this experimental investigation and followed with microscopic examinations of the stools. While the dysenteric symptoms, in such cases as they existed, were always promptly relieved and the entamoebae in both the acute and the latent cases always disappeared temporarily, the entamoebae in every case, except one, reappeared in the stools of the patient in from ten to fifteen days after treatment. In one case of latent infection the entamoebae disappeared from the stools of the patient after treatment and were absent for thirty days, when he was discharged from the hospital and passed from observation. A further study of the efficiency of ipecac and of the soluble salts of emetine in killing all of the entamoebae in the intestine of the patient, especially of latent cases, is greatly to be desired. The effects of varied doses, the administration by different methods, and especially the tests of prolonged and repeated treatment, controlled by daily stool examinations over long periods of time, should be investigated. Ipecac, especially its alkaloid emetine, is probably the most efficient drug that we possess for the treatment of entamoebic dysentery, but it is extremely important that a method of treatment be worked out that will permanently free the intestine of the patient from entamoebae in order to prevent relapses and to repress "carriers."

In consequence of the frequent failure of ipecac treatment as at present administered to kill all of the entamoebae in the intestine of infected persons, treatment should always be controlled by stool examinations. The usual routine examinations made during and immediately after treatment are useless, since the entamoebae almost always disappear temporarily after treatment. The examinations should be made at frequent intervals for some months after treatment, and if the entamoebae reappear in the stools the treatment should be repeated. With this precaution it is believed that relapses, so common in entamoebic dysentery, can be prevented.

The prophylaxis of entamoebic dysentery in many, if not most, parts of the Tropics has been based upon the erroneous conceptions concerning the etiologic agent of this disease. In consequence of the cultivation and infection experiments of Kartulis (1891), Celli and Fiocca (1894), Musgrave and Clegg (1904), Noc (1909), Greig and Wells (1911), Gauducheau (1912), Chatton and Lalung-Bonnaire (1912), and others, together with gross carelessness of investigators in the identification of species of amoeboid organisms, the opinion has been widely held, at least in the Far East, that, if not all amoebae living
in water and other external sources are capable of living para-
sitically in the intestine of man and of producing dysentery, at
least the pathogenic species is capable of living and multiplying
indefinitely outside of the body of its host. Such a characteristic
of Entamœba histolytica would be unique among pathogenic
microorganisms, and would, indeed, constitute entamœbic dys-
entery the most formidable disease of mankind and the least
amenable to prophylaxis. Not only the water, but everything
in the Tropics, even the air, contains amœbæ, motile or encysted
in greater or lesser numbers, and efficient preventive measures
against this disease would be practically impossible.

On the other hand, the experimental determination that enta-
mœbic dysentery is caused by one species of amœboid organism
only, and that this species is a strict or obligatory parasite which
cannot multiply outside of the body of its host, profoundly limits
the prophylactic problem of this disease; indeed, reduces it to
almost, but not quite, the same level as that of other intestinal
infectious diseases, such as bacillary dysentery, typhoid fever,
and cholera. Every case of entamœbic dysentery, under these
conditions, must arise directly or indirectly from some preceding
case of entamœbic dysentery, and the prophylactic problem be-
comes that of protecting the well from cases of the disease, the
sanitary disposal of the dejecta of the diseased, and the detection
and treatment of "carriers" of the pathogenic entamoeba.

Every acute case of entamœbic dysentery is constantly passing
in his stools greater or smaller numbers of Entamœba histolytica;
but in dysenteric stools the entamœbæ are all in the motile stage,
in which they are probably less resistant to external influence
than any other intestinal organisms. They not only will not
live, but even disintegrate within a few hours after being passed
in the faeces. It is also probable that in this stage they are
incapable of surviving passage through the normal stomach, but
are destroyed by the acidity of its contents. Of the 4 men who
ingested the motile Entamœba histolytica in my experiments, 3,
or 75 per cent, became infected; but these infections were secured
under the most favorable circumstances, large numbers of the
organism being ingested, together with magnesium oxide to
neutralize the acidity of the stomach. It is unfortunate that
some of these men were not fed the entamœbæ without neutraliz-
ing the acidity of the stomach contents, in order to determine
experimentally the possibility of infecting with the motile stage
under natural conditions. Darling (1913) states that infections
invariably fail when only the motile (trophozoite) stage of Enta-
mœba histolytica is fed to kittens. Shirota (1912) makes a
similar statement as a result of his experience. The purpose of my experiments as performed was to obtain parasitization and to secure the most favorable conditions possible for infection with any other organisms that, associated with the entameba, might be an etiologic factor in producing dysentery. In consequence of the extremely feeble resistance of the motile Entamoeba histolytica to external influences, it is not considered that cases of acute entamebic dysentery are an important source of infection.

On the other hand, it is believed that chronic and latent cases of this disease are the chief, if not the exclusive, source of infection in endemic regions, first, because of their relative prevalence; secondly, because this condition persists indefinitely; thirdly, because their infection is unsuspected; and, fourthly, because these "carriers" are constantly passing in their stools, often in enormous numbers, the resistant, encysted stage of Entamoeba histolytica.

From the results of my experimental infections it appears that 78 per cent of persons parasitized with Entamoeba histolytica become "contact carriers" of the parasite. For every case of dysentery obtained in these experiments, there were 5 cases of latent infection; and of the 4 cases of dysentery, 2 cases were chronic, and the 2 acute cases became "convalescent carriers" of Entamoeba histolytica. In the examination of 101 healthy men in Bilibid Prison, who had not been used for experiments, 9, or 8.9 per cent, were found to be "carriers" of Entamoeba histolytica. These men had all been in the prison for years, and it is consequently probable that the percentage of "carriers" was lower than would be found outside.

While acute entamebic dysentery lasts only days or weeks, the chronic and latent infections persist indefinitely. None of the 20 experimentally infected nor the 9 naturally infected men has ceased to be a "carrier" of Entamoeba histolytica, although some of them have been under observation for over two years. The longest time of which I have an accurate record of a man carrying Entamoeba histolytica is two years and four months, and it still appears in his stools in undiminished numbers. Moreover, as we have seen, the ordinary routine treatment of such "carriers" may not permanently remove the parasites from the intestine.

These "carriers" are constantly passing in their stools the resistant, encysted stage of the pathogenic entameba. As has been stated previously in another connection, in 930 stool examinations of men known to be parasitized with Entamoeba histo-
lytica, the entamœbæ were found 664 times, or in 71.39 per cent of these examinations. The majority of these examinations were of "carriers" who showed no dysenteric symptoms, but who were passing the encysted entamœbæ, often in enormous numbers, in their formed stools. These encysted entamœbæ, while incapable of multiplication or other vital activities outside of the body of their host, are resistant to external influences and are consequently capable of maintaining their vitality for some time outside of the body and of passing uninjured through the stomach of their host. Observations on the resistance of the encysted stage of Entamœba histolytica have not been as numerous in these experiments as could be desired. However, experiments were made with the cysts of Entamœba histolytica kept two days and with cysts of Entamœba coli kept ten days at tropical temperature. In both cases the cysts were kept moist. Parasitization was obtained in every case with this material. Darling (1913) put fæces containing cysts of Entamœba histolytica in 10 volumes of sterile tap water for three days. He was unable to infect 2 kittens or to find any cysts of the entamœbæ after this treatment, and concluded that the cysts disappear when in contact with water for this length of time. I have no data on the effect of drying upon the vitality of the cysts. Schaudinn (1903), however, infected kittens with fæces containing Entamœba histolytica air-dried for six weeks. On the other hand, Darling (1913) failed to infect 2 kittens with fæces containing cysts of this entamœba that had been dried in air for seven weeks. With regard to the resistance of the cysts of entamœbæ to the gastric juices in passage through the stomach, the following data were secured in these experiments. Of 12 men who ingested encysted Entamœba coli without neutralizing the acidity of the contents of their stomachs, 11 became parasitized; and of 6 men who ingested encysted Entamœba histolytica under similar conditions, all become parasitized.

The knowledge of the part which these "carriers" of Entamœba histolytica probably play in the spread of entamœbic dysentery, together with the ease and certainty with which such "carriers" can be detected by microscopic examination of their stools, makes the prophylaxis of this disease relatively simple. It is believed that it would be possible, were it practicable, to eradicate this disease from any region by a systematic examination of stools and the treatment or isolation of all persons found to be carriers of Entamœba histolytica. In the absence of such thoroughgoing prophylactic measures, a sanitary disposal of all fæcal matter should be insisted upon and household "carriers" of Entamœba
**histolytica** should be eliminated. Native household servants who cook and handle food, who are usually more or less uncleanly in their habits, and some of whom are carriers of *Entamoeba histolytica*, are believed to be one of the chief sources of infection of white persons residing in the Tropics; and, as a most essential prophylactic measure, stool examinations should be made of all such servants, and those found infected should be discharged or subjected to treatment.

Equally important is the matter of personal prophylaxis. On account of the relatively long incubation period of the disease and the frequent occurrence of chronic and latent infections, it will usually be possible to anticipate with treatment an attack of dysentery. If persons residing in endemic regions should have frequent stool examinations made by a competent protozoologist and, if at any time parasitization with *Entamoeba histolytica* be discovered, should undergo treatment, it is believed that it would rarely be necessary for a person to suffer from entamoebic dysentery. A stool examination made once a month would ordinarily be sufficient to anticipate an attack of dysentery. Such a procedure would constitute a most efficient method of personal prophylaxis.

**PART VI. SUMMARY AND CONCLUSIONS**

By Ernest Linwood Walker

This investigation was undertaken to determine experimentally the etiologic relationship of different species of amoeboid organisms to endemic tropical dysentery. It has consisted of 60 feeding experiments with the different species of *Amoeba* and *Entamoeba* that have been implicated in the production of this disease.

These experiments differ from those hitherto performed (1) in the number of comparative tests made of different species; (2) in that the experiments have been more carefully controlled and especially in that the species of amoeboid organism fed to, and recovered from, the experimental animal in every case have been determined; and (3) in the fact that the experiments have been made not upon the lower animals but upon man.

A. Twenty feedings of cultures, representing 13 strains and 8 species of *Amoeba*, isolated from the Manila water supply and other nonparasitic sources, from the stools of healthy persons or persons suffering from diseases other than dysentery, and from dysenteric stools, have been given to 10 different men, with the following results:

1. The *Amoeba*, when ingested by men, can usually be recovered in cultures from their stools on Musgrave and Clegg's medium during the first few days after feeding, but never subsequently.
2. Microscopic examination of the stools of men after ingesting cultures of amœbæ have been invariably and constantly negative.

3. None of the men who ingested cultures of amœbæ have developed dysentery.

4. Therefore, the following conclusions appear to be warranted:
   a. The cultivable amœbæ are incapable of living parasitically in the intestinal tract of man.
   b. The amœbæ, when obtained in cultures from stools, intestinal contents, or liver-abscess pus, are derived either from cultural contaminations or from encysted amœbæ which have been ingested with water or food and have passed unchanged through the intestinal tract.
   c. The cultivable amœbæ are nonpathogenic, and consequently play no rôle in the etiology of endemic tropical dysentery.

B. Twenty feedings with 5 strains of *Entamoeba coli* have been given to 20 different men with the following results:

1. Cultures on Musgrave and Clegg's medium of the stools of men who have ingested *Entamoeba coli* have been invariably negative.

2. On the other hand, *Entamoeba coli* has been found microscopically, after a short incubation period, in the stools of every man who became parasitized, and the entamoebæ have persisted in the stools of these men for an indefinite time.

3. Of the 20 men who ingested *Entamoeba coli*, 17 became parasitized at the first feeding and 3 who did not become parasitized were reserved as controls.

4. The incubation period of *Entamoeba coli*, as determined by these experimental parasitizations, varies from one to eleven days, with an average of 4.7 days.

5. None of the 17 men experimentally parasitized, nor the 3 nonparasitized controls, have developed dysentery.

6. From these results, the following conclusions appear warranted:
   a. *Entamoeba coli*, unlike the *Amœbæ*, is a strict or obligatory parasite and cannot be cultivated on Musgrave and Clegg's medium.
   b. *Entamoeba coli* is nonpathogenic, and consequently plays no rôle in the etiology of endemic tropical dysentery.

C. Twenty feeding experiments with *Entamoeba histolytica* have been made on 20 volunteers, with the following results:

1. Cultures on Musgrave and Clegg's medium of the stools of men who have ingested *Entamoeba histolytica* have been invariably negative.

2. Microscopic examinations, on the other hand, have shown *Entamoeba histolytica*, after a short incubation period, in the stools of every man who became parasitized, and the entamoebæ have persisted in the stools of these men for an indefinite time.

3. Of the 20 men who ingested *Entamoeba histolytica*, 17 became parasitized after the first feeding, 1 required 3 feedings before becoming permanently parasitized, and 2 who did not become parasitized at the first feeding were reserved as controls.

4. The incubation period of *Entamoeba histolytica* in these experimentally parasitized men has been from one to forty-four days with an average of nine days.

5. In these experiments it has been possible to obtain:
   a. Encysted "*Entamoeba tetragesia*" exclusively in the stools of men who had ingested motile *Entamoeba histolytica* only.
b. Motile *Entamoeba histolytica* exclusively in the dysenteric stools of men who had ingested "tetragena" cysts only.

c. An alternation of "tetragena" cysts and motile *Entamoeba histolytica* several times repeated in the stools of a man who had ingested "tetragena" cysts only and having attacks of dysentery alternating with normal stools.

6. Of the 18 men experimentally parasitized with *Entamoeba histolytica*, 4, or 22.2 per cent, have up to the present time developed entamebic dysentery.

7. The incubation period of the dysentery in these experimental infections has been twenty, ninety-five, eighty-seven, and fifty-seven days, respectively, with an average of 64.8 days.

8. No cases of dysentery have developed in men who ingested *Entamoeba histolytica* from an acute case of entamebic dysentery, from a liver abscess, nor in the 2 men who ingested *Entamoeba histolytica* but who did not become parasitized with the *entamoeba*.

9. All of the experimental dysenteries have been obtained after ingesting *Entamoeba histolytica* from normal stools of "carriers." In 2 of the cases the infection was from "contact carriers" who had not, and have not subsequently, developed dysentery, and in one of the latter cases three hundred seventy-one days and the passage through 2 "contact carriers" intervened between the case of natural and the case of experimental entamebic dysentery.

10. No cases of spontaneous entamebic dysentery have occurred in this ward during the period of these experiments.

11. In consequence of the results obtained in these experimental infections of men with *Entamoeba histolytica*, the following conclusions appear warranted:

a. *Entamoeba histolytica*, like *Entamoeba coli* and in contrast to the *Amœba*, is a strict or obligatory parasite and cannot be cultivated on Musgrave and Clegg's medium.

b. "*Entamoeba tetragena*" Vierck is identical with *Entamoeba histolytica* Schaudinn, and "tetragena" cysts are developed in the life cycle of *Entamoeba histolytica*.

c. The large percentage of latent infections obtained in these experiments is wholly consistent with our clinical and pathological experience with entamebic dysentery.

d. *Entamoeba histolytica* is the essential etiologic factor in endemic tropical dysentery.

D. Information believed to be of the greatest value for the diagnosis, treatment, and prophylaxis of entamebic dysentery has been obtained in this experimental investigation.

1. Since it has been determined that *Entamoeba histolytica* is the specific etiologic agent, it will be possible to make an accurate laboratory diagnosis of entamebic dysentery.

2. The distinction between the pathogenic *Entamoeba histolytica* and the harmless *Entamoeba coli* having been established, there will no longer exist an excuse for the indiscriminate treatment of all persons who show entamebe in their stools.

3. The relatively long incubation period of this disease and the ability to diagnose latent infections make it possible to anticipate with treatment an attack of entamebic dysentery.
4. Since there is evidence that ipecac treatment, which is very efficient in relieving attacks of entamœbic dysentery and causing the entamœba to disappear temporarily from the stools, does not always kill all of the entamœbae in the intestine, treatment should always be controlled by stool examinations for Entamoeba histolytica. By this precaution, relapses, so common in entamœbic dysentery, can be forestalled.

5. The following data have been acquired upon which to base a rational prophylaxis of entamœbic dysentery:
   a. Entamoeba histolytica is the essential etiologic agent in the disease.
   b. The specific entamœba is an obligatory parasite, and cannot propagate outside of the body of its host.
   c. The motile forms of this entamœba, which are passed in the bloody mucous stools in acute dysentery, quickly die and disintegrate and are probably, under natural conditions, incapable of withstanding passage through the human stomach.
   d. In consequence of the relatively long incubation period of entamœbic dysentery, the prevalence of chronic and latent infections, and the frequent failure of treatment to kill all of the entamœbae in the intestine, “carriers” of Entamoeba histolytica are common in endemic regions.
   e. These “carriers” are constantly passing in their stools large numbers of the resistant, encysted stage of Entamoeba histolytica.

6. These facts make it probable that “carriers” of Entamoeba histolytica constitute the chief, if not the sole, agents in the dissemination of entamœbic dysentery.

7. Prophylactic measures should, therefore, be directed toward “carriers” of Entamoeba histolytica, and should include the following:
   a. The identification of “carriers” of Entamoeba histolytica by the microscopic examination of the stools of convalescents, household servants, and other suspects or persons whose employment or associations make them particularly dangerous to the public health.
   b. The sanitary disposal of faeces.
   c. The treatment, controlled by microscopic examination of their stools, of all “carriers” of Entamoeba histolytica.

8. Since the incubation period of entamœbic dysentery is usually long and latent infections are common, the most efficient personal prophylactic measure is frequent stool examinations, as an index for treatment, of all persons residing in endemic regions.

LITERATURE REFERRED TO IN THE TEXT


IDEM. Entamoeba hominis e Amoeba coli Lœsch. Studio biologico e clinico. Annali Vigiene sperimentale (1897), 5, fasc. 1.


CHATTON, E., et LALUNG-BONNAIRE. Amibe limax (Vahlkampfia n. g.) dans l'intestin humain. Son importance pour l'interprétation des amibes de culture. Bull. Soc. path. exotique (1912), 5, 135–143.


CRAIG, C. F. Observations upon amoebae infecting the intestine, etc. Am. Med. (1905), 9, 854, 897, 937.


CRAIG and ASHURN. Quoted by Craig (1913).


FANTHAM (1912). Personal communication quoted by Craig (1912).


Dysentery and liver abscess in Bombay. Scientific Memoirs by Officers of the Medical and Sanitary Depts., Gov. of India. N. S. No. 47, 1911. [Reference: Centralbl. f. Bakt. etc., Ref. (1912), 52, 73–74.]


MCCARRISON. Quoted by Craig (1913).


IDEM. Weiterer Beitrag zur Kenntnis der Entamœben. Ibid. (1912), 26, 241-249.


WELLMANN, CREIGHTON. Quoted by Craig (1913).


ILLUSTRATIONS

(From water-color drawings by Teodosio S. Espinosa)

PLATE I

The figures in Plate I are all drawn from fixed and stained preparations
at the magnification of Zeiss \( \frac{1}{2} \) oil-immersion objective, ocular 3, and
tube length of 160 millimeters, and with the aid of a camera lucida.

FIG. 1. Motile form of a typical Amœba, cultivated from the Manila water
supply. Note the small size, central arrangement of the chromatin in the nucleus, and the contractile vacuole.

2. Encysted form of the same species of Amœba. Note the small size
and single nucleus with central arrangement of the chromatin.

3. Motile form of Entamœba coli from the stool of a healthy person.
Note the dense, granular structure of the cytoplasm, the relatively large amount of chromatin and its peripheral arrangement in the nucleus.

4. Encysted form of Entamœba coli, from the stool of a healthy person.
Note the large size, the relatively thick cyst wall, the 8 ring-form nuclei, and the absence of "chromidial bodies."

5. Motile form of Entamœba histolytica, from the stool of an acute case of entamoebic dysentery. Note the reticulated structure of the cytoplasm and the scanty chromatin in the ring-form nucleus.

6. The "tetragna" type of motile Entamœba histolytica, from a chronic case of entamoebic dysentery. Note the structure of the nucleus. It contains a heavier peripheral ring of chromatin—a part of which is detached from the nuclear membrane—than in the typical histolytica; and there is a central karyosome, consisting of a central granule surrounded by a circle of chromatin granules.

7. The pre-encysted stage of Entamœba histolytica, from a "carrier" case. Note the small size, dense cytoplasm, and heavy peripheral ring of chromatin in the nucleus, which causes it to resemble a small Entamœba coli.

8. Encysted form of Entamœba histolytica, from a convalescent case of entamoebic dysentery. Note the small size, the cyst wall, the 4 ring-form nuclei, and the "chromidial body."

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PLATE I. TYPICAL EXAMPLES OF AMOEBA AND ENTAMEBA.
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EXPERIMENTAL BALANTIDIASIS

By Ernest Linwood Walker

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Seven plates

Balantidiasis is the intestinal parasitization of man with the ciliated protozoan, Balantidium coli Malmsten, which may give rise to a chronic diarrhoea or a fatal dysentery. The first case reported was by Malmsten at Stockholm in 1857. Strong (1904) was able to collect from the literature 125 cases of balantidiasis. Since then and up to the present time 12 cases, of which the literature is available, have been reported. Therefore, in the fifty-six years following the discovery of the parasite only about 137 cases of infection of man have been reported in medical literature. This makes it appear that balantidiasis is a comparatively rare infection, and consequently of more scientific interest than practical importance.

In the Philippine Islands, however, parasitization with this protozoan appears to be relatively prevalent. The first case described here was by Strong in 1904. Subsequently a few cases were reported, notably 3 fatal cases with necropsy by Bowman (1909 and 1911). Willets (1913) found 2 cases in the examination of 400 stools in the Batanes Islands, north of Luzon, and I found 2 cases in the examination of 48 stools at San José, Mindoro. Thirteen cases have been observed at the Philippine General Hospital. In the Bilibid Prison 35 cases have been found in the last two and a half years, an average of more than 1 a month. From March 4 to March 25 of the present year, a period of twenty-one days, 8 new cases of parasitization with
this protozoan were discovered. Moreover, on account of the infrequent appearance of the parasites in the stools of infected persons and the absence of clinical symptoms in many of the cases, it is probable that parasitization with Balantidium coli is frequently overlooked in the routine examination of stools.

The geographical distribution of this infection is wide, and appears to be influenced by certain sanitary conditions rather than by climate. Cases have been reported in Russia, Sweden, Finland, Germany, Italy, North America, South America, Cuba, Africa, Sunda Isles, Cochin China, and the Philippine Islands. It is noteworthy that most of the known cases have occurred in Russia and Scandinavia, cold countries, on the one hand, and, on the other hand, in a tropical country, the Philippine Islands.

Balantidiasis in man is characterized clinically by the appearance of Balantidium coli in the stools and, in a certain proportion of the cases, by a diarrhoea or dysentery.

However, the appearance of the parasites in the patient’s stools is very irregular; they may be absent for weeks at a time, and they may at any time be found only in very small numbers and, consequently, frequently are discovered only when attention is attracted to the stools by the appearance of a persistent diarrhoea or of dysenteric symptoms.

The dysenteric symptoms are likewise an extremely inconstant manifestation of the infection. Of the 132 human cases of which I have been able to obtain access to the literature, 130 had diarrheal or dysenteric symptoms; but it is probable that the majority of these cases were brought to the attention of the physician by the dysenteric symptoms and that the parasites were found only in the attempts to discover the etiology of the symptoms. Of the 57 cases of parasitization so far reported in the Philippine Islands, only 11 have shown diarrhoeal or dysenteric symptoms. In other words, many of the infections are latent.

The mortality in 111 cases according to Strong (1904) was 32, or 29 per cent. The primary cause of death in some of these cases may have been other than the balantidiasis; on the other hand, many of the patients passed from observation and their ultimate fate was unknown. It is probable that if the patients parasitized, but not infected with the parasite, be included the percentage of mortality would be considerably lower, while if only those patients in which there was an actual invasion of the tissues by the balantidia be considered, the death rate from balantidal infection would be materially increased. The mortality in 57
recent cases in the Philippines has been up to the present time 4, or 7 per cent, but most of these are latent cases without symptoms.

The pathological changes in balantidiasis, as reported in the literature of 40 necropsies, showed ulcerations of the large intestine in 36, chronic catarrh in 3, and in 1 case the condition of the large intestine was not given. The ulcers are said not to be characteristic. The old ulcers are described as blackish or slate-colored and the recent ulcers are frequently slightly undermined and irregular. The mucosa between the ulcers in usually reddened and hæmorrhagic. In only 1 case (Peterson, 1873) has an ulcer been reported in the ileum. The parasites were found in the contents of the large intestine at necropsy in 21 of the cases. In all of these cases the ulcerative lesions were probably complicated by secondary bacterial invasion.

Histological studies of the lesions in balantidiasis have been made in a few of the fatal cases, notably by Strong and Musgrave (1901), Solowjew (1901), Bowman (1909 and 1911), and Bel and Couret (1910). The principal changes recorded are catarrh of the mucosa, cell degeneration and necrosis, polymorphonuclear infiltration (probably due to secondary invasion of the lesions by bacteria), hypertrophy of the vessels, hæmorrhages, round cell infiltration, eosinophilia, and penetration of the parasites into the sound tissues underlying the ulcers. The balantidia were found in the submucosa, muscularis, in the mesocolic lymph glands, and in the blood vessels and lymph spaces, lying singly or in groups.

Unlike infections with Entamoeba histolytica, liver abscess does not appear to be a common complication in balantidiasis; but Stockvis (1884) reported a case in which the balantidia were found in the sputum of the patient and were believed to have come from a liver abscess which had ruptured into the lung. The patient recovered, therefore no necropsy was obtained.

Besides man, monkeys and pigs are known to be naturally parasitized with balantidia.

Brooks (1902) reported an epizootic of dysentery among the apes in the New York Zoological Park due to balantidial infection. Noc (1908) found a case of balantidial dysentery in Macacus cynomolgus at the Pasteur Institute in Saigon, Indo China. Brumpt (1909) reports 6 cases of natural parasitization of Macacus cynomolgus with balantidia in Indo China.

Balantidia were first observed as parasites of the domesticated pig by Leuckart (1861 to 1863). They have been found by
Stein (1862), Ekecrantz (1869), and Wising (1871) in the pigs in Sweden, by Grassi (1882) in Italy, by Rapchevski (1882) in Russia, by Railliet (1886) and by Newmann (1888) in France, by Stiles (Strong, 1904) in the United States, and by Strong (1904) in Manila. The examination of the faeces of a somewhat limited number of pigs here convinces me that a large proportion of them in the Philippine Islands are parasitized with this protozoan. The balantidium found in the intestine of the pig is generally considered not to produce any symptoms or lesions in its host.

The question of the identity or nonidentity of the balantidium found parasitic in the pig with Balantidium coli of man has an exceedingly important bearing on the endemiology and prophylaxis of balantidiasis.

Wising (1885) and Grassi and Calandrucio (1888) were of the opinion that the balantidium of the pig is a species distinct from Balantidium coli of man. Wising states that the latter species is smaller and does not become encysted, while the pig balantidium is larger and usually appears in the faeces in the encysted stage. Grassi and Calandrucio drew their conclusions from the fact that they were unable to infect themselves with the balantidium of the pig by the ingestion of the encysted parasites. On the other hand, recent authors are inclined to the opinion that the balantidium of the pig and of man are one and the same species, but no special evidence is advanced to support this view.

In consideration of the close domestic relations existing between the pig and natives of the lower class, the fact that the pig is the chief scavenger in these Islands, the prevalence of balantidium as a parasite of the pig, and the fact that this parasite is passed more or less constantly in the faeces of the parasitized pig in the resistant encysted stage, make the identity of the 2 species particularly important in explaining the prevalence of infections of man with Balantidium coli in the Philippine Islands.

The experimental infection of animals with Balantidium coli has been attempted by many investigators. Brumpt (1909), who found cases of natural infection of apes with Balantidium coli in Indo China, reports that he was able to pass one of these strains through 6 healthy monkeys, to parasitize a monkey with balantidia obtained from the faeces of a pig, and to parasitize pigs with the strain of balantidium from the monkey. The incubation period of the parasite varied from two to seventeen days. A diarrhœa developed in 1 of the parasitized monkeys.
and 1 of the pigs, and the stools of the pig contained blood and
balantidia filled with red blood corpuscles. At the necropsy
performed on the parasitized monkey, balantidia were found
in the large intestine from the cæcum to the anus, but there were
no lesions. In the necropsy of the infected pig the author
states that lesions were present in the large intestine which
were identical with those described by Strong, Askanazy, and
others in human cases. No histological examinations appear
to have been made in any of Brumpt’s experimental infections
to determine the etiologic relation of the balantidia to the lesions.

On the other hand Ekecrantz (1869), Wising (1871, 1885),
Rapchevski (1880), Lavrovskaya (1890), Afanasyeff (1891),
Casagrandi and Barbagallo (1896), Chigayeff (1898), Valayeff
(1898), Zhegaloff (1899), Chichulin (1900), Strong (1904), and
Bowman (1911) have failed in their attempts to infect animals
(cats, rabbits, dogs, pigs, monkeys) with Balantidium coli. Casagrandi
and Barbagallo state, as a conclusion from numerous
experiments, that the parasites could sometimes live in the
intestine of cats if a catarrhal condition was first produced, but
that they were incapable of producing independent disease in
the intestine. Bowman injected fresh faeces from a case of
severe infection in man many times into the rectum of monkeys
suspended by the lower extremities in order that none of the
infective material could be evacuated. He performed a colotomy
on another monkey and injected 20 cubic centimeters of a balan-
tidial stool on two occasions into the colon. And, finally, tissue
from an ulcer containing balantidia was inserted beneath the
mucosa of the colon of a monkey and sutured in place. In every
case the results were negative; the parasites were never found
in the faeces of the experimental animals.

The following infection experiments on monkeys were under-
taken to discover the reason for the discrepancy in the results
of Brumpt and of other authors in their attempts to infect
animals with Balantidium coli, to determine the identity or non-
identity of Balantidium coli suis ¹ with Balantidium coli hominis,
and to obtain further information on certain obscure points in
the etiology, pathology, and endemiology of balantidiasis of man.

The monkeys used in these experiments were healthy indi-
viduals of the common species found on the Island of Luzon, name
undetermined. Some of them had been in captivity for some

¹ Suis or hominis is attached to the specific name throughout this paper
for convenience in distinguishing the pig and the human strains and not
for the purpose of indicating taxonomic varieties of Balantidium coli.
time, being kept in rooms in the animal house; others were freshly purchased by this Bureau and were presumably recently captured. No spontaneous infections with Balantidium coli have ever been discovered in the large number of monkeys that have been used in the biological laboratory. Owing to the fact that in infections of monkeys with this protozoan the parasites rarely appear in the stools, it was considered useless to attempt to control their freedom from natural parasitization by microscopic examination of their stools. The monkeys were kept in individual cages throughout the course of the experiments.

These monkeys were either fed or injected rectally with the faeces containing the balantidia, depending upon the stage of development of the parasites. If the balantidia were encysted, as was more frequently the case when they came from the pig, a portion of the faeces containing the cysts was mixed with the food of the monkey. On the other hand, motile balantidia, which are more frequently found in human stools, were injected rectally, since it is believed that the parasites in this unprotected stage are incapable of withstanding passage through the stomach. The diarrheal stool, diluted if necessary with physiological salt solution, was given as a high rectal injection through a rectal tube attached to a large syringe. Repeated feedings were given in many cases in order to insure parasitization of the experimental animal, which could not be readily determined by stool examinations on account of the infrequent appearance of the parasites in the faeces, and because the monkeys sometimes refused to eat the infective food. That repeated feedings are not necessary, however, to secure parasitization of monkeys with balantidia is proved by the fact that several animals which were fed or injected rectally only once became parasitized.

Balantidium coli suis was used in more of these experiments than Balantidium coli hominis because human cases that showed a sufficient number of the parasites in their stools were not frequently obtainable, and, moreover, it was especially desired to determine the parasitism and pathogenesis of the balantidium of the pig for the monkey.

Following the feedings or rectal injections, the stools of the monkeys were saved daily, or at frequent intervals, and examined macroscopically for diarrheæa or dysentery and microscopically for the presence of balantidia.

At the beginning of the investigation it was planned to kill the experimental animals at regular intervals in order to study the progress of the infection, but this intention was interfered
<table>
<thead>
<tr>
<th>Monkey No.</th>
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<tr>
<td></td>
<td>Superficial ulceration; hyperaemia; polymorphonuclear infiltration; no balantidia.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Hyperaemia; punctiform haemorrhages; eosinophile infiltration of mucosa; balantidia in mucosa and blood vessels of submucosa.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>Catarrhal exudate; no balantidia</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>27</td>
<td>Catarrhal exudate; minute superficial ulcers; polymorphonuclear infiltration; no balantidia.</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>Catarrhal exudate; no balantidia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>29</td>
<td>None made</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>...do</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Balantidia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Balantidia in mesenteric lymph glands; no cellular reactions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>None made</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>33</td>
<td>...do</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>34</td>
<td>Diphtheritic exudate; slight polymorphonuclear infiltration; no balantidia.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>Haemorrhagic exudate; some destruction of epithelium; no balantidia.</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>36</td>
<td>None made</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>37</td>
<td>...do</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>38</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Slight haemorrhagic exudate; eosinophile infiltration; no balantidia.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>Slight diphtheritic exudate; punctiform haemorrhages; eosinophile infiltration of mucosa.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>41</td>
<td>None made</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>42</td>
<td>...do</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Congestion of mucosa; eosinophile infiltration; no balantidia.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>44</td>
<td>None made</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>...do</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No.</td>
<td>Date of feeding or injection</td>
<td>Method of injection</td>
<td>Source of inoculation</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------</td>
<td>---------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>11</td>
<td>Sept. 31 and Oct. 7 and 8, 1912</td>
<td>Feeding</td>
<td>Rectal</td>
</tr>
<tr>
<td>12</td>
<td>Oct. 8, 1912</td>
<td>Rectal injection</td>
<td>Rectal</td>
</tr>
</tbody>
</table>

**Table I.—The experimental parasitization of monkeys with Balantidium coli and Balantidium coli innocuous.**
with by the fact that many of the parasitized monkeys did not become infected and by the death of a number of the monkeys during the course of the experiments. The deaths of these monkeys were probably due in most cases to the close confinement in small cages and to improper food. In consequence of these unforeseen complications and in consideration of the fact that the symptoms and pathology of the later stages of infection with Balantidium coli have been adequately studied in human cases, it was decided to confine the investigation to the parasitization and early stages of infection which are in need of elucidation.

Post-mortem examination, with special reference to lesions in the large intestine and to the presence of balantidia in the intestinal contents, was made of each monkey that died or that was killed after feeding or rectal injection with balantidia. In every case where a colitis or ulcerations of the large intestine were present, pieces of the tissues were fixed and subjected to a histological examination for cellular changes and for the presence of balantidia.

These experiments are summarized in Table I.

Of the 13 monkeys fed encysted balantidia from the pig, 12 became parasitized; and of the 4 monkeys that received rectal injections of motile balantidia from the pig, none became parasitized. Only 1 monkey was fed encysted balantidia from man and he did not become parasitized. Four monkeys received rectal injections of motile balantidia from man, and of these 2 became parasitized. Therefore 12, or 70.6 per cent, of the monkeys fed or injected with Balantidium coli suis; and 2, or 40 per cent, of the monkeys fed or injected with Balantidium coli hominis, became parasitized. The smaller percentage of monkeys parasitized with the balantidia from man may be due to several causes: to the smaller number of experiments with material from this source, that the infective material contained fewer balantidia, and to the fact that most of the experiments with the human strains were with motile forms administered by rectal injection.

In pigs parasitized with balantidium the parasites appear rather constantly in the encysted stage in the formed stools, and often in enormous numbers. On the other hand, in parasitized man the balantidia are rarely found in the encysted stage in formed stools, but usually only in the motile stage in diarrhoeal stools, and then often at irregular intervals. In monkeys, Table II shows the number of stool examinations made on different days of each animal that became parasitized and the number of positive and negative findings for balantidia.
Table II.—Showing the number of times Balantidium coli was found in the stool examinations of parasitized monkeys.

<table>
<thead>
<tr>
<th>Monkey No.—</th>
<th>Days under observation</th>
<th>Total number of stool examinations</th>
<th>Positive</th>
<th>Negative</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>152</td>
<td>44</td>
<td>2</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>36</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>114</td>
<td>58</td>
<td>12</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>44</td>
<td>16</td>
<td>3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>88</td>
<td>55</td>
<td>1</td>
<td>54</td>
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<td>29</td>
<td>55</td>
<td>54</td>
<td>17</td>
<td>37</td>
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<tr>
<td>30</td>
<td>73</td>
<td>39</td>
<td>3</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>42</td>
<td>16</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>54</td>
<td>22</td>
<td>0</td>
<td>22</td>
<td>Balantidia found at necropsy.</td>
</tr>
<tr>
<td>35</td>
<td>13</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>50</td>
<td>33</td>
<td>2</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>50</td>
<td>35</td>
<td>7</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>50</td>
<td>35</td>
<td>13</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>38</td>
<td>26</td>
<td>3</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>38</td>
<td>25</td>
<td>1</td>
<td>27</td>
<td>Balantidia found on day after feeding.</td>
</tr>
<tr>
<td>43</td>
<td>35</td>
<td>23</td>
<td>5</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

The small number of stool examinations made of some of the monkeys was due to my absence from Manila. With but few exceptions, the stools were formed and contained only encysted balantidia; when the stools were soft or diarrheal, the balantidia were sometimes found in large numbers in the motile stage. In most of the positive examinations the balantidia were few in number, often only one or several to a cover slip. It would appear from these results that monkeys parasitized with balantidia from either the pig or man show a condition, with reference to the appearance of the parasites in the stools, more closely resembling man than the pig; that is, the balantidia appear rarely and in small numbers in the formed stools of the parasitized animal. In consequence of this the incubation period of the parasite, that is, the time elapsing between feeding or injecting the infectious material and the appearance of the balantidia in the stools of the parasitized animal, is of little significance.

These experiments, therefore, prove that monkeys are readily parasitized with either Balantidium coli hominis or Balantidium coli suis. Furthermore, they make it evident that the apparent failure of every previous investigator, with one exception (Brumpt, 1909), to parasitize animals—at least monkeys—probably has been due to the infrequency, and often total failure,
Walker: Experimental Balantidiasis

of the balantidia to appear in the stools of the experimental animal. And they demonstrate that *Balantidium coli suis* behaves, with reference to its appearance in the stools of the parasitized monkey, as does *Balantidium coli hominis*.

Monkeys 10, 35, and 43, two of which had and 1 of which had not become parasitized with the balantidia, had a diarrhoea or slight dysentery just before death; but, as will be seen from the post-mortem examinations, the dysentery was probably due to other causes than the balantidium infection. None of the other monkeys had diarrhoea or dysentery during the time they were under observation. However, as will be seen from the histological examinations of infected monkeys, the ulcerative process of this infection is probably extremely chronic, and consequently no dysenteric symptoms would be expected in these early stages of infection.

Post mortem, a number of the monkeys, whether killed or dying naturally, showed a colitis and sometimes ulcerations, which histological examination showed not to be due to balantidium infection. These lesions, at first misleading, were soon cleared up by the histological study of the tissues, and have served the useful purpose of comparison with the lesions produced by *Balantidium coli*, and for determining the part played by lesions of other etiology in the entrance of the balantidia into the tissues of its host.

A comparison of the gross lesions in the intestine of monkeys having an early balantidial infection with those having a colitis due to other causes has shown that the balantidial infection is characterized chiefly by the inconspicuousness of the lesions, which consist simply of reddened areas of the mucosa with or without punctiform haemorrhages, sometimes so slight as to be overlooked, but no exudate or ulcerations; while the colitis of other etiology usually presents a catarrhal, diphtheritic, or haemorrhagic exudate, frequently associated with ulcerations.

One, or 50 per cent, of the 2 monkeys parasitized with *Balantidium coli hominis* showed balantidia in the tissues post mortem. The negative animal had been parasitized only thirteen days when it died, while the one which showed balantidia in the tissues had been parasitized forty-two days when the post-mortem examination was made. Of the 13 monkeys parasitized with *Balantidium coli suis*, 1, or 7.7 per cent, showed balantidia in the tissues post mortem. This is a smaller per cent of infections than with the balantidium from man, but it is to be borne in mind that the series of animals parasitized with the latter
variety is very small from which to draw conclusions. The time of parasitization before necropsy of the monkeys that did not become infected with the balantidium from the pig varied from thirty-four to one hundred fifty-two days, and in the infected monkey it was thirty-eight days.

These experiments, therefore, prove that both Balantidium coli hominis and Balantidium coli suis are capable of invading the tissues and becoming tissue parasites of the monkey. They further show that only a small proportion of the monkeys parasitized with balantidia from either source become infected; that is, show the parasites in the tissues within the period of time (from thirteen to one hundred fifty-eight days) during which these animals were under observation. However, it is probable, since in these early infections little or no gross lesions are apparent, because only a few balantidia may have entered the tissues, and since the sections made can include only an infinitesimal part of the whole intestine, that more of these parasitized monkeys were really infected. It is also to be borne in mind that every one of the animals parasitized would be liable sooner or later to become infected. The condition is exactly similar to that found to prevail in entamœbic dysentery, in which only about 22 per cent of men experimentally parasitized became infected and developed dysentery (Walker and Sellards, 1913).

Histological examination of sections of the large intestine of the monkeys parasitized with Balantidium coli and showing a colitis has disclosed well-marked differences between those which did and those which did not contain balantidia in the tissues. The latter show either a catarrhal or diphtheritic exudate or ulcerations, usually associated with polymorphonuclear leucocyte infiltration of the mucosa and submucosa. On the other hand, sections of the intestine of cases of these early stages of infection with balantidia show the epithelium intact, except for mechanical injury due to entrance of the balantidia or to minute hæmorrhages, but no exudate or ulcerations. There is more or less congestion of the blood vessels and the tissue infiltration, which is slight, is of round cells and eosinophiles.

Therefore, the lesions and cellular reactions in balantidial colitis, in the early stage before complicated by secondary bacterial invasion, are characteristic and are distinguishable from those of colitis due to bacterial infection. Moreover, these cases have demonstrated that lesions in the intestinal epithelium from bacterial infection or other causes not only are not necessary for
the entrance of *Balantidium coli* into the tissues, but that in no one of the relatively large number of parasitized monkeys in which such lesions existed have the balantidia taken advantage of them to enter the tissues.

In their entrance into the tissues of the intestine the balantidia pass through the healthy epithelium. No necrosis or ulcerations of the epithelium are apparent, only a pushing aside of the cells or, at most, a mechanical rupture of the epithelium exists. In every case the entrance was effected through the epithelium between, and in no case within, the tubules. In monkey 10, parasitized for thirty-eight days with *Balantidium coli suis*, some of the balantidia are just penetrating the epithelium and others are in the mucosa between the tubules and, to a less extent, in the submucosa and in the blood vessels of the submucosa (Plates I, II, and III). In this case they were not found in the muscularis. In monkey 31, parasitized for forty-two days and, according to my assistant who performed the necropsy, showing no intestinal lesions, the balantidia were found in the muscularis and in the mesenteric lymph glands (Plates IV and V).

Therefore, these experimental infections have shown that both *Balantidium coli hominis* and *Balantidium coli suis* are capable of penetrating the sound intestinal epithelium and of wandering widely in the sound tissues of the mucosa, submucosa, muscularis, blood vessels, and mesenteric lymph glands.

In these early infections the balantidia are found singly or in scattered groups, evidently in the process of migration rather than of active multiplication in the tissues. In consequence, there is as yet little injury to the tissues beyond some mechanical rupture; and usually even this is wanting; the parasites, which are capable of amœboid movements, pass between the cells like migrating leucocytes. Moreover, there is in these early cases little cellular reaction in the vicinity of the single parasites and no extensive cellular infiltration of the tissues. At a later period an active multiplication of the balantidia takes place in the tissues, forming nests or colonies (Plates VI and VII) which, by their multiplication, aided probably by the secretion of a ferment, produce cellular reactions, necrosis of the tissues, and finally open ulcerations, which are advanced by the secondary invasion of intestinal bacteria.

That *Balantidium coli* is able, without the aid of bacteria, to produce abscesses and ulcerations of the intestine of infected
man, I am able to demonstrate with some sections of a human case of balantidiasis. The intestine from which these sections were obtained is from the necropsy of one of Bowman's (1909–1911) cases. In certain sections of this material, which is heavily infected with balantidia, I have been so fortunate as to find, in addition to open ulcerations, closed balantidial abscesses situated in the thickened submucosa, which lie under the sound mucosa and entirely surrounded by sound tissues, and which are, consequently, probably free from intestinal bacteria. An early stage of such an abscess is shown in Plate VI, consisting of a small cavity filled with balantidia and the infiltration of the surrounding tissues with mononuclear cells. Plate VII shows a part of an advanced abscess, which is too large to be shown in one field of even the low power of the microscope. Examined with high magnification the "pus" of this abscess is seen to consist, not of polymorphonuclear leucocytes, but of cell detritus and mononuclear cells only. The tissues about the abscess show round-cell infiltration, but no polymorphonuclear leucocytes. The absence of polymorphonuclear leucocytes in the "pus" and in the tissues surrounding the abscess confirms the opinion that the abscess is free from bacterial infection. Both the abscess and the sound tissues surrounding it contain many Balantidium coli.

Therefore, it having been demonstrated that balantidia are capable of penetrating the sound intestinal epithelium, that they do not invade secondarily the lesions due to bacteria, and that in the submucosa the balantidia are able to produce abscesses which later extend through the mucosa and become open ulcers, it would appear that the primary etiologic relation of Balantidium coli to balantidial dysentery had been proved.

SUMMARY AND CONCLUSIONS

1. Parasitization of man with Balantidium coli is relatively common in the Philippine Islands.
2. The balantidia appear in the stools of parasitized individuals only at irregular intervals, and consequently infections, unless accompanied by clinical symptoms, may frequently be overlooked.
3. A large proportion of the pigs in and about Manila are parasitized with balantidia.
4. Balantidia are passed in the resistant encysted stage more or less constantly in the stools of parasitized pigs.
5. Morphologically Balantidium coli suis is identical with Balantidium coli hominis.
6. Forty per cent of 5 monkeys fed or injected rectally with *Balantidium coli hominis* became parasitized.

7. Seventy and five-tenths per cent of 17 monkeys fed or injected rectally with *Balantidium coli suis* became parasitized.

8. Monkeys parasitized with either *Balantidium coli hominis* or *Balantidium coli suis* show the parasites in the stools only at infrequent intervals.

9. Only a small proportion of the parasitized monkeys became infected. Of 2 monkeys parasitized with *Balantidium coli hominis*, 1, and of 12 monkeys parasitized with *Balantidium coli suis*, 1, showed the parasites in the tissues post mortem.

10. The early lesions of the intestine of monkeys infected with *Balantidium coli* consist only of a slight hyperæmia with or without punctiform hæmorrhages.

11. Histological examination of the tissues of monkeys recently infected with *Balantidium coli* show changes, notably vascular dilatation, minute hæmorrhages, round-cell infiltration and eosinophilia, which distinguish them from lesions of bacterial origin.

12. *Balantidium coli* was never found entering the tissues through the lesions in 10 parasitized monkeys having a colitis or ulcerations due to bacteria or other causes.

13. In those monkeys in which infection took place, the balantidia entered the tissues through the sound intestinal epithelium.

14. *Balantidium coli* can produce bacteriologically sterile abscesses in the submucosa of an infected intestine.

15. *Balantidium coli* is the primary etiologic factor in the symptoms and lesions of balantidial dysentery.

16. The latency prevalent in balantidiasis of man is due chiefly to the fact that the patient, although parasitized, is not infected with *Balantidium coli*, but in part to the chronicity of the ulcerative process in infected cases.

17. Every person parasitized with *Balantidium coli* is liable sooner or later to develop balantidial dysentery.

18. *Balantidium coli suis* is identical with *Balantidium coli hominis*.

19. The domesticated pig is the chief source of infection in the balantidiasis prevalent in the Philippine Islands.

20. Therefore, efficient prophylactic measures against balantidiasis in the Philippine Islands should be directed against these animals, which should be confined and not allowed to run in yards and dwellings.
LITERATURE CITED

AFANASYEFF, M. M. Novieshiye uspiekhi bacteriologii zaraznikh bolieznii i. parazitologii. Kaendar dyla vrachii 16°, St. Petersburg (1891), 177.


IDEM. A case of dysentery caused by Balantidium coli with coincident filarial infarction of the spleen. Ibid. (1911), 6, 147-153.


CHIGAYEFF, N. F. Sluchai yazvennovo vospaleniya tolstikh kishek s balantidio colii v isprazhneniyakh. Russkii Vratch, St. Petersburg (1898), 19, 1441.


LEUCKART. Die menschlichen Parasiten (1861-1863), 1, 146, 744.


NEWMANN. Traité des parasites et des maladies parasitaires des animaux domestiques (1888).

NOG, F. Un cas de dysenterie a balantidium chez le Macacus cynomolgus. Compt. rend. Soc. biol. (1908), 64, 878-880.

PETERSON, O. V. Nya fall af Balantidium coli. Upsala Läkaref, Förh. (1873), 8, 251.


VLAYEFF, G. Sluchai yazvennovo katarra tolstikh kishek s prisutstvieem v isprazhneniyakh balantidii coli. Russkyi Vratch, St. Petersburg (1898), 19, 140.


ILLUSTRATIONS
(From photomicrographs by Charles Martin)

PLATE I. Section of the large intestine of monkey 11. A single Balan-
tidium coli suis under the healthy intestinal epithelium. Note
the mechanical rupture of the epithelium which the parasite
has apparently caused in entering the tissues, the absence
of polymorphonuclear leucocytes, and to the extreme left the
punctiform haemorrhage with exuding red blood corpuscles.

II. Section of the large intestine of monkey 11. Three Balanti-
dium coli suis in the deeper part of the mucosa. Note that
the balantidia are in the tissues between, and not within, the
tubules and the nature of the cellular reactions.

III. Section of the large intestine of monkey 11. A single Balanti-
dium coli suis in a blood vessel of the submucosa.

IV. Section of a mesenteric lymph gland of monkey 31. Several
Balantidium coli hominis in the edge of the glandular tissue.

V. Section of mesenteric lymph gland of monkey 31. A single
Balantidium coli hominis in the center of the gland, only a
part of which is shown in the figure. Note the cross section
of a blood vessel adjacent to the balantidium.

VI. Section of the large intestine of a man dead from balantidial
dysentery. An early stage of a balantidial abscess in the
submucosa. Note the small cavity filled with balantidia and
the infiltration of the surrounding tissues with mononuclear
cells.

VII. Section of the large intestine of a man dead from balantidial
dysentery. A part of an advanced balantidial abscess in the
submucosa. The abscess is entirely surrounded by sound
tissues. Note the abscess cavity and necrotic material and
the balantidia and mononuclear cell infiltration in the sur-
rounding tissues.
PLATE I. SECTION OF LARGE INTESTINE OF MONKEY 11, SHOWING A SINGLE BALANTIDIUM COLI SUIS UNDER THE HEALTHY MUCOSA.
PLATE II. SECTION OF LARGE INTESTINE OF MONKEY 11. SHOWING THREE BALANTIDIUM COLI SUIS IN THE DEEPER PART OF THE MUCOSA.
PLATE III. SECTION OF LARGE INTESTINE OF MONKEY 11. SHOWING A SINGLE BALANTIDIUM COLI SUIS IN A BLOOD VESSEL OF THE SUBMUCOSA.
PLATE IV. SECTION OF A MESENTERIC LYMPH GLAND OF MONKEY 31, SHOWING SEVERAL BALANTIDIUM COLI HOMINIS IN THE EDGE OF THE GLANDULAR TISSUE.
PLATE V. SECTION OF MESENTERIC LYMPH GLAND OF MONKEY 31, SHOWING A SINGLE BALANTIDIUM COLI HOMINIS IN THE CENTER OF THE GLAND.
PLATE VI. SECTION OF THE LARGE INTESTINE OF A MAN DEAD FROM BALANTIDIAL DYSENTERY.
PLATE VII. SECTION OF THE LARGE INTESTINE OF A MAN DEAD FROM BALANTIDIAL DYSENTERY.
THE INFLUENCE OF COMPENSATED SALT MIXTURES ON THE DEVELOPMENT OF POLYNEURITIS GALLINARUM AND BERIBERI

By R. B. Gibson

(From the Laboratory of Physiology, College of Medicine and Surgery, University of the Philippines)

Four plates

Recent work on the etiology of beriberi has shown that this disease develops because of the absence from the diet of some substance or substances necessary for the normal nutritive process of the body. Thus Strong and Crowell have shown that the disease may occur in man under the most favorable hygienic conditions with exception in regard to diet. That beriberi in man may be caused by limited diets which do not include polished rice is evident from the observations of Axel Holst on the occurrence of the disease on Norwegian ships; of Little on the existence of beriberi on the coasts of Labrador and Newfoundland, where white wheat flour is the chief article of diet in certain seasons; and finally of Lovelace that cases have occurred in Brazil. Beriberi, then, is a subject of world-wide interest. Furthermore, the study of this disease promises to do much toward clearing up some of the important problems in the physiology of nutrition.

The observations of Fletcher and of Fraser and Stanton have shown that diets consisting chiefly of polished rice are the common cause of beriberi in the Orient. If the white rice, however, was replaced by the rough rice, the disease did not develop. Substitution of rough rice for the white article and additions to the dietaries of the native military forces and public institutions in the Philippine Islands have eliminated beriberi

1 This Journal, Sec. B (1912), 7, 271.
4 Ibid. (1912), 59, 2134.
5 Lancet (1907), 1, 1776.
6 Ibid. (1909), 1, 451; Studies from Institute for Medical Research. Federated Malay States (1909), No. 10.
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from these organizations.\(^7\) Shibayama,\(^8\) however, states that Japanese laborers, eating fresh unpolished rice, have developed the disease; and Strong and Crowell\(^9\) have observed mild symptoms of beriberi in men fed chiefly on Philippine red rice and also on white rice with an added alcohol extract of the polishings. Schaumann,\(^10\) in the course of an extensive study, found that the addition of dried egg white to maize fed to 3 rabbits did not prevent the development of polyneuritis. Eight doves receiving rice, desiccated egg white, and sodium chloride died even before the controls. Five doves were fed on boiled rice to which had been added 1 per cent of a mixture made up of potassium carbonate, 30 grams; sodium chloride, 3 grams; anhydrous sodium sulphate, 2 grams; calcium carbonate, 3 grams; magnesium carbonate, 4 grams; and iron oxide, 3 grams; no protective action was obtained. Grijns\(^11\) had previously found that sodium or iron carbonate and that Röhmann's\(^12\) salt mixture were without effect in beriberi. Schaumann also tried the effect of various compounds containing phosphoric acid. Calcium phosphate, glycero-phosphate, phytin from rice polishings and yeast nucleic acid, and phosphatids were without, or had but slight, protective properties. However, yeast, testicle, rice polishings, mongo beans, peas, and bran prevent the development of polyneuritis. Schaumann concluded that in as much as these substances are rich in phosphoric acid the absence of some phosphorus-containing substance in the diet is responsible for beriberi. Similar conclusions are reached from the consideration of the composition of the diets of sailors who were suffering from ship beriberi and from the analyses for phosphates, sulphates, and urea in the twenty-four-hour urines of these patients in the course of therapeutic feeding with the above substances. In the experiments, Schaumann employed doves, rabbits, guinea pigs, rats, dogs, monkeys, and goats.

Fraser and Stanton\(^13\) studied the relation of the phosphoric acid content of rice to beriberi production for different rice samples. Using fowls\(^14\) which they found do not develop polyneuritis when fed on rough rice or on white rice to which the

\(^7\) Chamberlain, *This Journal, Sec. B* (1911), 6, 133; Heiser, *ibid.* (1911), 229.


\(^9\) Loc. cit.


\(^11\) Cited from Schaumann, *loc. cit.*

\(^12\) *Allg. med. Zentr.-Zeitg.* (1903), No. 1; (1908), No. 9.

\(^13\) Studies from Institute for Medical Research. *Federated Malay States* (1911), No. 12.

\(^14\) Eijkmann, *Virchow's Arch.* (1897), 148, 523.
polishings had been added, they showed that a definite relationship actually existed. The total phosphoric acid content may be used as an indicator of the extent to which rice has been milled or polished, and therefore of its beriberi-producing qualities. These investigators further showed that the protective principle in the rice polishings was soluble in alcohol or in 0.3 per cent hydrochloric acid. Phytin, which comprises 32.5 per cent of the substances soluble in these reagents, was without protective properties.

Aron and Aron and Hocson\(^5\) found that nitrogen equilibrium could be maintained on a diet consisting chiefly of white rice in metabolism experiments lasting over a few days. The phosphorus balance became positive in beriberi cases or in the normal metabolism experiments when rough rice, rice polishings, or phytin were added to the diet. They also examined the phosphorus content of 28 samples of rice, for the most part native, and found that the phosphorus content within narrow limits is determined by the grade of milling. Teruuchi,\(^6\) however, has recently concluded that the phosphoric acid metabolism is not altered in beriberi.

From a consideration of the diet on which beriberi developed among the Philippine Scouts, Kilbourne\(^7\) suggests that the disease may be due either to deficient phosphates or to a disproportion of calcium and magnesium. Chamberlain, Bloombergh, and Kilbourne\(^8\) concluded that the food supplied was deficient in potassium and phosphorus. Furthermore, analyses of white and unpolished rice showed that the latter contained about twice as much phosphoric acid and two and one-half times the potassium as did the former. In experiments with fowls, the addition of potassium chloride, of phosphoric acid, or of both was without effect. Starvation experiments induced neuritis as evidenced chiefly by histological examination.

Chamberlain and Vedder\(^9\) fed each of 4 groups of fowls on white rice, plus 0.07 gram of potassium diphosphate, potassium citrate, potassium carbonate, and magnesium phosphate, respectively. This amount was used because it is slightly in excess of the amount of each of these salts present in 5 grams of rice polishings, which are protective. The fowls developed neuritis. These authors also found that the residue of the alcoholic extract

\(^5\) This Journal, Sec. B (1910), 5, 81; Ibid. (1910), 98; Ibid. (1911), 6, 361.
\(^6\) Verhandlungen der Japanischen Pathologischen Gesellschaft (1912), 32.
\(^7\) This Journal, Sec. B (1910), 5, 127.
\(^8\) Ibid. (1911), 6, 177.
\(^9\) Ibid. (1911), 251.
of rice polishings, when taken up in water, contains practically no phosphoric acid compounds, but still prevents polyneuritis. The protective substance was capable of dialysis through a semi-permeable membrane. The same authors confirm the earlier observations of Pol that extract of the mongo bean is protective.

Wieland reports analyses of mice which had been fed on rice. He found that such animals were no poorer in phosphorus than the controls. It would seem from these experiments that the etiology of the disease was not associated with deficiency in the phosphorus supplied to the organism.

Andrews has made some very interesting observations on infantile beriberi. Analyses were made of the milk obtained from 11 women whose infants had died of beriberi, as confirmed by necropsy. The figures show the milk in these cases to be scant, but some of them seem normal so far as protein, fat, and carbohydrate are concerned. Both the calcium and phosphoric acid content of the samples analyzed were above the normal. Puppies, allowed to suckle these women, died. Symptoms and necropsy agreed entirely with those of the infants dying of infantile beriberi.

Chamberlain, Vedder, and Williams found that arginine, histidine, asparagine, and other amino-acids, lipoids of the lecithin group, choline, and extract of onions were without protective action against polyneuritis in fowls. The neuritis-preventing principle was shown to be insoluble in ether. In a subsequent paper, Vedder and Clark state that fowls receiving 10 grams of meat or potatoes or 5 cubic centimeters of cow’s milk per day, with polished rice, receive partial protection only. Peas or peanuts given with the rice prevent the development of polyneuritis.

Funk has reported the apparent isolation by phosphotungstic acid precipitation of the alcohol-soluble protective substance from yeast, rice polishings, and other foodstuffs. He considers this substance a pyrimidine derivation of the composition $C_{17}H_{49}N_2O_7$, and melting at 233°. Suzuki, Shimanura, and

20 Ibid. (1911), 395.
22 Arch. f. exper. Pathol. (1912), 69, 293.
23 This Journal, Sec. B (1912), 7, 67.
24 Ibid. (1912), 39.
25 Ibid. (1912), 423.
26 Journ. Physiol. (1912), 45, 75.
Odaki 27 also obtained a phosphotungstic acid precipitable protective substance from the alcoholic extract of rice polishings. Vedder 28 has not succeeded in isolating this substance in experiments as yet reported. In the same paper, Vedder reports incidentally experiments on fowls with rice and Röhmann’s salt mixture which are negative. The paper appeared while the present work was under way. Vedder and Clark 29 suggested the occurrence of at least two “vitamines” in the alcoholic extracts of the rice polishings to account for the types of polyneuritis gallinarum which they distinguished.

Osborne and Mendel 30 have recently published a comprehensive study of the rôle of the individual proteins in nutrition. They have followed the growth changes in young rats when fed on a single highly purified protein, along with fat, carbohydrate, and a salt mixture having the same composition as the salts in milk. The experiments show that normal growth can occur only when the protein component of the diet is complete as regards the amino-acids obtained on cleavage. Mere maintenance, or even nitrogen starvation, results when an incomplete individual protein is fed. The food in Osborne and Mendel’s experiments contains no purines or nucleic acid. In the case of the vegetable proteins, such as edestin, there is no organic phosphorus, yet normal growth was obtained even with a diet consisting of edestin, starch, sugar, and salts. There is apparently no physiological necessity for organic phosphorus, lipoid, or organic iron (in more stable form than the citrate).

According to Osborne and Mendel, then, provided sufficient calories are fed as fat or carbohydrate, but two dietetic factors are necessary—a chemically complete protein and a physiologically balanced salt mixture. It would seem that the development of polyneuritis in fowls ought to be prevented when white rice is fed with properly balanced mineral ingredients, provided the rice protein is nutritively adequate.

Deficiency in the protein may be excluded. The chief protein of the rice 31 is oryzenin, a glutelin. Suzuki, Yoshimura, and Fuji 32 have determined some of the amino-acid cleavage products of this protein. Their analyses indicate that the protein is

28 This Journal, Sec. B (1912), 7, 412.
29 Loc. cit.
to be regarded as "complete," although the presence of tryptophane was not determined. However, I have prepared some oryzenin, and found that it gives the Hopkins-Cole reaction. Karl Thomas claims to have shown, too, that the rice proteins have over four-fifths the nutritive efficiency of casein.

Analyses of polished rice show a varying ash content of from about 0.45 to 1 per cent, although proportionally the mineral ingredients are fairly constant. For comparison with Osborne and Mendel's experiments, I have calculated the inorganic constituents per kilogram of rice as follows:

**Table I.—Salts per kilogram of food.**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Osborne and Mendel's experiments</th>
<th>Rice I</th>
<th>Rice II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>Grams 0.45</td>
<td>0.12</td>
<td>0.20</td>
</tr>
<tr>
<td>Ca</td>
<td>0.7</td>
<td>0.30</td>
<td>0.74</td>
</tr>
<tr>
<td>Mg</td>
<td>6.1</td>
<td>0.18</td>
<td>0.33</td>
</tr>
<tr>
<td>Na</td>
<td>8.0</td>
<td>0.80</td>
<td>1.82</td>
</tr>
<tr>
<td>K</td>
<td>10.0</td>
<td>3.20</td>
<td>6.68</td>
</tr>
<tr>
<td>PO4</td>
<td>12.4</td>
<td>0.004</td>
<td>0.09</td>
</tr>
<tr>
<td>Cl</td>
<td>0.9</td>
<td>0.03</td>
<td>0.066</td>
</tr>
<tr>
<td>SO4</td>
<td>0.13</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>Citric acid</td>
<td>10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiO2</td>
<td>0.0</td>
<td>0.12</td>
<td>0.64</td>
</tr>
</tbody>
</table>

* Per cent.

Most striking differences are observed. The rice is deficient in calcium, and there is, relative to the element, a much greater proportion of magnesium. Sodium and potassium chloride are low. The total amount of the mineral constituents is low. The salts certainly seem far from being properly balanced to maintain normal physiological activity over relatively long periods of time. The presumable absence of mineral salts of the organic acids, in the white rice, might be considered as a contributing factor to the development of beriberi; particularly is this point significant for polyneuritis gallinarum, since the end product of nitrogenous katabolism, uric acid, is eliminated as the urate. In as much as certain salts of the organic acids are soluble in alcohol, part of the protective effects of the alcoholic extracts of

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34 Kellner, Uchiyama, and Yamada, Die landw. Versuchsstationen (1892), 41, 295.
the rice polishings may be due to this factor. In fact, qualitative tests have shown that calcium, potassium, and some organic acid, other than lactic acid, are present in such extracts of the rice polishings.

It is probable that the balanced inorganic constituents of Osborne and Mendel's ration are not as well adapted for fowls as for mammals. Still, in the experiments reported in this paper, the attempt has been made to supply the several salts to the rice fed to fowls with particular reference to calcium and the salts of some organic acid. While the experiments have not been successful in this respect, the results are of sufficient interest to warrant publication. Some experiments with monkeys, one of which developed an almost typical case of beriberi, are included.

**PREPARATION OF THE SALT MIXTURES**

The rice employed (Philippine No. 1) had an ash content of 0.47 per cent. A stock salt mixture was made with rice flour, but otherwise prepared in essentially the same manner as described by Osborne and Mendel. When the stock salt mixture was added to about two and one-half times the weight of cracked rice, 1 kilogram of the food would have approximately the composition for the mineral ingredients recommended by Osborne and Mendel if the analyses for "Rice I" can be taken as representative. In the calcium experiments, the carbonate was neutralized with hydrochloric acid or with both hydrochloric and lactic acids (the latter in amount equivalent to the citric acid in Osborne and Mendel's experiments). In two cases, half the above amount of lactic acid, as sodium lactate, was fed with the polished rice.

For 980 grams of rice flour there were used CaCO₃, 53 grams; MgCO₃, 5.5 grams; K₂CO₃, 50.8 grams; Na₂CO₃, 54.3 grams; Fe-citrate, 1.7 grams; HCl (sp. gr. 1.20), 137.7 cubic centimeters; H₂SO₄ (sp. gr. 1.84), 2 cubic centimeters; H₃PO₄ (85 per cent), 19.3 cubic centimeters; and citric acid, 40 grams. In the calcium chloride experiments 54 grams of CaCO₃ were neutralized with HCl and added to 1 kilogram of rice flour; and for the lactate experiments a mixture of 30 grams of CaCO₃ neutralized with lactic acid and of 24 grams of CaCO₃ neutralized with HCl, per kilogram of rice flour, served as the stock salt mixture.

**EXPERIMENTAL METHODS**

Well-grown young male fowls were used in the experiments. These were kept in individual cages with alberine stone floors and provided with a perch. The cages were cleaned daily. In the earlier experiments, the stock rice-salt mixture was made
into a granulated paste with cracked white rice and water, and the amounts eaten by each fowl recorded. In subsequent experiments, the fowls were fed when they refused to eat the rice provided. It was found that they would eat voluntarily sometimes as much as 85 grams of rice per day, although many fowls may be maintained upon an average of 35 grams of rice. The chickens were weighed every morning before feeding, and the condition of each noted. Fowls dying were necropsied, and the sciatic nerve removed to study the degenerative changes, in as much as Clark has stated that microscopic evidence of polyneuritis may be observed even a week after the rice feeding has been started.

THE EFFECTS OF FEEDING WHITE RICE WITH THE COMPENSATED SALT MIXTURE

Three fowls were fed on the white rice used in the subsequent experiments for controls. These developed polyneuritis on the nineteenth, twenty-second, and twenty-ninth days, respectively. Examination of the sciatic nerves by the Marchi method showed typical and pronounced Wallerian degeneration in each case.

Three more fowls were fed on white rice plus the stock salt preparation. These developed neuritis on the thirty-seventh, thirty-seventh, and thirtieth days, respectively. Degeneration was typical in certain fibers in one case, typical but not pronounced in the second, and very pronounced in the third instance.

The experiment is summarized in Table II.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First day.</td>
<td>Seventh day.</td>
</tr>
<tr>
<td>2</td>
<td>do</td>
<td>1,174</td>
<td>1,210</td>
</tr>
<tr>
<td>3</td>
<td>do</td>
<td>985</td>
<td>1,061</td>
</tr>
<tr>
<td>4</td>
<td>Salt mixture</td>
<td>1,064</td>
<td>1,062</td>
</tr>
<tr>
<td>5</td>
<td>do</td>
<td>1,156</td>
<td>1,060</td>
</tr>
<tr>
<td>6</td>
<td>do</td>
<td>1,130</td>
<td>1,176</td>
</tr>
</tbody>
</table>
The addition of the compensated salt mixture, then, has not prevented the development of polyneuritis in fowls. In the experiments reported above, however, the onset of the disease seems to have been slightly protracted, and the degenerative changes in the nerves were less pronounced than in the controls.

EXPERIMENTS WITH CALCIUM CHLORIDE AND WITH LACTATES

As has been stated, as compared with Osborne and Mendel's salt mixture, the rice is notably deficient in calcium. Accordingly, 3 pairs of fowls were fed on rice with the addition of the calcium lactate and calcium chloride, and with the calcium chloride alone.

One fowl, allowed to eat voluntarily, progressively increased in weight from 1,035 to 1,212 grams on the calcium lactate and calcium chloride mixture, but on the fiftieth day developed neuritis. Histological examination of the sciatic nerve showed typical degeneration in a few nerve fibers, along with many nuclei of the embryonic or regenerating type in the fiber sheath. (These histological findings and their significance for the question of regenerative changes in nerve fibers are to be discussed by Clark in a paper shortly to be published.) The other fowl of this pair, which received the calcium chloride rice only, gradually dropped in weight from 1,165 to 744 grams, and died of general physical weakness, there being no symptoms of neuritis. Histological examination of the sciatic nerve gave only one or two fibers showing the typical degeneration. These and the other experiments are given in Table III.

The calcium lactate-chloride fowl of the second pair lost weight after the second week, developed chicken pox on the thirty-second day of the experiment, and practically recovered from this but was found dead on the forty-seventh day. There were no symptoms of neuritis. At necropsy, numerous nodules were found in the wall of the intestine, which on section seemed to inclose some animal parasite. Histological examination of the right sciatic nerve showed fibers in the preparation typically degenerated. The calcium chloride fowl gradually fell in weight from 1,076 to 940 grams developing chicken pox on the twenty-seventh day, when the daily weight fell rapidly. This fowl practically recovered from the pox, but gradually grew weaker, and was killed on the thirty-sixth day. Neuritis was questionable, and probably had not developed. Only a very few fibers of the sciatic showed the Wallerian degeneration. Both the above chickens were allowed to eat voluntarily.
The third pair of fowls was given 60 grams of the calcium rice mixture per day, and, when the food was unconsumed, was fed the balance. The calcium lactate-chloride fowl gained rapidly in weight from 1,170 to 1,295 grams on the twenty-first day of the experiments; then the weight dropped slightly, and again rose to 1,260 grams on the thirty-second day of the experiment, when neuritis developed. Examination of the sciatic nerve showed moderate although typical degeneration. The calcium chloride fowl of this pair gained in weight from 1,167 to 1,300 grams for the first two weeks, and then gradually lost weight, seemed sick on the twenty-first day of the experiment, and died on the twenty-fifth day. Neuritis symptoms were questionable, and the sciatic fibers showed but little degeneration. It is probable that these two fowls received too much calcium, in as much as they were forced to consume almost twice the food per diem as was eaten by the other two pairs after the first few days of the experiment; there was, however, no evidence of hæmorrhages into the tissues of the œsophagus between the crop and the muscular stomach or of ulceration of the mucosa of the latter, which I have observed in chronic calcium poisoning in some feeding experiments not reported in this paper.

Two fowls were given one-half the amount of lactic acid fed in the above experiments, but in the form of sodium lactate. One fowl gained rapidly in weight from 1,055 to 1,200 grams on the twentieth day of the experiment, often voluntarily eating 75 grams of rice per day. The body weight did not fall below 1,150 grams until the thirty-seventh day of the experiment, when he ate little and seemed sick. The salt mixture was changed so that the fowl received twice the former amount of lactic acid, half as sodium and half as the calcium lactate, and half the amount of calcium chloride as was received by the calcium lactate-chloride chickens in the above experiments. He was given, or fed in part, 60 grams of this rice and salt mixture per day, and rapidly improved in weight and condition. On the sixtieth day, when the fowl weighed 1,187 grams, incipient neuritis was evident. The fowl was killed on the sixty-second day. Examination of the sciatic nerve showed most profound degeneration, nearly every fiber being involved. Numerous nuclei of the embryonic nerve fiber type were found in histological preparations of the sciatic. The second sodium lactate fowl was started simultaneously with the other, and rapidly gained from 1,040 to 1,160 grams the first week. Subsequently, he ate much less, and the body weight dropped to 1,047 grams on the twenty-first day.
When fed by hand, the weight was increased to 1,092 grams on the thirtieth day; the fowl, however, seemed slightly sick, and died suddenly on the thirty-third day from some cause not revealed at necropsy. There was no evidence of degeneration on histological examination of the sciatic nerve.

A striking incidental observation is found in that the fowls, which received the lactate, developed brilliant red erect combs and wattles and a fine plumage. This is in marked contrast with the results obtained in the other rice-fed fowls. These differences are shown in the accompanying reproductions of photographs of the chickens.

The fact that the lactate chickens have not lost weight, and in particular have put on weight even when the experiments have extended over relatively long periods, is important. This finding suggests that a closer symptomatic relationship exists between polyneuritis gallinarum and beriberi in man than the evidence heretofore available has permitted to be accepted. From these experiments, it would seem that the administration of calcium salts or of lactates has prolonged the period required for the development of the polyneuritis. Furthermore, it seems that the fowls will not survive for long the administration with the rice of the calcium as the chloride only. Regeneration processes, as evidenced by the presence of embryonic nuclei in the nerve fibers of one fowl receiving calcium lactate and of a second on sodium lactate and then calcium lactate, are most suggestive.

EXPERIMENTS WITH MONKEYS FED ON WHITE RICE AND SALTS

Schaumann found that a monkey, fed on rice, lost appetite, and developed a paralysis of the lower extremities and progressive marasmus. Degeneration of many nerve fibers was evident. Aron obtained a somewhat similar result with 3 monkeys fed on white bread. Shiga and Kusama observed in a monkey at first an increase of appetite followed after thirty-seven days by a loss of appetite, and subsequently by a loss of the patellar reflex and paralysis of the lower extremities. The animal died ten days later. There was degeneration of the peripheral nerves and the cells of the anterior horn, atrophy of the musculature, etc.; the heart showed a dilatation hypertrophy. Nagayo and

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87 Loc. cit.
88 Loc. cit.
89 Loc. cit.
TABLE III.—Rice fed with CaCl₂, Ca-lactate, and Na-lactate.

<table>
<thead>
<tr>
<th>No.</th>
<th>Material fed</th>
<th>Weight of fowl on the—</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day</td>
<td>7th day</td>
</tr>
<tr>
<td>-----</td>
<td>--------------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>Ca-lactate</td>
<td>1,035</td>
<td>1,058</td>
</tr>
<tr>
<td>2</td>
<td>Ca-chloride</td>
<td>1,165</td>
<td>1,025</td>
</tr>
<tr>
<td>3</td>
<td>Ca-lactate</td>
<td>1,068</td>
<td>1,075</td>
</tr>
<tr>
<td>4</td>
<td>Ca-chloride</td>
<td>1,076</td>
<td>1,014</td>
</tr>
<tr>
<td>5</td>
<td>Ca-lactate</td>
<td>1,170</td>
<td>1,225</td>
</tr>
<tr>
<td>6</td>
<td>Ca-chloride</td>
<td>1,167</td>
<td>1,196</td>
</tr>
<tr>
<td>7</td>
<td>Na-lactate</td>
<td>1,055</td>
<td>1,150</td>
</tr>
<tr>
<td>8</td>
<td>do</td>
<td>1,040</td>
<td>1,152</td>
</tr>
</tbody>
</table>

* Mild attack of chicken pox.

* Food changed from Na-lactate to half Na-lactate and half Ca-chloride and lactate rice.
Fujii fed 6 monkeys on cooked white rice. Of these, 2 died of simple inanition, 3 of inanition with scurvy, and the sixth in thirty-four days of inanition with symptoms of beriberi. In the sixth monkey the pain sense and patellar reflex were depressed but present during the last days; the heart was somewhat dilated, but not hypertrophied to any great extent; the lungs were congested and edematous, and there was degeneration of the cells of the anterior horn of the cord and of the peripheral nerves.

Six recently trapped monkeys of the common Philippine species, *Pithecus syrichta* (Linnaeus), were obtained and placed in separate cages. These were fed on rice which had been boiled until soft in a relatively large amount of distilled water; the rice was washed two or three times, and then water strained off through gauze. To the rice fed to three of the monkeys there was added a salt mixture made up according to the method Osborne and Mendel, except that rice flour was used instead of the lactose. A little banana was added to the rice when cooked to flavor it, as it was found that the monkeys were refusing to eat after the first few days of the experiment.

On the forty-second day of the experiment, 1 of the salt-fed monkeys, whose weight had fallen from 1,341 to 1,289 grams, became edematous. The edema was especially noticeable in the face. On the following day the edema was more striking. The third day the edema had largely disappeared, but the monkey was evidently sick, and was irritable when touched. On the forty-sixth day of the experiment, the animal was lying on its side and evidently dying. At necropsy, the body seemed poorly nourished, with the viscera normal except for a slight gastritis. The right heart was greatly dilated, the ventricle wall being very thin. The heart appeared as if double apexed. The lungs seemed normal. There was no excess of fluid in the body cavities, but the tissues seemed wet when cut. Histological examination of the sciatic nerve, as may be seen in the accompanying photomicrograph, showed typical Wallerian degeneration (Plate IV).

The other 5 monkeys became progressively marasmic. There was no marked difference in the development of the conditions in the salt-rice and the rice groups. One of the monkeys fed on rice alone died on the eighty-sixth day of the experiment. At necropsy the monkey was found to be rough-haired, very poorly nourished and apparently starved, somewhat jaundiced,

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89 Verhandlungen der Japanischen Pathologischen Gesellschaft (1912), 39.
but with no obstruction in the common or cystic duct, and without any special evidence of beriberi. The sciatic nerve, however, was found on histological examination to show typical degeneration.

The experiment was discontinued after one hundred twenty days, when all the remaining animals, then in very bad condition, were returned to a mixed diet.

It has been shown that the attempt to keep monkeys on the salt mixture added to water-extracted rice has not sufficed either to maintain the weight of the animals or to prevent the development of an almost typical case of beriberi in one instance. The experiments again serve to show the resistance of monkeys to white rice as compared with man.

SUMMARY AND CONCLUSIONS

The addition of a compensated salt mixture to white rice fed to fowls has not prevented the development of polyneuritis gallinarum. However, the onset of the disease seems to have been slightly protracted, and the degenerative changes in the nerves were less pronounced.

Fowls fed on white rice and lactates, and in particular calcium lactate, have maintained body weight, even when the experiments have extended over relatively long periods. This fact suggests that a closer symptomatic analogy may exist between polyneuritis gallinarum and beriberi in man than the evidence available has hitherto permitted to be drawn. The administration of calcium salts or lactates prolonged the period required for the development of neuritis, though the fowls did not long survive the diet of rice and calcium chloride. The lactate-fed fowls developed brilliant combs and a fine quality of plumage in contrast with all other chickens employed in these experiments. Regenerative processes, as evidenced by the discovery of embryonic nuclei, were suggested on examination of the nerve fibers of 2 fowls which had received calcium lactate.

The attempt to keep 3 monkeys on an approximately compensated salt mixture and white rice failed either to maintain the body weight of the animals or to prevent the development of an almost typical case of beriberi in 1 case. These experiments, with the 3 controls, demonstrate again the resistance of monkeys to white rice as compared with man.

Osborne and Mendel have shown that, with an exactly physiologically balanced salt mixture, rats may maintain their weight and even grow to maturity when fed on pure protein with carbohydrate or carbohydrate and fat. However, it is evident that
under normal circumstances of life such conditions do not obtain for the individual except in the early stages of its development. Accessory factors must play a rôle of extreme importance in normal dietaries, as shown by Hopkins who found that a given increment of growth in young rats is attained with much greater economy when a ration of milk is added to the artificial mixture of casein, carbohydrate, lard, and salts. The feeding experiments which I have carried on again emphasize the existence of such accessory factors for normal nutrition.

Since this paper was written, Clark has made a study of the so-called "embryonic nerve fiber." The results are in part based on the examination of the nerves of the "calcium lactate" fowls and a full description of these nerves will be given in Clark's paper. He concludes that the "embryonic nerve fiber" is a stage of advanced degeneration rather than a regenerative phenomenon.

I have shown that the calcium lactate has prolonged the onset of symptoms of the polyneuritis. Clark believes that the longer course of the disease has permitted degeneration to occur in certain fibers of the sciatic to a degree much more intense than has hitherto been observed for fowls fed on rice alone; for instance, a degeneration of the type observed after a nerve is cut. The ordinary rice neuritis, then, must be of a relatively mild type, as is further evidenced by the rapid recovery, often in two or three hours (Funk), subsequent to the administration of the "vitamine" preparations.

"Journ. Physiol. (1912), 44, 425."
ILLUSTRATIONS
(Photographs by Cortes)

PLATE I

Fig. 1. Fowl fed on the rice and calcium chloride, after thirty days.
2. Fowl fed on the rice and calcium lactate and chloride, after thirty days.

PLATE II

Fig. 1. Fowl fed on the rice and calcium chloride, after thirty days.
2. Fowl fed on the rice and calcium lactate and chloride, after thirty days.

PLATE III

Fig. 1. Fowl fed on 60 grams of the rice and calcium chloride per day, after thirty days.
2. Fowl fed on 60 grams of the rice and calcium lactate and chloride per day, after thirty days.

PLATE IV

Microphotograph of the sciatic nerve of the monkey which died with beri-beri symptoms after forty-six days on a diet of boiled rice with the salt mixture (Marchi preparation).
Fig. 1. Fowl fed on the rice and calcium chloride, after thirty days.

Fig. 2. Fowl fed on the rice and calcium lactate and chloride, after thirty days.

PLATE I.
Fig. 1. Fowl fed on the rice and calcium chloride, after thirty days.

Fig. 2. Fowl fed on the rice and calcium lactate and chloride, after thirty days.

PLATE II.
Fig. 1. Fowl fed on 1 gram of the rice and calcium chloride per day, after thirty days.

Fig. 2. Fowl fed on 60 grams of the rice and calcium lactate and chloride per day, after thirty days.

PLATE III.
PLATE IV. MICROPHOTOGRAPH OF THE SCIATIC NERVE OF THE MONKEY WHICH DIED WITH BERIBERI SYMPTOMS AFTER FORTY-SIX DAYS ON A DIET OF BOILED RICE WITH THE SALT MIXTURE (MARCHI PREPARATION).
AN UNUSUAL DISEASE PREVAILING IN EPIDEMIC FORM AT
BUHI, AMBOS CAMARINES, P. I.

By MARSHALL A. BARBER

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

On October 17, 1912, Dr. Segundo Isaac, acting district health officer of Ambos Camarines, reported the presence of an unusual disease prevailing at Buhi, Ambos Camarines Province, Luzon. His report, in summary, is as follows:

Eight deaths were reported to me as caused by this disease from August 15 to date. It is suspected that some of the deaths were due to other diseases. Of these 8 fatal cases, 5 were thoroughly investigated:

Zeila Juareg: A girl of 13 years. The morning of October 3 she noticed a red tumor on her breast. This tumor was hot, somewhat hard, and gave very little pain. On the next day she felt slight pain in it. She died on October 5 with subnormal temperature.

Maria Sabinorio: A girl 14 years of age. She felt slight pain in the breast on the morning of October 6, the pain extending later in the day to the stomach. Little pain was felt until her death the next day at 2 o'clock in the afternoon.

Justa Balectora: On September 26 she was pricked by a small piece of bamboo. Inflammation of the part followed. The lesion was soft and white in the center with some infiltration and inflammation. On the 28th inflammation extended to the forearm and the elbow. At night she suffered headache and pain in the bowel. She died on the morning of the 30th, not suffering great pain.

Severino Oliceres: On October 4 he was pricked on the hand by a bamboo thorn. A slight inflammation followed which disappeared after a little treatment. The night of October 7 a swelling appeared on the breast, not painful but somewhat hard and red. The next day the patient felt a slight pain in the bowel, and died at 3 o'clock in the afternoon.

Esperanza Arcilla: A girl 2 years of age. She had a very small nodule in the leg. It was cauterized, and disappeared; but after a while another nodule appeared near it. This was treated the same way and also disappeared, but was followed by a third in the same region. After two days she died with the same symptoms as the above-mentioned cases.

Other patients suffering with this disease are likely to recover. In almost all of the fatal cases the patients died after a very short illness and with very little pain. The disease begins in the skin, extends to other parts of the body, and death follows with subnormal temperature.

Almost all the people of the town declare that this disease is, in all respects, similar to the disease that is causing great mortality among carabaos.

As a measure for its suppression, instructions were given to the president for the isolation of the infected persons and the disinfection of the premises.
I was detailed to Buhi to investigate this disease, and remained there from October 30 to November 4, 1912, inclusive. At the time of arrival the severity of the epidemic had abated, and there were no more of the rapidly fatal cases. However, some 18 persons were seen who were suffering from a type of inflammation which they alleged was of the same nature as that of the earlier fatal cases. There were 3 or 4 fairly severe cases, and 1 case, a woman of 90 years, died November 3.

The patients were questioned as to the history and symptoms of the disease, and some of them were visited on 3 or 4 successive days. Temperature and pulse were taken of some 13 of the more severe cases. Blood, and, where obtainable, pus was examined microscopically, by culture, and in some cases immediately inoculated into guinea pigs. In 1 case cultures were made of blood from a vein of the arm.

The patients were for the most part adults, although there was one 7 years old and another 2 years and 8 months. There were 11 females and 7 males.

As to the lesions, the one common to all cases was swelling, often but not always painful, usually rather diffuse, but in some cases well defined and varying from a small lump to a tumor the size of half an orange. There was no tendency to localize in the joints or in the inguinal or axillary glands. The locality of the lesions was as follows: arm, or hand and arm, 7; foot, 3; leg below knee, 2; thigh, 1; face, 2. The lesions were usually located on one side or the other with no tendency to right and left symmetry.

As a rule there was no tendency of the lesion to come to a focus with pus formation, unless some surgical or cauterizing remedy had been applied; but in 2 cases not thus treated abscesses had formed. In about half the cases the lesion was said to have begun at some definite point on the skin and to have spread from it. In 4 cases the starting point was described as a pimple, and in 1 as "like an ant bite" on the finger. In 5 or 6 cases the lesion was said to have begun at an extremity, usually the fingers, and to have extended up the arm or leg. Three cases showed a vesicular eruption over the lesion.

The temperatures of 13 of the more severe cases were taken by mouth, some of them on 3 or 4 successive days. In 6 cases there was a temperature of 100° or over, in one, 102°; the rest ranging from 99°.5 to normal, and exhibiting no more temperature than would be expected in a patient suffering from a minor inflammation.
As to laboratory findings, pus, where obtained from a superficial lesion, showed either pure Staphylococcus aureus or Staphylococcus pyogenes with Streptococcus. In pus obtained from a deep abscess avoiding surface contamination, a pure culture of Streptococcus was obtained. Microscopical examinations of blood smears from nonpurulent lesions were negative, and cultures from this source and from blood taken from the vein of an affected arm were also negative. All guinea pig inoculations of pus or blood were negative. Plague and anthrax could be definitely excluded.

The duration of the cases was from two or three days to several weeks, and all cases seen by me recovered except one, a woman of 90, who had an abscess on the ankle.

The people on being questioned maintained that a disease like the one observed by me had not been known in Buhi before, and certainly the occurrence of so many cases in a small population and the fact that they followed an epidemic of fatal cases would argue something unusual. There is no conclusive evidence that the disease seen by me was a milder form of the earlier epidemic; but the testimony of the people, the similarity in the lesions, and the fact that the one immediately followed the other point to that conclusion.

The only positive laboratory findings were pyogenic cocci, and it is possible that many of the milder and perhaps of the earlier fatal cases were due to pyogenic infection. The fact that the disease often began at a definite point in the skin, sometimes a minor lesion, and extended from it, would support this view. But one would not expect a pyogenic infection to take an epidemic form, unless in a people whose resistance had been lowered by exceptional hardships. The location of the town of Buhi is healthful, and the physical condition of the people was generally good at the time of my visit; but there had been an extended drought earlier in the season. There was no foundation for the belief that this disease had any connection with that of carabaos.

Inquiry has been made among persons who have had a wide medical experience in the Philippine Islands, but none have observed a similar epidemic. Dr. A. G. Sison of the Philippine General Hospital mentioned a cellulitis, probably pyogenic in origin, known among the people of Manila and the northwest provinces as "culebra" or "snake," because of its tendency to progress from an extremity upward. There is a slight fever and a vesicular eruption over the lesion. This disease does not occur in an epidemic form.
It is recognized that the provisional explanation of the cause of the Buhi epidemic is unsatisfactory, and the pyogenic bacteria may be associated with some other agent which is the real cause of the disease. However, it seemed worth while to give a brief account of the observations made by Doctor Isaac and by me, in the hope that they may be of some use to those who have seen, or may encounter, a similar disease.¹

¹It may be of interest to note the nature and variety of remedies used for this disease by the people of the village. Application of carbolic acid, petroleum, silver nitrate, tincture of iodine, and charcoal of coconut shell; and poultices of garlic, mustard, and of the leaves of the following plants: anonang, betel-nut palm, dao tree, cotton, and calumpinag. Also cupping with a cow’s horn was practiced, burning with a lighted cigarette, and binding with a string to arrest the progress of the inflammation.
THE INFECTION OF ACHLYA WITH VARIOUS MICROORGANISMS

By Marshall A. Barber

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Three plates

In a previous paper, I described a method of inoculating microorganisms and other substances into the vacuoles or protoplasm of living cells; and, in a later article, gave some of the results obtained by such inoculations into fish molds, various algae, and the larvae of a gnat. In this paper essentially the same method of inoculation has been used; but the host has been grown in pure culture, thus making it possible to add various media or other substances to the culture and to do away with bacteria other than the ones inoculated.

Achlya, one of the fish molds, was chosen for these experiments because of its large filaments and ease of cultivation.

Pure cultures were easily obtained by the method of single-cell isolation described by me in various publications. Insects were placed in water containing algae, in the usual manner of obtaining cultures of fish molds. After a crop of zoospores had formed, a mass of them in the resting condition was transferred to a hanging drop. With a fine capillary pipette, single zoospores were separated from the mass, freed from bacteria by successive washings in droplets of sterile water, picked up with a fresh pipette, and each one transferred to a test tube containing distilled water plus a small quantity of glucose broth. The whole process of isolation of a series by this method requires only a few minutes. Or, a mass of zoospores, partially freed from bacteria, may be deposited in one end of a long hanging drop of sterile water. These spores, at first invested with a membrane, burst the membrane within two or three hours and swarm to the other end of the drop. They may be picked

1 Journ. Infect. Dis. (1911), 8, 348.
2 Ibid. (1911), 9, 117.
with the capillary pipette while swarming in the nearly or quite bacteria-free end of the drop, or after they have come to rest and germinated. Either method enables one to obtain a culture from a single zoöspore, but the latter sometimes facilitates freeing them from bacteria. Before finally transferring to the test tube, each isolated zoöspore may be examined in a small droplet of water with a higher power in order to make sure of the absence of bacteria.

In the absence of zoöspores or resting spores in the pure culture, one may make subcultures by transferring the mycelium grown in the liquid medium to sloped agar tubes. When growth is established there, new cultures may be made from detached pieces of agar containing living hyphae.

For purposes of inoculation it is necessary to have a thrifty mycelium in a hanging drop of nutrient medium. For obtaining this there are several convenient methods.

From a young subculture growing in a thin layer of agar in a Petri dish one may cut with sterile knife or platinum spatula small blocks of agar from the margin where growth is most vigorous. These are transferred to the sterile cover glass, and, if desired, fresh nutrient medium is added to the margin of the block.

Another method would be by transferring bodily a small mycelium to a liquid medium in a hanging drop.

Zoöspores in this species were formed much less freely in pure culture than in ordinary water cultures with bacteria. But sufficient zoöspores for hanging drops or for making subcultures were obtained from a three to ten days' growth in test tubes containing about 10 cubic centimeters of distilled water with 0.05 to 1 cubic centimeter of 1 per cent glucose broth added to each tube. In one culture, glucose broth, made 5 + acid and added in the proportion of 0.1 cubic centimeter to 5 cubic centimeters distilled water, gave an abundant crop in from two to three days. Apparently some degree of starvation facilitates the formation of zoöspores in this species, since a vigorous growth in undiluted glucose broth or on sterilized insects in water usually failed to produce them.

The best mycelia for the purposes of these experiments were obtained from the large resting spores formed vegetatively at the tips of hyphae. These usually occurred abundantly in old cultures in distilled water plus glucose broth. These are separated from the mycelium with the platinum loop, and when settled to the bottom of the test tube may be transferred by a pipette to a hanging drop. From this drop one or more are
selected and transferred to a second cover for the permanent culture. These spores produce in a few hours a thrifty mycelium with hyphae larger than those from zoöspores and more suitable for inoculation. Abundant zoöspores were obtained within twenty hours by sowing one of these resting spores in a hanging drop, consisting of the original fluid in which the resting spores had formed. This fluid was somewhat enriched by nutriment from the mycelium crushed in the process of separating the resting spores. No extended experiments were made to determine the conditions which favor the formation of various types of reproduction, since the primary object was simply to get material suitable for inoculation.

The following is an example of one series of isolations:

May 21. Resting zoöspores from the tip of a zoösporangium of Achlya were separated, washed, and transferred to tubes of distilled water plus a few drops of glucose broth. Tube 1 received 16 spores; tube 2, 6 spores; tube 3, 3 spores; tube 4, 4 spores; tubes 5, 6, 7, 8, and 9, each 1 spore.

May 23, all showed growth of Achlya except tube 8, which remained sterile. All were free from bacteria except tubes 1 and 2 which had received the masses of 16 and 6 spores. Tube 7 developed zoöspores after four days' growth at room temperature; but zoöspores could not be detected in any other. Five of the pure cultures, including tube 7, developed oögonia and antheridia after six days' growth, and the 2 contaminated ones developed them some time later. The addition of undiluted 1 per cent glucose broth to 2 tubes, 1 containing zoöspores the other none, brought about a luxuriant vegetative growth with few or no zoöspores or oögonia.

Both liquid and agar media were used for hanging drop cultures. In making these a large cover, about 38 by 65 millimeters in size, was sterilized and placed on the glass box used in isolating microorganisms. A rectangular or ovoid barrier of sterile paraffin was usually made on the underside of this cover by melted paraffin blown from the tip of a bent pipette. Within this inclosure the medium was placed, and the spore or mycelium planted in it. Such cultures may be grown for days over a moist chamber. This chamber should be 0.5 centimeter or more deep, else branches of the mycelium may grow to the bottom of the cell and introduce bacteria into the culture.

Liquid culture media may be readily withdrawn from such cultures and a new medium or any other liquid substance added by means of a sterile capillary pipette bent at the tip.

It is possible to inoculate a hypha so that all growth will
take place inside of the infected mycelium and none in the medium around it; or, if desired, to have one kind of organism growing in the host and another kind outside. To avoid inoculating the medium outside of the plant some special precautions are necessary. A hanging drop of the bacterium is made outside of the paraffin barrier, and beside it is placed a drop of sterile water or agar. After supplying the pipette with the necessary dose it is withdrawn from the bacteria and passed through the water or agar drop to remove from its surface any adhering bacteria. After inoculating the fungus the tip must be withdrawn very slowly and cautiously in order to prevent the forcing out of any of the injected bacteria by the cell pressure. In case some bacteria are left outside in the medium, it is often possible to remove them with an ordinary capillary pipette. If too numerous or scattered to be removed in this way, their growth may be restricted or their effects diminished by frequently withdrawing the old medium and substituting new, or by adding some specific serum unfavorable to the development of the bacterium.

In the following experiments, bacteria pathogenic to animals were inoculated in order to test their effects on plant protoplasm. Of motile forms, Bacillus pyocyaneus and the vibrios of Asiatic cholera were tested; and of the nonmotile, the bacillus of dysentery, Shiga-Kruse type, and the bacillus of bubonic plague.

With Bacillus pyocyaneus some 14 successful inoculations were made, the majority in pure cultures of Achlya in hanging drop at a room temperature of from 27 to 31° C. Under these conditions infection usually followed the inoculating of even very small doses, and the death of the filament occurred within twenty-four hours. Not only the hypha at the point of inoculation become infected, but any branches not plugged off from the inoculated filament soon became swarming to their finest endings with actively motile bacilli. Noninfected filaments often remained in good condition in the same hanging drop. The cause of the death of the infected filaments was, apparently, primarily due to exhaustion of nutriment by the bacteria, since the protoplasm of the host often remained living and motile when the bacilli were so numerous as to form practically an emulsion in the vacuole. Bacillus pyocyaneus grown outside the mycelium in an agar hanging drop may be tolerated two or three days.

After the death of the filament the bacteria continued to grow, packing the filament with densely crowded masses, and often bursting through the wall and forming masses usually at the tips of branches. (Plate I.) The whole infected filament takes
on a yellowish brown color and contrasts sharply with the surrounding healthy portion. The bacteria which burst out are in part living and may contaminate the medium.

*Bacillus pyocyaneus* is more rapidly destructive of the fungus than any other organism tested. The death of the infected filament may be delayed some days by keeping at refrigerator temperature, but the bacteria remain living and multiply on removal to a higher temperature.

Specific rabbit serum, agglutinating *Bacillus pyocyaneus* in a dilution of at least 1 to 200, immediately clumped bacilli outside the filaments, but had no effect on those inside, even in dead filaments where the cellulose wall of the host alone intervened between the serum and bacilli. Undiluted serum was discharged from a pipette against such walls with no effect on the motility of bacteria inside. This serum had no immediately harmful effect on healthy *Achlya* filaments.

Inoculation with the vibrios of Asiatic cholera gave results similar to those with *Bacillus pyocyaneus*, except that infection was less sure and proceeded more slowly. The contents of *Achlya* cells are evidently a favorable medium for the growth of cholera. This is shown not only by successful infection, but by the fact that vibrios lying outside are immediately attracted to a spot where the pricking of a hypha has allowed cell contents to escape.

An infected hypha after death became packed with vibrios in much the same way as with *Bacillus pyocyaneus*, and similar extrusions from burst places occurred, but less strikingly than in the case of *Bacillus pyocyaneus*. (Plate II.) Some 10 successful inoculations were made with cholera, all in *Achlya* cultivated in artificial media.

The experiments with agglutinating serum were repeated with cholera. Infected hyphae, both dead and living, were treated with a rabbit serum agglutinating in a dilution of at least 1:500. Any vibrios outside were immediately clumped; but, as in the case of *Bacillus pyocyaneus*, the cellulose wall appeared to be an effective barrier against the passage of the agglutinins. In one experiment, a one-half dilution of the strongly agglutinating serum was injected directly into an infected hypha by means of a fine capillary pipette. The vibrios inside were immediately agglutinated, although they had been unaffected by the same dilution applied outside. The serum-injected hypha lived for at least three-fourths of an hour after the inoculation, as shown by the movement of its protoplasm. Infected cells, even though living and with actively motile protoplasm, had a diminished turgidity;
and it was much more difficult to inject a substance into them than into healthy cells.

In order to obtain a more rigid test of the resistance of this plant to serums, the fungus was planted in a soft medium consisting of agar one-half and the strongly agglutinating serum one-half. The mycelium grew well, and after one or two days' growth was inoculated with cholera vibrios. Within five hours the inoculated filament was swarming with actively motile vibrios, and the next day the filament and its branches were dead with vibrios penetrating to the finer endings. A portion of the medium removed with a capillary pipette and applied to a hanging drop of cholera vibrios caused immediate clumping. It is evident from this experiment that infection with high motility of vibrios goes on unchecked, even when the host is grown in a highly agglutinating medium.

To summarize, the cellulose wall of the fungus is apparently an effective barrier against the agglutinins of both cholera and Bacillus pyocyaneus serums.

Somewhat different results were obtained as regards the permeability of this plant for acids. Since Achlya will tolerate a degree of acidity in the medium decidedly harmful to cholera vibrios, it was possible to determine if the progress of an infection might not be altered by changes in the reaction of the medium.

As a protocol a detailed description of one of this series of experiments is given:

April 4, morning. A small mycelium grown from a resting spore was placed in a hanging drop of the water of condensation of alkaline agar to which a small quantity of nearly neutral glucose broth had been added.

April 4, 5.30 p. m. Above medium removed and cholera peptone plus about 0.1 volume of glucose broth substituted.

April 5, 10.15 a. m. (room temperature 28°.6). Inoculated with a fresh culture of cholera vibrios.

April 5, 11.00 p. m. Filament infected, with motile vibrios present. Withdrew alkaline medium and substituted an acid medium consisting of distilled water 5 parts and 1 per cent glucose broth, 5 + acid, 3 parts. A previous test had shown that cholera vibrios were unable to grow in a hanging drop of this medium.

April 6, 9.30 a. m. (temperature 28°.3). A portion of the inoculated filament is dead, but with few vibrios in it. Branches 1 and 2 are living and contain vibrios with little or no motility.
Actively motile vibrios added to the hanging drop immediately clumped and became motionless.

April 6, 12.10 p. m. Withdrew medium and added fresh of the same kind. This was repeated at 7 p. m. the same day.

April 7, 8.30 a. m. Branch 1 still living and healthy with thick protoplasm. The length of the branch has nearly doubled since the second day of infection. Few vibrios are present inside of filament, none of them motile.

In order to determine if the vibrios could be revived, the medium was withdrawn and one substituted which contained but 1 part of the acid glucose broth in 8 of water. A previous test had shown that vibrios multiplied freely in this fluid with active motility.

April 7, 4.45 p. m. The protoplasm in branch 1 is apparently more actively motile. Few vibrios within it and these tending to clump. None outside in that part of the culture.

April 8, 8 a. m. (temperature 29°.8). A segment in the middle of branch 1, including about 0.4 of the whole length, is still living with protoplasm actively motile. Withdrew medium and added fresh of the same kind.

April 8, 7 p. m. The living portions of the filament are growing into the dead portions. No vibrios found inside on examination with 1/4 oil immersion lens.

April 9, 7 a. m. Portion of branch still alive.

April 9, 3.30 p. m. Branch apparently dead. No vibrios found inside, but are multiplying in the outside medium.

Summary.—An infection of this filament took place, but was checked apparently in response to treatment with an acid broth. The filament lived four days with conditions under which non-treated infected filaments have usually died within twenty-four hours. The final death of the filament—like that of the controls—was evidently due to causes other than the cholera infection.

In another experiment a plainly infected filament was apparently “cured” in the same way. In other experiments infection had gone so far that the vacuole of the filament was well filled with actively motile vibrios. Here infection could be checked, and the vibrios made to clump by acid treatment; but in those filaments which survived treatment the vibrios sometimes became active again. In some cases acid-treated vibrios inside living filaments took on monstrous forms, some becoming thick spirals and some amoeboid in form while still retaining their motility. In one infected filament the protoplasm of the
host massed into a sphere containing no or very few vibrios, took on a wall, and later grew into the infected part, now dead. It was found more difficult to infect filaments in even a slightly acid medium.

In summary, Achlya filaments in which cholera infection is not too much advanced may apparently be "cured" by the addition of a weakly acid medium, but the wall and protoplasm of the living plant evidently offers some resistance to the passage of the acid. Vibrios inside were less affected than those without, and highly infected filaments were difficult to cure.

The dysentery bacillus inoculations were made with an old culture of the Shiga-Kruse type. Some four successful infections were accomplished. While the bacilli grew well, in some cases filling the filament for a considerable distance from the point of inoculation, the destruction of the host was much less rapid than in the case of the motile bacteria. In one experiment an infected filament lived nearly, if not quite, five days at high room temperature. During at least three days of this time the bacilli were numerous in the host; but its protoplasm continued to move, though in contact with dense masses of bacteria. In another case an Achlya, taken directly from a water culture among algæ, was inoculated. A portion of the infected mycelium lived six days after infection.

Bacilli of bubonic plague also grew well in the living filaments of Achlya. In one experiment a highly virulent culture, the first twenty-four hours' growth from an infected guinea pig, grew well, and formed chains which extended far from the point of inoculation and into neighboring branches. The hypha inoculated and other parts of the mycelium in connection with it died within twenty-four hours. After the death of the host rhizoid-like filaments from neighboring hyphæ surrounded and penetrated the infected filament apparently obtaining food from the disorganizing mass of bacilli. A similar attraction of masses of bacteria for Achlya was seen in dysentery infections. This behavior of Achlya does not prove that the bacteria possess no toxins for Achlya, since toxins, like agglutinins, may be unable to pass the cellulose wall. However, in dysentery at least, there is little evidence of the formation by bacteria inside the wall of toxins destructive to the host.

Some interesting results were obtained by the inoculation of spores of an Aspergillus isolated from moldy bread. A single spore was inoculated into the vacuole of a filament of Achlya growing in pure culture in a hanging drop of glucose agar. In
twenty-four hours the spore had germinated and put forth a long hypha which grew rapidly within the host and formed septa.

On the second day after inoculation the *Aspergillus* mycelium had branched extensively, spreading through the infected hypha and far into its branches. The invading hyphae tended to follow the wall of the host, lying in the layer of protoplasm. The *Achlya* was still living with motile protoplasm two days after inoculation, but the protoplasm was becoming scanty. In many places branches of the *Aspergillus* grew through the wall of the still living host and penetrated the surrounding medium. (Plate III.)

Here the *Aspergillus*, apparently an ordinary saprophyte, traverses the wall of the living host from within outward in the same way that a true parasite enters. The penetration of the wall is accomplished in both cases with little or no loss of contents or immediate disturbance of the protoplasmic circulation of the host. No case was observed of *Aspergillus* branches outside penetrating a new *Achlya* filament.

During the second day the *Achlya* filament died, filled with the mycelium of the *Aspergillus*. There were no bacteria in the neighborhood, and the death of the host was apparently due to exhaustion of food. One or two days later branches of the *Aspergillus* coming from the *Achlya* filament formed fruit stalks and spores.

In this experiment we have a saprophyte, or certainly a plant not normally parasitic on *Achlya*, behaving much like a parasite when its spores are mechanically introduced into the other plant. Yeast plants also grew well in the hyphae of *Achlya* growing in pure culture. Both fungus spores and yeast plants are comparatively difficult to inoculate, since one must use a pipette of relatively large size in order to admit these cells into it, and such a pipette must necessarily make a large opening in the wall of the filament inoculated.

**SUMMARY**

I. *Achlya* growing in pure culture is readily infected with *Bacillus pyocyaneus*, cholera vibrios, or the bacilli of dysentery and of bubonic plague, when inoculated into the cell cavity.

II. *Pyocyaneus* or cholera-infected filaments usually die within twenty-four hours after inoculation. Filaments infected with dysentery bacilli sometimes survive five or six days at a room temperature of from 27° to 31°.

III. Yeast plants and spores of *Aspergillus* also develop in
Achlya, the latter behaving much like a true parasite when inoculated under these conditions.

IV. Parasitism rather than intoxication apparently plays the larger part in these infections.

V. Aside from the occasional walling off of a less infected portion, Achlya shows little power of protecting itself against an infection introduced within the filament.

VI. An infection with cholera may be delayed, or, when not too far advanced, entirely arrested by the application of weakly acid media outside the filaments.

VII. Agglutinins of both cholera and Bacillus pyocyaneus in specific serums apparently fail to penetrate in the slightest degree the cellulose wall of the fungus.
ILLUSTRATIONS

Plate I. A mycelium of Achlya infected with Bacillus pyocyaneus. The infected filaments show dark in the photograph because packed with bacilli which took the stain (carbol fuchsin) deeply. The dark rounded masses are composed of bacteria forced out during the process of growth.

II. A mycelium of Achlya infected with vibrios of Asiatic cholera. The dark-staining filaments are packed with bacteria, and a few extruded masses of bacteria are shown.

III. A filament of Achlya infected with Aspergillus. The outgrowth of filaments of the Aspergillus through the walls of the host is shown at the end and the side of the Achlya filament.
PLATE I. A MYCELIUM OF ACHLYA INFECTED WITH BACILLUS PYOCYANEUS.
PLATE II. A MYCELİUM OF ACHLYA INFECTED WITH VİBRIOS OF ASIATIC CHOLERA.
PLATE III. A FILAMENT OF ACHLYA INFECTED WITH ASPERGILLUS.
ACUTE MALIGNANT GLANDERS IN MAN

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One plate

There is a form of acute malignant rapidly fatal glanders in man, the importance of which is not sufficiently emphasized in the current literature of the subject.

The four appended condensed case records form the basis for the present discussion of this subject.

INCIDENCE

Human glanders, except in certain parts of Russia, is a rare enough disease to make individual case reports of interest because the diagnosis often, if not usually, is not made early in the disease. For example, Meyer and Crohn^1 state that only 9 cases were reported in the city of New York during two years. When one considers the very contagious character of this infection, together with the very close association between human beings and horses, particularly in the Philippine Islands and most other tropical countries, it is surprising that human glanders is not more frequently encountered. The four cases from Manila would suggest a greater incidence than is generally recognized in this country.

CASE I. ACUTE MALIGNANT GLANDERS—DURATION OF ILLNESS FOURTEEN DAYS—DEATH

Patient 599.—Filipino, male, 18 years of age, coachman by occupation. Admitted to the hospital complaining of very high fever, severe pains in the joints, and headache. Family history and history of previous diseases unimportant.

Present illness.—Eight days before admission, patient was taken suddenly ill with fever, headache, and nausea with occasional vomiting. Within twenty-four hours, joint pains developed, starting in the right knee and spreading rapidly to nearly all of the important joints of the body. These pains were severe and acute in character, and a considerable amount of edema in and about the joints developed very rapidly. No external wound nor primary lesions of the mucous membrane were discovered.

On admission to the hospital on the eighth day of his illness, the patient was very ill; he had high fever and a rapid pulse, a general toxic appearance, and his joints were swollen, painful, and tender to the touch. On this date a single superficial pustule about 1 centimeter in diameter was noticed on the left side of the neck, and within forty-eight hours a considerable number of these lesions were found scattered over the body, particularly on the face and chest. The lesion was a particularly typical one (Plate 1). It appeared suddenly and developed rapidly, starting as a vesicle, but within a few hours became pustular. It was superficial in character, umbilicated, and surrounded by a marked inflammatory zone. In appearance these lesions very closely resembled ecchyma. They varied in size from 5 millimeters to 1 centimeter in diameter, were easily broken, and Bacillus mallei was cultivated from the contents practically in pure culture.

The only important clinical laboratory findings in this case was the blood. The leucocytes, on the day of admission, were 7,800 with

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Three days after admission and when the eruption was fully established, there were 9,000 leucocytes with

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On the twelfth day of the disease, the patient's general condition was much worse. The vesicular eruption had extended to the face and trunk. Some of the vesicles were umbilicated, a few were pustular. A clinical diagnosis of glanders was made by one of us (Musgrave), and the diagnosis was confirmed by the usual laboratory methods two days later.

The patient suffered intensely with joint pains during the two following days. The fever ranged from 38° to 39.5° C.; there were frequent irregular chills and great prostration and toxæmia. Death occurred on the fourteenth day of the disease.

**Autopsy Report**

*Anatomic diagnosis.*—Papulo-pustular eruption of skin; glanders; multiple small abscesses of pectoral muscles; large abscesses near ankles; pyarthrosis; pyæmia; beginning abscesses and posterior congestion and oedema of lungs; subserous ecchymosis of heart; fatty and albuminous degeneration of kidneys; acute exacerbation of splenic tumor.

*Pathologic diagnosis.*—Body of fairly well-nourished Filipino; rigor mortis moderate; skin of face, chest, abdomen, and extremities show numerous, but not conglomerate, papulo-pustular eruptions, averaging 1 centimeter in diameter, raised about 2.5 to 3 millimeters above the surface. They are dull gray in color, and very few are umbilicated; all contain a gray mushy pus, emitting an odor suggestive of mice stools. Stray ulcers
are seen where a papulo-pustule has ruptured; the edges of these ulcers are slightly beveled, neither punched out nor undermined; their bases are covered with gelatinous sloughs. The palms and soles are free; the post-cervical lymphatic glands are numerous and faintly palpable. There is no scar on the penis; the mucous membrane appears to be normal.

The subcutaneous fat is moderate in amount and dry. The recti muscles are dark red; the pectoral muscles have numerous abscesses containing a gelatinous pus, measuring 1 centimeter deep and 5 centimeters wide. On dissecting away the skin, many of the pustules can be seen on the subcutaneous tissues, some having penetrated them. Large abscesses 4 by 3 by 2 centimeters are observed beneath the skin on legs, arms, and thighs. The joints of knees and ankles contain the same gray gelatinous pus, which in some places is fluid in character.

The peritoneum is gray, dry, and lusterless.

The pericardium contains a few cubic centimeters of clear, dull yellow serum. The heart shows numerous punctate and conglomerate hæmorrhages near the auriculoventricular septum. The heart is large and flabby; it was kept for a museum specimen.

Lungs.—Both pleural cavities are dry; there are a few recent firm adhesions between the right apex and upper part of chest wall. Both lungs are heavy, boggy, and òdematous; they do not crepitate well. They are blue-gray anteriorly and darker posteriorly. There are numerous dull blue-black areas 5 centimeters in diameter scattered over their surface. Cut sections ooze dark blood and froth, being blue-gray in front and darker in the posterior two-thirds. Anterior mediastinal glands are enlarged and òdematous.

Kidneys.—Fatty capsule preserved, facial lobulations becoming obliterated. True capsules strip readily, leaving a dull yellow-red surface. Cut section is rather dry. The glomeruli and striations are invisible on the dull pale brown surface. Pyramids have lost their blue color and striations; same color as cortex. Suprarenals and ureter appear normal.

Liver.—Anterior border presents a blunt angle; color light brown, mottled with few large (3 by 4 centimeters) areas of dull yellow hue. Cut surfaces show a dull yellow color, oozing little blood, with lobulations obliterated. The dull yellow surfaces extend about 3 centimeters beneath the surface. Gall bladder is slightly distended with thick black bile.

The pancreas is dull pink and firm.

The stomach and intestines are moderately distended with gas. The mesenteric glands are large—more than 1 centimeter long—pale, dull pink, and firm.

CASE II. ACUTE MALIGNE NT GLANDERS WITH SUPPURATIVE ARTHRITIS—DURATION OF ILLNESS FOURTEEN DAYS—DEATH

Filipino coachman, twenty-five years of age, previously in good health. Family and personal history negative.

Present illness.—He was taken ill rather suddenly with chills and fever and aching pains over the body. Irregular temperature and chilly sensations continued for six days. Arthritic pains developed on the fourth day, first in the right wrist, and spread rapidly to other large joints.

Patient came into our service on the seventh day of the disease with diagnosis of malaria and acute rheumatism. Examinations on the day of admission showed two papulo-pustular lesions, one on the right shoulder
and one on the right side of the neck. The temperature was 39° C. There was considerable swelling of a number of the large joints which also were very painful and tender to the touch. Patient was already obviously in a septic condition, and, although no external lesions of any description nor ulceration of the mucous membrane could be found, the diagnosis of acute malignant glanders was made from the appearance of the above-mentioned pustules. This diagnosis was confirmed by obtaining *Bacillus mallei* in pure culture from the pustule.

The laboratory findings in this case were as follows.

The urine contained a small amount of albumin, and occasional hyaline and granular casts were found.

The leucocytes were counted on the day of admission and again four days later. The first count showed 7,000 and the second 8,500 with very little disturbance in the differential findings.

Culture from the left knee joint obtained by aspiration gave a pure culture of *Bacillus mallei*. The patient grew rapidly worse from the day of admission, and died on the fifteenth day of the disease.

Incomplete autopsy showed a general supplicative arthritis, fairly numerous rather superficial skin pustules similar to that described in the first part, with a few small abscesses in the lungs and a few in the kidneys.

**ADDITIONAL CASES OF ACUTE MALIGNANT GLANDERS IN MANILA**

Wherry reported 2 cases of this type of glanders. The following is abstracted from his report.

**CASE 1 (WHERRY)**

*History* (by Maj. Bannister, U. S. Army).—H. M., an American negro, 27 years of age, teamster, was admitted to the First Reserve Hospital on May 29, 1904, with a diagnosis of articular rheumatism. The patient had been ill for two weeks with chills, fever, and rheumatic pains.

While at the First Reserve Hospital he had a remittent temperature varying between 101° and 105° F., with an irregular morning rise and evening fall. A small pus sac was found posterior to the olecranon of the left arm. Aspiration yielded a few drops of lemon-colored serous liquid which, microscopically, contained numerous pus cells and a few capsulated rod-like bacteria. Later, a larger pus sac was found on the dorsum of the left foot.

*Sputum examination*.—May 30, mucopurulent, containing a few blood cells; no tubercle bacilli. *Blood* examination, May 29, 21,000 leucocytes; June 3, 23,400 leucocytes; no parasites. *Urinalysis*, May 29, 1,021; alkaline, trace of albumin; no casts, many leucocytes. June 2, 1,019; alkaline, no albumin; no casts, few leucocytes; bile.

*Diagnosis*.—Pyæmia; acute articular rheumatism, both elbows, knees, and ankles; supplicative inflammation of left elbow (type undetermined).

About June 6, he developed a cutaneous eruption which was considered to be that of smallpox, since a smallpox patient had been removed from an adjoining bed seven days before the eruption appeared on H. M. He was removed to the military smallpox hospital, and died on the following morning. The body was sent to the city morgue marked “suspected smallpox case.”

—Bureau of Government Laboratories, Manila (1904), No. 24.
Incomplete autopsy 987 (about twenty-four hours after death).—No autopsy was requested, but Dr. W. E. Musgrave and Dr. W. R. Brinckerhoff, who happened to be at the morgue, noticed the peculiar cutaneous eruption and brought pieces of the skin to the laboratory for further examination. Large areas of skin surface were covered with the numerous, closely set vesicles of miliaria, and among these were numerous pustules of varying size. Some of these pustules were capped by a vesicle, which in some instances showed a central depression giving an appearance of umbilication.

None of us thought of human glanders until a microscopic examination showed that pustules contained numerous bacteria morphologically resembling Bacterium mallei—rods about the length of, and somewhat thicker than, the tubercle bacillus, which stained irregularly with Löffler's methylene blue and lost the stain in Gram's method. No such bacteria were found in smears made from the abscess in the left ankle.

Bacterium mallei was isolated by the usual bacteriologic methods.

Histologic examination (by Doctor Brinckerhoff).—A section through one of the larger pustules (about 4 millimeters in diameter) shows a densely infiltrated area in the skin and subcutaneous tissues. This inflammatory exudate lies chiefly between the muscular layer and the Malpighian cells of the epidermis. The epidermis is raised to a considerable distance above its level in the adjacent normal skin. At the point where it leaves its normal level the deeply pigmented cells of the rete malpighi are seen to be greatly elongated, and just before it reaches its greatest elevation there is a splitting away of the horny layer, which, continuing to a similar point on the opposite side, leaves a space which constitutes the vesicular portion of the pustule.

Under a high-power lens the contents of the vesicle is seen to be composed of degenerated polymuclear leucocytes and cells from the stratum granulosum, nuclear fragments, and a granular detritus which represents the products of cell degeneration and coagulated serum. Beneath the area the cells of the rete show marked infiltration and vacuolation, many of their nuclei staining but faintly or not at all. The deeper infiltrated area is composed of a dense collection of more or less degenerated leucocytes and erythrocytes, degenerated epithelial cells, and nuclear fragments, many of which seem to have coalesced to form irregular, deeply staining masses of chromatin. Here some islands of degenerating epithelial tissue, probably the remains of papillary pegs, may be seen. No giant cells are present. A very prominent feature is the widespread and extensive destruction of the nuclei of the fixed and infiltrating cells. This varies from simple vacuolation to complete karyorrhexis. A number of normal polymuclear cells may be seen with their protoplasm filled with rounded or rod-shaped nuclear fragments. The blood vessels of the subcutis show great congestion. The underlying muscular and glandular tissues appear normal. There are no signs of proliferation—everywhere those of degeneration.

CASE II (WHERRY)

A. C., Filipino, 38 years of age, married, a clerk by occupation, was taken sick on October 2, 1903, with chills and fever. In December of the same year he had an abscess on the posterior portion of the right leg, which was opened by the physician who attended him. Again, in February, 1904, he had an abscess in the anterior and upper portion of the right side of the chest, which underwent resolution without operation. On June 27, 1904,
an eruption appeared on the face and later on the chest and abdomen. On the third day of the eruption the patient died. The patient was the owner of a stable where horses and vehicles were kept for hire; he had recently lost several horses from glanders.

**Autopsy.**—The body appears emaciated. A papulo-vesicular eruption is scattered over the skin surface. These lesions are most numerous on the face, back of the trunk and upper arms, buttocks and back of the upper part of the thighs, and more scattered over the chest and the abdomen. They are not distributed regularly, but are grouped with intervening areas of comparatively free skin, and vary in size from 2 to 3 and 6 to 8 millimeters in diameter. The smaller ones appear as shotty papules, while the larger ones are distinctly vesicular. Several of these vesicles show depressed areas, which give the appearance of being umbilicated, although more commonly their surfaces appear wrinkled. On section they are seen to be situated on a fairly well-defined, yellowish, firm nodular base, which extends into the subcutaneous tissue. The eruption on the face seems to be more advanced. On the forehead and cheeks the lesions appear as irregularly circular, raised, dark red and purple-colored plaques, about 1 to 1.5 centimeters in diameter. Some of these are rounded and nodular, others flat with depressed center and raised edges. A few present a reddish yellow, ulcerated center surrounded by raised edges. Two of these nodular plaques occur beneath the skin of the scalp, just above the upper margin of the forehead.

The tissues just to the right of the nose are so swollen as to close the right eye, which itself does not seem to be affected. A section through these swollen tissues reveals many discrete and confluent, yellowish and grayish, caseous foci, which are surrounded by congested and necrotic tissue and extend to the depth of about 2.5 centimeters from the skin surface. The alae nasi are thickened by similar nodules, and one can be seen on the mucous membrane of the upper lip, just to the left of the nasal fossa. A dirty, purulent discharge escapes from the nostrils. Upon opening the nares, the mucous membrane, especially of the right one, is seen to be ulcerated. The ulceration covers most of the mucous surface of the floor and walls of this nostril, and extends upon the inferior turbinate bone. Small, grayish or yellowish nodules may be seen projecting from the ulcerated surfaces. The right nostril is not affected to such a marked degree. The ethmoidal cells are filled with purulent matter. The right clavicle is much thicker than the left, but nothing of note is seen on section.

The thoracic cavity contains no fluid. Both lungs are bound to the thoracic walls by firm, fibrous strings of adhesions. The lungs are emphysematous anteriorly, and posteriorly show considerable hypostatic congestion. On palpation small nodules can be felt beneath the visceral pleura, which on section appear as pea-sized or smaller, circumscribed, grayish yellow areas of a confluent tubercular structure. None of them are capsulated or caseated, but some are surrounded by an irregular, reddish area of pneumatic consolidation. They seem to be limited to the pleural surface.

The bronchial glands appear normal on section. The trachea and bronchi are slightly congested, and are covered by a mucopurulent secretion. The heart muscle is rather pale on section, but otherwise the organ appears normal. The mucous membrane of the esophagus shows hyperplasia of the solitary follicles.

The liver is of about normal size, soft, and on section its markings are
indistinct. The spleen is somewhat enlarged, soft, and on section its pulp is diffusent. The kidneys are slightly enlarged, their capsules strip readily, and on section the cut surface is yellowish white and the cortical and medullary markings are very indistinct. The stomach and intestines were not opened. No further examination was made.

Bacteriologic examination (by Dr. W. R. Brinckerhoff).—The twenty-four-hour cultures on glycerin agar showed numerous barely visible, transparent colonies. In forty-eight hours these colonies became visible, and in pure cultures gave the biochemical reactions which have been described as characteristic of Bacillus mallei.

The histologic changes in the subcutis and lower layers of the cutis are similar to those described in case I. The process, however, is not so far advanced, for, although karyorrhexis is widespread and prominent, it is not so marked in the upper layers of the cutis, where the chief changes are a loss in the staining power of the nuclei and a general vacuolation of the cell protoplasm. The stratum corneum has not been split off and consequently the pustule is not covered by a vesicle.

Lung.—A section through one of the subpleural nodules (about 3 millimeters in diameter) shows, under a low magnification, an irregular area of consolidation characterized by intense infiltration of the pulmonary alveoli and marked congestion of the blood vessels of the alveolar walls and of the pleura covering the affected area.

Under a higher power the contents of the alveoli are seen to be composed chiefly of polymuclear and transitional leucocytes, a few lymphocytes, pigment-carrying cells, and a few large cells which lie, for the most part, near the alveolar walls and resemble desquamated endothelial cells. It is apparent that many of the cells in this area are undergoing degeneration and karyorrhexis, but not to so marked an extent as in the skin pustule of case I. This area is surrounded by pulmonary tissue which shows intense congestion and in which the alveoli are filled, for the most part, with extravasated blood, granules, and threads of fibrin, desquamated endothelial cells, and a few polymuclear and transitional leucocytes. Deeper within the section the alveoli appear normal.

In another field of the section several small foci of infiltration may be seen, situated at some distance beneath the pleural surface, and each is about the size of a single air cell. Their contents is composed of cells similar to those found in the larger focus, but karyorrhexis is not so marked a feature. One of these appears to have ruptured into an adjoining alveolus, and such a process may indicate the histogenesis of the larger foci. All the foci appear to be recent ones, and there are no signs of proliferation or encapsulation. No giant cells are to be seen.

DISCUSSION

Etiology.—The disease is caused by a general infection with Bacillus mallei, and consequently its spread must conform to the usual well-known methods of transmission of diseases caused by bacteria.

When this fact is considered together with the very great virulence of the organism both in human beings and in some of the lower animals, the comparative rarity of the infection in man
is difficult to explain, particularly in countries where the incidence of infection in animals is very high.

The habits and customs of coachmen in Manila, for example, and the close housing of man and horses and other animals, give the most favorable opportunities for the spread of the disease to man. If there is a natural immunity in man against the infection, this immunity certainly has a very irregular distribution and must be very strong in a large percentage of people, entirely absent in a few, and of moderate degree in practically none. Variation in virulence in the various strains of the bacillus does not answer the question because the disease practically always is fatal in horses, and, when man becomes infected, the disease usually is a particularly virulent one and cases of mild infection are almost unknown.

Pathologically the most striking peculiarity of B. mallei infection is the marked predilection of the organism for joints, lymphatics, and cutaneous tissue. In practically every instance there is a more or less extensive suppurative arthritis and suppurative lymphadenitis and a more or less extensive distribution of suppurative skin lesions.

Inflammatory areas and abscesses in other tissues and organs of the body frequently develop toward the end of the disease, but the principal and early and constant lesions are as above mentioned.

Symptoms.—The period of incubation is unknown. The onset is similar to that of a number of severe acute infections; usually, it is rather sudden with chill or chilly sensations with fever and indefinite aching pains. In some cases there is a prodromal period of indefinite symptoms and a more gradual development of fever. In still other cases the onset resembles that of pneumonia very closely, and it may not be possible positively to differentiate the two conditions until the absence of the expected consolidation is noted.

The fever usually runs a more or less irregularly remittent course, varying from 38° to 40° or even 41° at times.

The pain at first may be more or less general in character, similar to that usually seen in dengue. More often, however, from the first and in all cases after the disease is fully established the pains localize in the joints—particularly the larger joints—as the elbows, knees, ankles, etc.

The joints rapidly become swollen and painful and the skin glistening, as is seen in rheumatic fever. Still later, fluid accumulates, and in the course of a few days suppuration is found.
in one or more joints. The aspirated contents of the joints shows pure culture of *B. mallei*.

The lymphatic glands in various parts of the body and particularly around the primary focus of infection, when one is present, rapidly become swollen and tender and gradually suppurate, and in some instances, when the patient lives long enough, break down and cause open ulcers. In cases I and II that are reported here, there was, however, striking absence of enlargement of the lymphatic glands that could be detected by physical examination, and there was but slight lymphadenitis. Exception is made of the mediastinal glands which were found at autopsy slightly enlarged and oedematous.

The skin lesion is a very striking and characteristic one. The lesions never are exceedingly numerous, and sometimes only a few will be found until toward the end when they usually become very numerous. They appear one or a few at a time, and, while showing a predilection for the face, neck, back, and chest, may be seen on any part of the body. The first skin lesions usually appear in from four to seven days after the onset of the disease, and others continue to appear throughout the course of the disease. However, we have seen one patient who did not have more than 20 of these lesions before the twelfth day of the disease.

The lesions appear at first as simple superficial papules which rapidly enlarge and soon become vesicles, then pustules, and then break down and become open sores. The lesions always appear to be superficial, although careful examination reveals a surrounding area of infiltration, and section shows that the infiltration extends through all layers of the skin.

During the vesicular stage, the superficial raised character of the lesion resembles that seen in impetigo contagiosa. Cultures from the skin lesions usually show pure culture of *B. mallei*.

One very striking clinical feature of acute glanders is the marked depression and general appearance of serious illness that develops early in the disease and continues throughout its course. The patients gradually sink into unconsciousness a few days before the end.

Diagnosis.—The disease frequently is not diagnosed during life and rarely during its early stages. This, too, in spite of the fact that the diagnosis is very easy to make clinically and its confirmation by laboratory methods a simple and easy procedure.

The reason why the diagnosis is not more often made is due to the rarity of the infection. Most physicians never have seen
a case, and it, therefore, is not included in the routine mental pictures that one examines in connection with cases of fever, rheumatic pains, and skin lesions.

Glanders is most frequently mistaken for dengue fever, rheumatic fever, syphilis, or typhoid fever, and less frequently for pleurisy, pneumonia, and certain skin diseases, particularly impetigo contagiosa, pemphigus, or eczema.

During the early stages before the appearance of the characteristic skin lesion, the clinical picture of glanders may resemble dengue or rheumatic fever very closely, but careful examination will justify distinction even at this stage. Dengue may be eliminated positively and absolutely by the well-known blood picture of this disease.

The mode of onset, the character of the pains, the fever, and the blood picture of glanders and rheumatic fever may be indistinguishable, and, as it often happens in acute glanders cases that there is no visible primary lesion, the diagnosis may remain in doubt until suppuration in the joints or the skin lesions make the diagnosis clear. The value of early blood cultures as a diagnostic method in glanders needs further study.

The only excuse for confusing glanders with the skin diseases mentioned is in the similarity of the local lesions. The pronounced constitutional manifestations of glanders should obviate this mistake more often than it does.

The prognosis in this form of glanders is bad. The disease probably is a general infection practically from the beginning and rapidly becomes a virulent pyaemia.

There is no known treatment that has any curative properties or that influences the course of the disease further than to alleviate some of the distressing symptoms that supervene before unconsciousness develops.
ILLUSTRATION

Plate I. Appearance of skin in acute malignant glanders in man.

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PLATE I. APPEARANCE OF SKIN IN ACUTE MALIGNANT GLANDERS IN MAN.
NOMA IN THE PHILIPPINE ISLANDS WITH REPORT OF A CASE ENDING IN RECOVERY

By C. M. Reyes

(From the Clinics of the College of Medicine and Surgery, University of the Philippines, and the Philippine General Hospital, Manila, P. I.)

One plate

The problems of epidemic diseases and intestinal parasitic infections have received a great deal of attention from writers on tropical conditions, but literature on the more common clinical diseases is not so voluminous, and very little mention of the incidence of noma in hot countries is to be found. Yet it is a fact that noma does exist and makes its ravages among children in tropical climates; it is spoken of as being fairly common in Korea and Formosa, and it is occasionally met with in China. In India it is said to be especially frequent in adults.

It is a noteworthy fact that in the Philippines, at least, measles, which is the one eruptive fever most commonly followed by noma, rarely ever assumes the gravity it does in the more temperate zones, like the United States, for example. Whether this may account for the apparent rarity of the malady in this and other tropical countries, as judged by the scanty literature on the subject, is open to question.

The several cases of noma seen at the Philippine General Hospital were unfortunately seen too late in the course of the disease to justify surgical intervention. All such patients have succumbed to the effects of a most virulent sapremia. In the following case the disease developed while the patient was being treated for empyæma in the hospital, and is reported on account of the extreme rarity of recoveries from this fatal affection.

REPORT OF CASE

Patient.—A. O., 2½ years of age, male, born of Filipino parents.

History.—Patient was admitted to the hospital May 17, 1912, complaining of fever and cough of one month's duration. After examination, a diagnosis of empyæma on the left side was made and drainage instituted. The temperature gradually subsided, and the patient did well up to August, when he began to develop
a high, irregular temperature and was steadily failing, until by
the latter half of September it was deemed necessary to submit
him to a second operation by enlarging the old drainage wound.
Condition of the patient remained but little changed.

On October 1, 1912, the patient's upper lip was noticed to be
swollen and òedematous and further examination showed slight
necrosis of the mucosa opposite the insertion of the teeth. The
necrotic tissue was scraped, and the exposed surface cauterized
with pure phenol. By the next day the necrotic process had
extended to the inside mucosa of the lower lip and a small part
of the gums of both upper and lower jaws. Four front teeth
were removed because they were found to be so loose that there
was danger of their being swallowed. The same treatment as on
the previous day was instituted, plus frequent swabbing of the
parts with hydrogen peroxide, and later with a potassium per-
manganate solution.

On October 3, the skin over the upper lip became glossy, and by
the fifth day the gangrenous process had invaded new tissues
outward, so as to present a typical picture of cancrum oris—a
foul, ash gray, pultaceous mass, involving the entire upper lip
(Plate I, fig. 1).

It was impossible for the patient to take anything but liquids,
and his low state of health from the original trouble did not
warrant a more active surgical interference, which would have
necessitated general anaesthesia. The treatment outlined above
was continued except that iodine was substituted for phenol on
account of the exposed situation of the parts. The course of
the lesion was progressive, until by the tenth day the floor of the
nose also was involved, and the patient presented a pitiful ap-
pearance. The odor from the sloughing necrotic tissue was most
offensive.

There was no further extension of the process, a line of demar-
cation gradually formed, and by the twentieth day of the disease
the margins of the ulcer began to show a more healthy appear-
ance, and the general condition of the patient showed slight
improvement. The favorable local changes continued until
healing was complete, but the process had destroyed so much of
the upper lip that the middle of the upper jaw was left exposed.
The patient has not yet sufficiently recovered from his original
affection to justify a plastic operation (Plate I, fig. 2).

Examination of smears from the slough showed long spirilla
and spindle-shaped organisms, and blood culture gave *Staphylococcus aureus* and *albus*.

Noma is essentially a disease of children, especially under 10 years of age, but it has been met with in adults. Girls are more often affected than boys.

It usually follows or develops during the course of some debilitating sickness, like the eruptive fevers, particularly measles and typhoid. It has been met with after scarlet fever, whooping cough, bronchopneumonia, diphtheria, variola, etc. Unhygienic surroundings, underfeeding, and cachexia are strong predisposing causes, but it has been met with where the sanitary, hygienic, and dietetic conditions were most favorable. Usually sporadic in its appearance, the disease has been observed from time to time in epidemic forms, which, together with the fact that it seems to have a special predilection for the lining mucous membrane of the different orifices of the body, strengthens the belief that it is of a specific infectious nature. Formerly the malady was considered to be the result of some vasomotor or trophic disturbance, but in the light of the present bacteriologic researches it must be considered as an infectious disease due to a definite cause, and the isolation of the specific microorganism may be predicted with confidence.

Blumer and MacFarland, after studying a series of 16 cases, arrived at the conclusion that the disease begins primarily as a simple infection and terminates as a mixed infection, generally with a slim organism of the leptothrix type predominating. Weaver and Tunnicliffe, who have compiled the results of the bacteriologic studies of noma by several investigators, report the almost constant finding of polymicrobial cultures from the superficial areas of necrotic tissue, while the deeper portions showed long, thread-, or filament-like bacteria in almost pure culture, the bacteria being easily decolorized by Gram, but not growing on ordinary culture media.

The disease usually attacks the mouth and cheek, but may occasionally attack the vulva and anus (noma pudendi). Perhaps this may explain, in part, its greater frequency among girls.

It usually begins as a livid, swollen patch on the mucous membrane of one of the cheeks, followed by an inflammatory infiltration which is rapidly transformed into typical gangrenous tissue. The lesion soon spreads from the cheek, which becomes a foul, ashy gray, pultaceous slough, and the process often extends
to the adjacent soft parts and underlying bones, so that in the very rare instance of recovery a subsequent plastic operation becomes necessary.

The prognosis is bad, about 80 per cent of the cases succumbing within ten days. Much depends upon the promptness with which treatment is instituted. Neglected cases invariably are fatal.

REFERENCES

ILLUSTRATION
(Photographs by Cortes)

PLATE I

Fig. 1. Case of noma before recovery.
2. Same as fig. 1, after recovery.
Fig. 1. Noma before recovery.

Fig. 2. Noma after recovery.
CONCERNING VARIOLOID IN MANILA

BY P. M. ASHBURN, E. B. VEDDER, AND E. R. GENTRY

(The United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands)

Both the Director of Health and the physician in charge of San Lazaro Hospital have several times spoken of the disease resembling varioloid that appears in Manila with a certain amount of regularity during each hot season and have expressed their uncertainty as to its nature. As a precautionary public health measure the cases have been classified as modified smallpox and isolated and treated as such. Having been particularly anxious to obtain access to cases of smallpox for the purpose of making experimental inoculations in monkeys, it has been our fortune to see a number of cases, all of which have shown great similarity and which are characterized in general by very slight, or no, fever and constitutional disturbance and by the appearance on the face, scalp, trunk, and limbs of a vesicular eruption, the lesions of which vary from 1 to 5 millimeters in diameter, are at times unilocular, at times multilocular, that very rarely umbilicate or pustulate, and usually dry to form brown scabs by the third or fourth day. The scabs fall off, leaving a small, pale mark without pitting. The lesions when first seen appear as mere papules roughening the skin, without redness. On the second day they are apt to be clear vesicles surrounded by a small area of redness. The lesions are most common on the face; next on the shoulders and front and back of the trunk, where they are distributed about equally; they occur less commonly on the arms and legs, and least so on the palms, soles, and scalp. In the cases seen the lesions were always discrete. A few red spots not to be positively identified with the skin lesions have been seen on the palatal surfaces; a few patients have spoken of mild sore throat. No complications or sequelae have been observed. Some of the vesicles are elliptical in outline rather than round. The ages of the patients seen by us have varied from 12 months to middle life. The records show that there have been lately about 500 cases of "varioloid" in Manila per year, and Dr. A. P. Goff, physician in charge of San Lazaro Hospital, who has seen and treated the cases, states that most

1 P. M. Ashburn, major; E. B. Vedder, captain; and E. R. Gentry, lieutenant; Medical Corps, United States Army, members of the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands.
of them are covered by the above description, and that there has been no mortality due to the disease.

We are of the opinion that of the cases occurring between July, 1912, and January 25, 1913, only one was smallpox or varioloid; and that case undoubtedly contracted the infection in China, as the disease developed a week after his departure from Hongkong and two weeks after leaving Shanghai. The other cases we think were not smallpox because:

(1) The cases were uniformly mild, the lesions like those of chickenpox rather than smallpox; shotty induration, umbilication, and pustulation were of rare occurrence.

(2) Previous successful vaccination or previous smallpox did not influence the occurrence or severity of this disease.

(3) Absence of both previous vaccination and previous smallpox did not influence it.

(4) Attempts (7 in number) to infect monkeys by inoculation with fresh vesicle contents were uniformly unsuccessful. Had the disease been smallpox most of the monkeys should have shown successful inoculations.

As illustrating the absence of influence of both smallpox and vaccinia on the occurrence of this disease, the following table shows the facts in regard to 15 patients admitted from January 4 to January 29, 1913.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Date of admission.</th>
<th>Previous successful vaccination.</th>
<th>Previous smallpox.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>Jan. 4</td>
<td>Yes; 6 months ago</td>
<td>No.</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>9</td>
<td>Yes; since admission</td>
<td>Yes; 15 years ago.</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>16</td>
<td>Yes; 6 months ago</td>
<td>No.</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>17</td>
<td>No</td>
<td>No.</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>19</td>
<td>Yes; in 1911.</td>
<td>No.</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>18</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>20</td>
<td>Yes; 16 days ago</td>
<td>No; mother of case 1.</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>20</td>
<td>No</td>
<td>No.</td>
</tr>
<tr>
<td>9</td>
<td>13</td>
<td>20</td>
<td>Yes; 6 months ago</td>
<td>No.</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>20</td>
<td>Yes; 1 year ago</td>
<td>No.</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>20</td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>22</td>
<td>No; attempt 6 months ago</td>
<td>No.</td>
</tr>
<tr>
<td>13</td>
<td>17</td>
<td>22</td>
<td>Yes</td>
<td>No.</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>25</td>
<td>Yes; Jan. 18</td>
<td>No; daughter of case 6.</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>29</td>
<td>Yes; date unknown</td>
<td>No.</td>
</tr>
</tbody>
</table>

We are of the opinion that the disease in question is not smallpox or varioloid and that it is, in all probability, chickenpox; and that smallpox, except for an occasional imported case, is nonexistent in Manila. If our opinion be correct, the facts signify a notable achievement in preventive medicine, and it is unfortunate that the records do not show it forth.
REVIEWS

Epidemic | Cerebrospinal | Meningitis | by | Abraham Sophian, M. D. | formerly with New York Research Laboratory | Twenty-three illustrations | St. Louis | C. V. Mosby Company | 1913 | Cloth, pp. i-xv + 1-272. $3.00.

The author's large laboratory and clinical experience has enabled him to place before the medical profession a very valuable book on this subject. Several typographical errors detract from the appearance of the book.

J. A. J.

Tuberculin | in | Diagnosis and Treatment | by | Francis Marion Pottenger, A. M., M. D., LL. D. | medical director of the Pottenger Sanatorium for diseases of the lungs and | throat, Monrovia, California | with thirty-five illustrations, | including one plate in colors | St. Louis | C. V. Mosby Company | 1913 | Cloth, pp. 1-243.

This monograph is written by one who is so evidently an optimist that at times he seems to permit his enthusiasm to overbalance his judgment. It is on the whole a very good résumé of the treatment of pulmonary tuberculosis by vaccine therapy. The author lays commendable stress on the fact that this method of treatment is not one which lends itself to haphazard administration. In Plate I, figure 1, the explanatory legend to be correct should read "left" instead of "right," as it is the left eye depicted.

J. A. J.

Pellagra | History, Distribution, Diagnosis, Prognosis, | Treatment, Etiology | by | Stewart R. Roberts, S. M., M. D. | associate professor of the principles and practice of medicine, Atlanta College | of Physicians and Surgeons, Atlanta, Georgia; physician to the Wesley | Memorial Hospital; formerly professor of biology in Emory College | with eighty-nine special engravings | and colored frontispiece | St. Louis | C. V. Mosby Company | 1912 | Cloth, pp. 272. $2.50.

Since 1907 when Searcy reported an epidemic of pellagra among the inmates of the Mount Vernon Hospital for the Colored Insane in Alabama, many articles relative to various phases of the disease have appeared in American and European medical literature. A book reviewing all this work was greatly needed by the busy practitioner. Such a book is Doctor Roberts's, and the
work has been performed in a very thorough manner. The section upon History and Distribution which naturally includes synonymy is particularly well executed, a map of the world and one of the United States showing the geographical distribution of pellagra being given.

While discussing the subject in general, the volume deals with it more directly as found in the South Atlantic portion of the United States. Doctor Roberts has seen the disease in Italy, and hence writes at first hand of pellagrous conditions in that country. It is obvious also that he has been in close personal touch with many of the investigators who have contributed largely to our present knowledge of the disease.

Symptomatology should have been included in the title. This section together with that on diagnosis apparently embraces most of the author's personal observations. A complete bibliography of the important literature on the subject would have added to the value of the volume.

D. G. W.

Diagnostic Methods | Chemical, Bacteriological | and Microscopical | a textbook for students and practitioners | by | Ralph W. Webster, M. D., Ph. D. | assistant professor of pharmacological therapeutics and instructor in medicine in | Rush Medical College, University of Chicago; director | of Chicago Clinical Laboratory | Second edition, revised and enlarged | with 37 colored plates | and 164 other illustrations | Philadelphia | P. Blakiston's Son & Co. | 1012 Walnut Street | 1912 | Cloth, pp. i–xxxvi + 1–682. $4.50.

This book is designed for the use of students and practitioners, and it is to be commended for its marked lucidity and brevity in discussing laboratory diagnostic methods accepted by laboratory workers up to the date of its publication. Naturally it contains little that cannot be found in other publications on the same subject. The illustrations by Katharine Hill are excellent.

The portions of the volume which deal with the parasites of man are poorly executed; they are incomplete, and well-known rules of zoölogical nomenclature have been disregarded.

D. G. W.


The author in a clear and condensed manner presents a résumé of the results obtained by the use of chloride of lime as a general
disinfectant and as a sterilizing agent for water and sewage. Not the least point of value in this book is a large bibliography. The volume should be valuable to all public health officials.

J. A. J.

Laboratory Methods | with Special Reference to the Needs of | the General Practitioner | by B. G. R. Williams, M. D. | member of Illinois state medical society, American medical association, etc. | assisted by | E. G. C. Williams, M. D. | formerly pathologist of northern Michigan hospital for the insane, | Traverse City, Michigan | with an introduction by | Victor C. Vaughan, M. D., LL. D. | professor of hygiene and physiological chemistry and dean of the department | of medicine and surgery, University of Michigan, Ann Arbor, Michigan | Second edition | illustrated with forty-three engravings | St. Louis | C. V. Mosby Company | 1913 | Cloth, pp. 1-210. $2.50.

This little manual is exactly what the authors claim for it in the preface to the first edition, namely, a laboratory guide for the general practitioner. The fact that a prominent laboratory worker writes the introduction to the present edition speaks well for the merits of the book. It is moderate in price and should be of service to those for whom intended.

J. A. J.

The Narcotic Drug Diseases | and | Allied Ailments | Pathology, Pathogenesis, and Treatment | by | Geo. E. Pettey, M. D., | Memphis, Tennessee | member, Memphis and Shelby County Medical society, etc. [7 lines] | Illustrated | Philadelphia | F. A. Davis Company, publishers | 1913 | Cloth, pp. i-viii + 1-156. $5.00.


Cardio-vascular Diseases | recent advances in their anatomy, physiology, pathology, diagnosis and treatment | by | Thomas E. Satterthwaite, A. B., M. D., LL. D., Sc. D. | [11 lines, membership in societies, etc.] | [motto] | Lemcke and Buechner | 32 West 27th Street New York City | [no date of publication.] "Copyrighted 1913." Cloth, pp. 1-166, 80 text figs.
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BACTERIOLOGICAL OBSERVATIONS MADE DURING THE OUTBREAK OF PLAGUE IN MANILA IN 1912

By Otto Schöbl

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

One plate

During the recent outbreak of plague in Manila, I had the opportunity to make certain observations which are of interest. These observations were made in the examination of: (1) Specimens taken from patients and from dead bodies at autopsies, (2) samples of bloodsucking insects collected in houses where plague patients had lived, (3) rodents caught by trap or poisoned in the parts of the city where plague cases occurred from time to time, and (4) domestic animals suspected of plague infection.

BACTERIOLOGICAL EXAMINATION OF PLAGUE PATIENTS

In order to secure as early diagnosis as possible, the following procedure of investigation was adopted:

1. The bubo was aspirated by means of a sterile hypodermic syringe. The material thus obtained was placed in the water of condensation of an agar-slant culture tube.

2. At least 7 centimeters of blood were withdrawn from the cubital vein by means of another sterile syringe, and 5 centimeters of it were placed in an Ehrlemeyer's flask, containing 200 centimeters of neutral meat broth. The rest of the blood was emptied into a sterile tube, and used for agglutination tests.

Cultures obtained by this method were examined microscopically, and the growths on various culture media were studied. Gram stain, Löffler's methylene blue, and hanging-drop method were used. Polar-staining and chain formation in liquid media and the characteristic type of colony on the surface of agar were looked for. Animal inoculation was performed in every case, and the culture isolated from each case was identified by agglutination test, rabbit's immune serum being used.

The results of the bacteriological examination of a series of 24 patients are tabulated in the two following tables. Table I includes the fatal cases and Table II those cases which recovered.
### Table I.—Examination of fatal cases of plague.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Race</th>
<th>Sex</th>
<th>Age</th>
<th>Date of examination</th>
<th>Date of illness</th>
<th>Hours before death</th>
<th>Bubo</th>
<th>Blood</th>
<th>Skin</th>
<th>Sputum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sing Nu</td>
<td>Chinese</td>
<td>Male</td>
<td>(?)</td>
<td>July 11, 1912</td>
<td>48</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Asuncion Raymundo</td>
<td>Filipino</td>
<td>Male</td>
<td>15</td>
<td>Sept. 29, 1912</td>
<td>3</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Filo Almadas</td>
<td>do</td>
<td>do</td>
<td>39</td>
<td>Oct. 10, 1912</td>
<td>2</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Polycarpo Guzman</td>
<td>do</td>
<td>do</td>
<td>34</td>
<td>Oct. 22, 1912</td>
<td>3</td>
<td>23</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Jose Sarmiento</td>
<td>do</td>
<td>do</td>
<td>37</td>
<td>do</td>
<td>3</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Julian Gonzales</td>
<td>do</td>
<td>do</td>
<td>41</td>
<td>do</td>
<td>3</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Valeriano Buencamino</td>
<td>do</td>
<td>do</td>
<td>31</td>
<td>do</td>
<td>10</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pedro Nicomedes</td>
<td>do</td>
<td>do</td>
<td>30</td>
<td>do</td>
<td>2</td>
<td>106</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Regino Gulino</td>
<td>do</td>
<td>do</td>
<td>34</td>
<td>Oct. 24, 1912</td>
<td>2</td>
<td>106</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Martin Dimalanta</td>
<td>do</td>
<td>do</td>
<td>35</td>
<td>Oct. 23, 1912</td>
<td>3</td>
<td>255</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Roberto Obiso</td>
<td>do</td>
<td>do</td>
<td>25</td>
<td>do</td>
<td>1</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Juan Barceta</td>
<td>do</td>
<td>do</td>
<td>23</td>
<td>Oct. 24, 1912</td>
<td>3</td>
<td>37</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Yu Tum</td>
<td>Chinese</td>
<td>Male</td>
<td>14</td>
<td>do</td>
<td>2</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Augustin Monterey</td>
<td>Filipino</td>
<td>Male</td>
<td>29</td>
<td>Nov. 1, 1912</td>
<td>1</td>
<td>27</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Demetrio Fabraw</td>
<td>do</td>
<td>do</td>
<td>27</td>
<td>Nov. 23, 1912</td>
<td>4</td>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ambrosio Sobremonte</td>
<td>do</td>
<td>do</td>
<td>21</td>
<td>Dec. 7, 1912</td>
<td>6</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mateo Marcelo</td>
<td>do</td>
<td>do</td>
<td>22</td>
<td>Aug. 20, 1912</td>
<td>8</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alejandro Gita</td>
<td>do</td>
<td>do</td>
<td>23</td>
<td>Nov. 24, 1912</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Months.
### Table II.—Examination of plague patients who recovered.

|----------|----------------|------------|------|--------|----------------------|----------------------|--------|----------|-------------|----------| tion.     |
| 2. Dionisio Capate | Filipino   | Male   | 18   | 1912.  | Sept. 29            | 2                    | -      | -        | -           | 0        | 0         |
|          |               |          |      |        | Oct. 2              | 5                    | +      | +        | -           | 0        | 0         |
|          |               |          |      |        | Oct. 3              | 6                    | 0      | 0        | -           | 0        | +1:16     |
|          |               |          |      |        | Oct. 7              | 10                   | -      | -        | -           | 0        | 0         |
|          |               |          |      |        | Oct. 15             | 18                   | -      | -        | -           | +1:64    |           |
| 5. Alejandra Fisher | European | Female | 6    | 1912.  | Oct. 20             | 7                    | +      | +        | +           | 0        | 0         |
| 11. Gabriel Sevilla | Filipino   | Male   | 21   | 1913.  | Jan. 11             | 48                   | -      | -        | -           | 1:120    |           |
|          |               |          |      |        | Dec. 2              | (9)                  | +      | +        | +           | 0        | 0         |
|          |               |          |      |        | Dec. 5              |                      | 0      | 0        | 0           | +        |           |
|          |               |          |      |        | Dec. 16             |                      | -      | -        | -           | -1:80    |           |
| 19. Esteban Roa   | Filipino   | Male   | 15   | 1913.  | Dec. 16             | 23                   | -      | -        | -           | +1:60    |           |
|          |               |          |      |        |                      |                       |        |          |             |           |           |
|          |               | do     |      |        | Dec. 11             | 3                    | +      | +        | 0           | 0        |           |
|          |               |        |      |        | Feb. 11             | 33                   | -      | -        | -           | 0        | 0         |

**Note.**—The bubo in Nos. 2, 5, and 24 never opened spontaneously. The pus was aspirated at the time of the second, eventually third, examination. Nos. 11 and 19 opened spontaneously. A fistula formed along the canal which was caused by the puncture, and healed up in several weeks. Hard inguinal buboes of secondary order persisted in patient 19 at the time of second examination. No plague bacilli were found either in the bubo of the first or second order. Patient 20 had a considerable amount of pus in the inguinal primary bubo, but it was not opened until after the last examination.

The diagnosis of plague could be safely made from the microscopical examination of the liquid aspirated from the bubo in the majority of the cases. However, in certain instances the amount of the aspirated fluid being small and the bacilli very few, it was impossible to diagnose the case, especially when the cultures from the bubo were negative. Repeated examination of the patient was necessary under those conditions, but it happened in cases 22 and 23 that the patients died of plague before a second examination could be made. The smears and cultures from case 22 remained sterile, while the smears and cultures made from the swelling on the neck of patient 23 revealed the presence of pneumococci. Both patients died of
plague, as was ascertained by examination of the organs after death.

Two of the patients, cases 8 and 12, had numerous plague bacilli in the sputum at the time when the expectoration showed the presence of blood (twenty-three and one-half and eighty-two hours, respectively, before death). In 3 cases I was able to prove the presence of *Bacillus pestis* in the skin lesions, *intra vitam*, fifteen, twenty-two, and forty-eight hours, respectively, before death. In case 18 there was no doubt that the skin lesions, which covered the whole body and the face, were of secondary nature, as the patient died shortly afterward. It was undoubtedly a case similar to those reported by Gotschlich and Zabolotny.\(^1\) In the other two patients there was only 1 maculopapulous efflorescence on the foot in case 1 (with a corresponding femoral bubo) and 2 lesions of the same type on the arm and forearm in case 4 (with a corresponding axillary bubo). It is possible that these lesions were the original port of entry of infection. Numerous plague bacilli were found in the skin lesions of these cases, both microscopically and in culture.

The plague patients tabulated in Table II recovered. They were all treated with antiplague serum. While cases 5, 2, 19, and 24 appeared clinically to be rather severe, cases 2 and 20 were mild.

It can be seen from the table that the plague bacilli may not be detected in the enlarged gland at first (case 2) and that their presence may be revealed only after repeated examination of the bubo. It is also evident from the results of repeated examinations that the plague bacilli disappear from the infected gland in a comparatively short time, as a rule at the time when pus starts to form. Contrary to the findings in patients who died, distinct phagocytosis was noticed in the smears made from the aspirated liquid in those patients who recovered and who had been treated with serum soon after the onset of the disease. It is undoubtedly this process that clears the gland of the infectious agents.

The general opinion in regard to the presence of *Bacillus pestis* in the circulating blood seems to have been, as Thompson remarks, that “the bacillus is rarely to be found in the peripheral blood stream before the agonal stage.”\(^2\)

The Austrian Commission, using few drops of blood, found

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\(^1\) Kolle und Wassermann. Handbuch der pathogenen Mikroorganismen. Gustav Fischer, Jena (1908) 2, 521.

positive blood culture in 40 per cent; Calvert in Manila in 100 per cent when examined twenty-four hours before death; Choksy, Berestneff, and Mayr in 45 per cent; and Greig in 60 per cent. The Indian Commission examined 28 patients, and obtained positive blood cultures in 16 out of 23 fatal cases. Not a single positive blood culture was obtained from the patients who survived. The time of blood examination in positive cases was three and one-half to seventy-five and one-half hours before death. The amount of blood used was 1 cubic centimeter. Only 6 out of the 30 samples, which gave positive blood culture, were found positive by microscopical examination of blood smears. The following conclusions are based on these observations in regard to the septicæmic stage of bubonic plague: (1) "A severe septicæmia may be present at a comparatively early stage of the disease and for a considerable number of hours before death, and (2) the septicæmia may be of an irregular and fluctuating type." ¹

From the tables it will be seen that out of 15 patients examined by me, 14 gave positive blood culture; and of these 3 recovered. One blood culture revealed the presence of streptococcus in addition to Bacillus pestis. The results of the examinations tabulated in Tables I and II show, in agreement with the findings of the Indian Commission, the occasional early occurrence of plague bacilli in the blood stream, as the time of examination in the positive cases varied from one hour to one hundred six hours before death. In consideration of the ephemeral character of the septicæmic stage of plague, as evidenced by repeated blood cultures in the three patients who recovered, one can hardly avoid the impression that there is a certain degree of septicæmia in every case of plague. The possibility of detecting the bacillus in the circulating blood increases in proportion with the quantity of blood used for culture. The best chance to recover plague bacilli from the circulating blood seems to be in the stage of high fever and general prostration.

The phenomenon of agglutination of plague bacilli by the serum of patients was first observed by Wissokowitsch and Zabolotny in 1897 ² and later confirmed by the German Plague Commission. Vagedes, Klein, and others ³ pointed out the defects of the reaction as a diagnostic means. Aside from the technical difficulties, the reaction was found inconstant, and its

¹Ibid. (1907), 7, 395.
²Referred to in Kolle und Wassermann. Handbuch der pathogenen Mikroorganismen (1903), 2, 524.
occurrence was not noticed until the second week of the disease and even then only in low dilutions of the serum.

Although the recent work of Strong and of Strong and Teague has reduced the technical difficulties, the fact remains that positive agglutination of plague bacilli by the patient's serum cannot be obtained in the first week of the disease, and, therefore, the isolation of plague bacilli from the body of the patient is still the only quick and safe method of plague diagnosis.

Having utilized the technique devised by Teague, I have had no difficulty in performing the agglutination test in plague. The emulsion of plague bacilli, to be used for the test, was prepared by suspending young cultures of virulent plague bacilli, grown at 30°C., in salt solution and filtering the suspension through filter paper. No antiseptic was added nor heat applied. Serial dilutions of unheated patient's serum were mixed with equal amounts of bacterial suspension in small test tubes. Incubation at 35°C. followed. Controls, consisting of serial dilutions of normal human serum as well as bacterial suspensions without serum, excluded any possible error which might have been caused by spontaneous sedimentation of the bacterial suspension; while a parallel test with highly agglutinant serum facilitated the reading of positive results.

Altogether, 22 tests were performed on 15 patients, 11 of whom were fatal cases and 4 of whom recovered. In the negative reactions, the duration of the disease at the time of examination ranges from two to six days. The nonfatal cases showed slight agglutination from the sixth day on. From that day, the agglutination titer of the serum was found to rise, and the agglutinins persisted in the blood of convalescents up to the seventh week of the disease.

It must be borne in mind that the patients, who showed positive agglutination, had been vigorously treated with anti-plague serum. Nevertheless, in consideration of the low titer of the curative serum (dilution 1:32, agglutination positive; dilution 1:64, agglutination negative), the rise of the agglutinant power of the patient's serum in dilutions higher than 1:16 cannot be explained as wholly due to passive immunity, but rather to active immunity arrived at on the principle of simultaneous immunization.

*This Journal, Sec. B (1907), 2, 155.
*Ibid. (1912), 7, 194-201.
*It is hoped that it will be possible to examine some of the survivors for agglutination from time to time.
From the preceding observations the following conclusions are drawn:

1. The importance of blood cultures as a diagnostic means is evident from the fact that positive blood culture was obtained in practically every case that was examined in the febrile stage of the disease, even when buboes or signs of pulmonary involvement had not been detected clinically.

2. It is also evident that *Bacillus pestis* may be found in the circulating blood of the patients even in cases which subsequently recover.

3. The period of time during which *Bacillus pestis* circulates in the blood is evidently short and irregular.

4. Mixed infection may be encountered in plague septicæmia (*Streptococcus, Pneumococcus*).

5. The agglutination test is of no value for the diagnosis of plague, as it was found positive only in convalescents.

6. Phagocytosis of plague bacilli in the bubo was noticed only in patients who recovered after being vigorously treated with curative serum.

7. The presence of numerous plague bacilli in comparatively insignificant skin lesions during the life of the patient points to the possibility of direct transmission, while the fact that a patient without any apparent bubo, who is not so sick as to be detained from his daily occupation, may expectorate large numbers of plague bacilli, are facts of great importance with regard to the communication of the disease. It is obvious that the last-mentioned condition might, and very likely does, give rise to an epidemic of pneumatic plague if the atmospheric and sanitary conditions are favorable.

II. OBSERVATIONS ON THE TRANSMISSION OF PLAGUE BY BLOODSUCKING INSECTS

Judging from the data which have been collected from the literature on the transmission of plague (Table III), Simond seems to have been the first to call attention to the important part which bloodsucking insects, particularly fleas, play in the

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   - Report of Indian Plague Commission (1898-99).
transmission of plague. Although many investigators have been successful in demonstrating the presence of Bacillus pestis in the digestive system of bloodsucking insects, it was not until the experiments of Gauthier and Raybaud that the actual transmission of plague infection by fleas was convincingly proved. Ever since the exhaustive and conclusive experiments, which were carried out both under natural and artificial conditions by the British Plague Commission, and the work of Verbijtski, which antedates the British Commission, were presented, there has been no doubt that the transmission of plague by bloodsucking insects particularly by the fleas is one, although not the only, mode of spreading this disease. It is obvious, as Herzog correctly remarks, that the factors which are responsible for the spreading of plague must be considered individually in each epidemic and in various parts of the world as well. There is no doubt that the importance of any insect in the transmission of plague depends on its habits as well as on those of the host, be it either animal or man.

### Table III.—Insects found to contain Bacillus pestis.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Yersin</td>
<td>Flies</td>
<td>Laboratory infection</td>
<td></td>
</tr>
<tr>
<td>Nuttal</td>
<td>do</td>
<td>do</td>
<td>Negative by bite.</td>
</tr>
<tr>
<td>Do</td>
<td>Bedbugs</td>
<td>Experimental infection</td>
<td>Negative.</td>
</tr>
<tr>
<td>Do</td>
<td>Flea</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>Hankin</td>
<td>Ant's faeces</td>
<td>Fed on plague material</td>
<td></td>
</tr>
<tr>
<td>Do</td>
<td>Bedbugs</td>
<td>Plague hospital</td>
<td></td>
</tr>
<tr>
<td>Ogata</td>
<td>Flea</td>
<td>Plague rats</td>
<td></td>
</tr>
<tr>
<td>Simond</td>
<td>do</td>
<td>Plague rats, experimental</td>
<td>Positive.</td>
</tr>
<tr>
<td>Tindswell, 1900</td>
<td>do</td>
<td>Plague rats</td>
<td>Negative.</td>
</tr>
<tr>
<td>Tindswell, 1903</td>
<td>do</td>
<td>do</td>
<td>Do.</td>
</tr>
<tr>
<td>Kotte</td>
<td>do</td>
<td>Experimental infection</td>
<td>Do.</td>
</tr>
<tr>
<td>Gauthier and Raybaud</td>
<td>do</td>
<td>do</td>
<td>Positive.</td>
</tr>
<tr>
<td>Linton</td>
<td>do</td>
<td>Epidemic among pigs; harbored fleas; dead rats found.</td>
<td>Do.</td>
</tr>
<tr>
<td>Ziroli</td>
<td>do</td>
<td>Retained Bacillus pestis, 7-8 days.</td>
<td></td>
</tr>
<tr>
<td>British Commission</td>
<td>do</td>
<td>Repeated experiments</td>
<td>Do.</td>
</tr>
<tr>
<td>Verbijtski</td>
<td>Flea and bedbug</td>
<td>Experimental infection</td>
<td>Do.</td>
</tr>
<tr>
<td>La Bonadière and</td>
<td>Fly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthopolidee</td>
<td>Pediculus capitis.</td>
<td>Dead body of a plague case</td>
<td></td>
</tr>
</tbody>
</table>

During the recent outbreak of plague in Manila, several samples of bedbugs from the beds of the plague patients and dog fleas from a plague-infected house were collected and examined, but with negative result.

In spite of the fact that it adds nothing new to the question
of whether or not plague can be transmitted by fleas, since the question has been conclusively answered by the work of the Indian Commission, nevertheless the following observations of a small outbreak of plague among animals, the spreading of which was due solely to fleas, are of interest.

One wild rat was inoculated with strain Iloilo 3 of Bacillus pestis. The skin adjoining the root of the right ear was scarified, and a loopful of the culture was smeared on the scarified skin. The rat was found dead three days after the inoculation.

The cage containing the dead rat was immersed in kreolin solution. At autopsy the cervical glands were found slightly swollen, somewhat reddened, but no hæmorrhagic œdema of the surrounding tissue was noticeable. There was slight necrosis at the place of inoculation, showing superficial, purulent discharge. Clear effusion in both pleural cavities and one hæmorrhage in the pleura were found. The lungs were hyperæmic, but otherwise normal. The spleen was of somewhat darker color, but otherwise normal in size and appearance. The liver showed a slight degree of parenchymatous degeneration, the congestion making prominent the structure of the organ. The typical, although not constant, changes of the organ, which are characteristic of natural plague infection in rats, were absent. The kidneys were without macroscopic change. The lymph glands, with exception of the cervical nodes, were normal.

Examination of the rat’s fur revealed ectoparasites on the neck, under the chin, and back of the ears; these at the time of the examination apparently were dead. About 6 common rat fleas were found and identified as Laëmopsylla cheopis Rothsch. The parasites were immersed in sterile salt solution for three hours. When removed in a dry test tube, they began to move about sluggishly. The intestinal tract of these fleas contained blood.

Five of the fleas were crushed by means of sterile forceps, and inserted in a pocket under the shaved skin of a guinea pig. The animal died of plague within three days, showing considerable hæmorrhagic œdema around the place of inoculation, typical bilateral inguinal buboes, and characteristic changes in the spleen. Smears and cultures made from the bubo and spleen were positive for Bacillus pestis.

Another wild rat, which was in a separate cage in the same room where rat 1 had been kept, died twenty-four hours after rat 1. The two cages were at least 10 centimeters apart. Rat 2 harbored fleas of the same species as were found on rat 1.
Numerous severe bites were detected back of the ears and on the neck of the dead animal. The post-mortem findings were identical with those described in rat 1; that is, cervical buboes, pleural effusion, and slightly enlarged spleen.

It is well to remark that both rats had been kept in the same room for about six months. Fleas had never been noticed on our guinea pigs. During the time the rats had been kept in the plague house no irregular results were noticed in plague-inoculated animals. At the time the first rat was inoculated no other plague-infected animals were in the plague house, and since that time another building has been used for plague-infected animals.

The attached plan of the plague house shows the location and time of death of these and the other animals in this outbreak.

Two days after the death of rat 2 three guinea pigs, which were kept in separate cages in the same room, were found dead of plague (smears and cultures were both positive). Several fleas (*Laeomopsylla cheopis*) were found on the necks of these animals. They were collected and inoculated in the same way as the fleas from the first rat. The experimental animal, which was inoculated with the fleas, was killed and found to be infected with plague. The findings were local reaction, inguinal buboes, and typical spleen. Smears and cultures were positive for *Bacillus pestis*.

Although numerous healthy guinea pigs were examined in the same plague house, no fleas could be found at that time, only the 2 rats and the first 3 guinea pigs are positively known to have harbored fleas, the latter after the death of the rats and not before.

The gross lesions in these naturally infected guinea pigs were somewhat unlike those found in guinea pigs infected either by vaccination or by intraperitoneal or subcutaneous inoculation. All except one showed primary buboes on the neck with more or less extensive hemorrhagic edema extending in some cases over the thorax. There was little pleural effusion present; the spleen always showed typical changes of necrotic foci varying in size and number. In one instance similar foci were found also in the liver, large enough to be visible macroscopically. This was in a case where like changes were found in the lungs.

Only one of the guinea pigs showed an exception, in that the primary buboes were located in the inguinal region, with pelvic and axillary glands secondarily involved. These are the findings usually met with in guinea pigs artificially infected with plague.
by the vaccination method, if the lower part of the abdomen be chosen for inoculation. The reason for such a deviation from the findings in the rest of the guinea pigs may lie in the fact that this animal was almost completely deprived of hair by a skin disease.

It is of importance to mention the skin lesions which were found on the necks of the guinea pigs, particularly under the chin. Besides small red spots which appeared to be fresh flea bites, small, elevated, and fairly deep infiltrations partly covered with moist scab were found in the skin under the chin. Other animals showed changes usually found in the scarified skin of guinea pigs after artificial inoculation with plague material. The base of each cutaneous efflorescence was hæmorrhagic and oedematous.

A histological study of the tissues of these guinea pigs known to be naturally infected by plague fleas showed the following changes:

The cervical bubo.—The enlarged lymphatic gland was surrounded with a thickened capsule. Necrosis existed in the subcapsular part of the gland, where it formed an almost continuous circular zone, leaving the central part less changed. Smaller irregular necrotic foci were scattered throughout the section. Polymorphonuclears in various stages of disintegration were found throughout the section.

The lungs.—Very few blood extravasations were present in the alveoli; otherwise normal.

The spleen.—The capsule was thin. There were subcapsular hæmorrhages. The Malpighian bodies were somewhat enlarged, but of normal structure. Throughout the parenchyma irregular multiple necrotic foci were found, leaving but little of spleen tissue intact. Numerous polymorphonuclears which were present showed varying degrees of karyorrhexis.

The kidneys.—The outline of the cells was indefinite; a few miliary hæmorrhages existed in the cortical part of the organ.

The liver.—There was excessive congestion, fatty degeneration, and pigmentation of the cells. The capsule was slightly thickened.

The skin.—The epithelium was missing in one place in the section, and cellular infiltration extended from that place into the subepithelial layer of the surrounding skin. The same kind of infiltration reached deep into the skin, stripes of cellular infiltration penetrating into the tissue along the muscle fibers. There was no direct connection between the cellular infiltration and the follicles of the hair.

It may be well to describe in detail the time of death from plague among these and the other animals in this outbreak, as well as the time when the plague house was disinfected.

The first animal (rat 1) having been inoculated on August 27, in the afternoon, died of plague within three days (August 30). The second animal (rat 2) died twenty-four hours later.
Guinea pigs 3, 4, and 5 (see plan) were found dead on the morning of September 2; that is, two days after the death of rat 2 and three days after the death of rat 1.

The same day that the three guinea pigs were found dead of plague, rooms I, III, IV, and VI (see plan) were thoroughly disinfected. The floor, the ceiling, and the walls were sprayed with kerosene and lysol solution. The remaining animals in room VI were destroyed, and the cages disinfected. No animals were kept in rooms I, III, and IV at that time.

Three days after the death of animal 5, guinea pigs 6 and 7 were found dead of plague, while the next day guinea pigs 8 and 9 died. No death occurred on September 7, but the next two days each recorded two plague guinea pigs (10, 11, 12, and 13). On September 11, the last guinea pig died of plague in this outbreak. The whole building was then thoroughly disinfected. No plague-inoculated animals were kept in the rooms after the first sign of the epidemic. After September 11, no more cases of spontaneous plague infection were observed.

It will be noticed that the epidemic lasted eleven days after the first animal died and fourteen days after animal 1 was inoculated. Altogether, 14 animals out of at least 200 animals exposed died of plague.

No death occurred among rabbits, although these animals were distributed among the guinea pigs. In fact, 2 rabbits were surrounded by plague guinea pigs 8, 9, and 10 (see plan), but did not contract plague.

From the epidemiological standpoint it is interesting to know the dimensions and location of the cages in which the animals were kept.

Aside from the 2 rats which were confined in ordinary traps that stood on a table 80 centimeters high, the rest of the animals were kept in regular metal animal cages. The dimensions of the cages are: Fifty centimeters long, 36 centimeters broad, and 30 centimeters high. The cage stands on four legs each 10 centimeters long; the center of the bottom of the cage holds a drain opening 8 centimeters above the floor.

The majority of the cages in room II were located on the floor; some on the second shelf of a wooden rack. This last-mentioned arrangement, judging from the construction of the wooden frame, allowed a continuous passageway for the fleas to the second shelf of the racks. On the other hand, the deaths among the guinea pigs in room V were restricted to the cages standing on the floor, the majority of cages in that room being placed on tables 80 centimeters high.
Only a theoretical explanation can be given of the short duration and sudden cessation of the outbreak. One can assume with great probability that the first partial disinfection drove the fleas away from the primary source of infection, and that they traveled as far as possible. They finally settled in those guinea-pig cages which had not been molested by the first disinfection. Having no new supply of plague blood (all of the plague-infected guinea pigs having been removed, most of them before death), the fleas soon cleared themselves of plague bacilli. The peculiar feature of the outbreak; namely the failure to find fleas on the animals in rooms II and V, finds its explanation in the observation of the Indian Commission who found that the fleas “died or disappeared very rapidly.”

The following conclusions can be drawn from these observations:

1. The common rat flea (*Loemopsylla cheopis*) prefers the rat to the guinea pig.

2. In the absence of rats it will attack guinea pigs rather than rabbits.

3. The fleas which have sucked blood from rats or guinea pigs afflicted with plague septicaemia were found to harbor virulent plague bacilli inside of their bodies.

4. The transmission of plague infection by direct or indirect contact being excluded in our case, the fact that fleas of the same species and harboring plague bacilli were found on the rat and on the guinea pigs, the presence of flea bites on the rats and on the guinea pigs with positive findings of skin lesions on that part of the body where the fleas and flea bites were located, together with the anatomical picture of the findings in the guinea pigs, lead to but one explanation; namely, that the plague infection was transmitted by fleas.

**Observations on Animals Suspected of Plague**

Out of the several tens of thousands of rodents examined during the antirat campaign, we have found only two plague rats which showed the typical picture of natural plague infection in rat; that is, cervical buboes with surrounding edema, subcutaneous injection, pleural effusion, enlarged spleen, and such changes of the liver as are characteristic of natural plague infection in rats. Microscopically, large numbers of plague bacilli were found in these cases, and pure cultures of *Bacillus pestis* were recovered from the spleen. Histological examination of internal organs, particularly that of the liver, confirmed the bacteriological findings. The remainder of the plague rats
exhibited only two of the signs of plague infection; namely, bubo and œdema of the surrounding tissue, and eventual hæmorrhages.

Besides plague infection, a great number of rats showed purulent conditions from causes other than plague. Abscesses of the lungs were frequently met with, and cervical or axillary buboes are not uncommon in Manila rats. Various pyogenic bacteria were found in the pus of such abscesses. Of the less common was Bacillus pyocyaneus and the pneumobacillus of Friedländer. Chronic plague was excluded in these cases since the animal inoculation failed to produce plague infection.

More than half of the rats examined harbored parasites in their organs. Echinococcus tenixformis was found in the liver of practically every gray rat, while a small Ascaris and Tænia diminuta were not uncommon in the intestines. Two rats were found to have sarcosporidiosis, 2.6 per cent showed rat leprosy, and 7.4 per cent trypanosomiasis. One tumor of the mammary gland and one tumor in the axillary region were encountered, while one tumor of the large curvature of the stomach proved to be a chronic inflammatory tumor due to parasites. One peritoneal tumor in a rat (Mus decumanus) gave the impression of a malignant tumor on account of the mililiary dissemination in the peritoneum. It was found to consist of muscle and spindle-cell sarcomatous tissue. Ectoparasites were very seldom noticed, on account of the method of collecting the rats. When present, they were mites and fleas.

In the naturally infected plague rats the rigidity of the fresh cadaver was pronounced. The primary bubo was in every case cervical. Cervical glands were enlarged and hæmorrhagic with slight œdema of the surrounding tissue. The subcutaneous injection extended over the neck and chest. The inguinal glands were small and pigmented. The lungs were collapsed, and showed hæmorrhagic foci. The spleen was slightly enlarged, firm, and dark red. The liver was rather large, firm, pale red, with shade of yellow, which was caused by minute yellowish foci thickly scattered throughout the tissue and visible through the capsule. The kidneys were hyperæmic. The intestines were without change. The serous membranes were pale with no hæmorrhages.

Histological examination of the tissue of naturally infected plague rats showed the following changes:

Liver.—The structure of the organ was well marked; the veins dilated, trabeculae slightly compressed, nuclei well stained, and few of the liver
cells showed vacuoles. Small foci, most numerous under Glisson’s capsule, were scattered throughout the organ; they varied in size, but were not larger than a miliary tubercle. The small necrotic foci were found to consist of few necrotic liver cells. The center of the larger foci was formed by degenerated and necrotic liver tissue, surrounded by round-cell infiltration. Polymorphonuclears were also found in the zone of cellular infiltration. There was a slight degree of haemorrhage in each focus. Epitheloid cells and large vesicular cells with several nuclei were to be found. The foci, mentioned above, were sharply demarcated from the surrounding liver tissue, which appeared to be intact.

Spleen.—The structure was well preserved, the capsule thin. The Malpighian bodies were normal as to the elements of which they consist. Cells with pyecnotic nuclei were scattered throughout the organ, and vesicular cells with small, deeply stained, excentrically located nuclei were present. Polymorphonuclears were found in the tissue in considerable numbers. No localized necrotic foci could be found in sections through the spleen.

Cervical glands.—The blood vessels were considerably distended. A few haemorrhages and polymorphonuclears were present. Eödema of the capsules and surrounding tissue existed. Part of the gland was necrotic.

Lungs.—The blood vessels were distended. The alveoli contained homogeneous masses and blood. There were numerous subpleural haemorrhages. The bronchi were collapsed, and contained mucus.

Kidneys.—The cortical part showed subdued structure; the epithelial cells had an indefinite outline and occasionally showed vacuolization. The medular part was better preserved. There were miliary subcapsular haemorrhages. A few small foci were scattered throughout both medular and cortical parts. They consisted of round-cell infiltration.

NATURAL PLAGUE INFECTION IN A CAT

The experiments of the German Plague Commission proved that cats showed considerable resistance to plague infection as cutaneous and subcutaneous inoculations failed to infect them. According to the Austrian Commission, cats develop submaxillary buboes if fed on plague material. They are said by Albrecht and Gohn 9 sometimes to recover. Out of four cats fed on plague material two died of plague, one showing submaxillary the other mesenterial buboes. Virulent plague bacilli were found in the discharge from the nose and also in the fæces of cats which apparently did not become infected after having been fed on plague material.

One case of spontaneous plague infection of a cat was recorded by Thompson 10 in Sydney.

W. Hunter 11 in Hongkong made observations on cats suffer-

9 Über die Beulenpest in Bombay im Jahre 1897. (1897), II B, II C.
11 Lancet (1905), I, 1064.
ing from plague infection. The author also undertook a few experiments, and arrived at the following conclusions:

1. Cats suffer from plague.
2. The disease may be acute or chronic.
3. The type of the disease is septicæmic.
4. The animals may occasionally play a part in the dissemination of plague.
5. In plague-infected areas cats probably become infected through rats, which they devour as food.
6. In plague-infected districts possible plague infection in cats is of great importance from a domestic point of view.

On November 27, 1912, a sick cat was brought to the laboratory for examination. It was reported that the animal was found in a warehouse in which dead rats had been found some time previously. The rats were not examined. In the morning of the 30th, the cat was found dead in the cage where it had been kept under observation. The following are the post-mortem findings:

The animal was a fairly well-nourished female.\(^{12}\) The subcutaneous tissue, pericardium, mediastinum, and mesenterium contained considerable amounts of fat.

The subcutaneous tissue of the neck showed œdema and small hæmorrhages. The submaxillary tissues were swollen on both sides. When the fasciae and superficial muscles of the neck were removed, enlarged glands were found on both sides. These were closely attached to the submaxillary salivary glands. The surrounding tissue was œdematous, but no hæmorrhages were noticed in the vicinity of the enlarged glands. Upon section the glands were found to be necrotic, and upon pressure a thin purulent liquid escaped. There were no hæmorrhages within the glands. Several enlarged lymph nodes, smaller in size, could be followed down the neck on the left side. The lymph nodes in the axillæ as well as in the groins and peribronchial nodes were normal. The mesenteric glands were slightly enlarged and reddened.

The lungs were slightly collapsed. A clear, sanguinous, slightly coagulated effusion was observed in both pleural cavities. The tissue of the lungs showed considerable œdema and hypostasis. The bronchi and pharynx showed no changes, the mucous membrane being pale and thin.

The heart was normal.

The spleen was enlarged, of light red color, with follicles slightly prominent.

The stomach contents was blackish in color; there were no hæmorrhages or ulcers in the mucosa.

\(^{12}\) The cat was the mother of 4 kittens which were about 3 weeks old at the time the cat was delivered for examination. They were kept under observation for several weeks, but showed no signs of plague infection.
The liver was somewhat enlarged. The organ showed prominent structure, the centers of the acini being red, the periphery lighter in color.

The kidneys were slightly enlarged and pale. The capsule peeled off easily, the venæ stellatae were prominent, the surface smooth; there were no hæmorrhages. The cortex was increased in breadth and was of the same color as the surface; the pyramids were darker in color. The organ was of fragile consistence.

Suprarenals were normal, as were also intestine and bladder.

The histological findings were as follows:

*Bubo.*—The capsule of the gland was œdematous. The whole gland as seen in cross section had undergone necrosis, except a few foci which still showed cellular structure.

*Lungs.*—The alveoli were filled with homogeneous masses, containing but few degenerated epithelial cells and leucocytes. The blood vessels were dilated, particularly in the subpleural part of the organ. In some places capillary mycotic emboli with subsequent hæmorrhage were encountered. The large blood vessels and bronchi were normal.

*Salivary gland.*—Those glands attached to the primary bubo showed the normal structure of a combined mucous and serous gland.

*Liver.*—There was considerable congestion. The centers of the acini showed parenchymatous and fatty degeneration. The cells on the periphery of the acini exhibited typical fatty infiltration. The large blood vessels and small ducts were without change.

*Kidney.*—The cells of the kidney showed various degrees of degeneration, ranging from parenchymatous to fatty infiltration. There were a few capillary hæmorrhages and hyaline casts present.

*Suprarenals.*—These showed slight degeneration.

*Spleen.*—This organ showed congestion, a few hæmorrhages, and bacterial emboli; otherwise normal.

The bacteriological examination of the material from this cat gave the following results:

1. *Smears*:
   a. From the buboes showed degenerated leucocytes, many lymphocytes, and numerous bacteria, some of which resembled *Bacillus pestis* in their polar staining.
   b. From the spleen showed numerous plague-like, polar-stained bacilli. Round involution forms were present.

2. * Cultures*:
   a. From the buboes were badly contaminated with *Bacillus coli* and *Bacillus pyocyaneus* colonies.
   b. From the spleen: A few scattered colonies of *Bacillus pyocyaneus* developed on the surface of the agar. Between the large colonies a scanty growth of dewy appearance was noticed. Smears made from this growth revealed plague-like bacilli of the cultural type, showing a few club-shaped involution forms. Subcultures were made in order to secure pure culture. They showed a pure growth of *Bacillus pestis* as indicated by the morphology of bacilli and shape of the colonies. Agglutination with plague-immune serum was positive.
3. Inoculation experiments (vaccination method):
   a. One guinea pig was inoculated with the material from the left bubo, another one with material from the right bubo. They died of plague on the third and fifth days, respectively.
   b. One guinea pig was inoculated with the material from the spleen. It died of plague on the third day.
   c. One guinea pig was inoculated with material from the nostrils obtained by swab. The animal survived, showing no indication of plague.
   d. One guinea pig was inoculated with material from the rectum obtained by swab. It died of plague on the fifth day.

Although plague infection among cats is apparently a rare occurrence, the fact that cats may contract the disease in spite of the high degree of resistance to plague infection has to be considered from the hygienic standpoint.

To appreciate the important rôle which cats may play in the spreading of the disease one need only consider the close contact of these animals with rats on one side and human beings on the other. It is also a well-established fact that not only plague-infected cats, but also those which have devoured plague-infected material and remained apparently normal, may excrete plague bacilli which have retained their full virulence.
ILLUSTRATION

Plate I. Animal house.
SOME EXPERIMENTS ON THE INOCULATION OF MONKEYS WITH SMALLPOX

By P. M. Ashburn, E. B. Vedder, and E. R. Gentry

(The United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands)

Seven charts

I. EXPERIMENTS WITH VESICLE CONTENTS FROM A CASE OF DISCRETE SMALLPOX

On December 4 the case of a Dutch traveler who had contracted smallpox in China came under observation. This case was a very typical discrete smallpox in a man whose general condition was excellent and who had been successfully vaccinated in childhood (about 1884) and revaccinated with doubtful result about 1900. At the time of admission he was moderately covered with discrete lesions, those on the upper part of the body being good vesicles, those on the feet and legs not quite mature. He was in the eighth day of the disease. Vesicle contents drawn into capillary tubes was used to inoculate 5 monkeys. Other vesicle contents in capillary tubes was preserved for later use.

Monkey 21.—This animal, an unvaccinated female, was inoculated on the belly on December 5. The temperature (101°.4 F. at time of inoculation) rose steadily until the 11th when it reached 104°.8 F., dropping by the morning of December 12 to 95°.4 F. The animal died on the 12th, the cause of death being sepsis from a large abscess below the jaw, which was doubtless due to an injury received in fighting with other monkeys before the inoculation. The sites of inoculation on the belly showed waxy scabs, not to be definitely described as "takes."

Monkey 5.—A large male monkey, that had been successfully vaccinated in October, was inoculated at 6 sites on the abdomen on December 4 with fresh vesicle contents. No local lesions resulted. There was, however, a moderate rise of temperature

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Chart 1—Temperature chart of monkey 5.
on the third day, followed by a drop, and a second rise on the sixth day, with almost continuous elevation to the seventeenth day. The chart (chart 1) is attached. We call attention to the probability of this rise being due to variola sine eruptione, the eruption being absent because of the protection afforded by the vaccination in October.

Monkey 19.—A rather small unvaccinated male was inoculated at 5 points on the abdomen on December 4 with fresh vesicle contents. The temperature chart (chart 2) is attached, and attention is invited to its resemblance to chart 1.

On December 8 it was noted that all of the points of scarification were reddened. On the 10th the areas of redness were more widely extended and marked, and the belly wall about them was deeply indurated. On the 11th dry scabs were forming and the induration was slightly less. On the 12th a papule was noted on the scalp; on the 13th 5 papules were found on the scalp and legs. By the 15th a fairly profuse eruption of small vesicles and pustules, some of them ruptured, was seen on the face, arms, legs, and about the anus, while the lesions on the abdomen had further subsided, the induration and swelling about them greatly lessened, and the sites of inoculation were marked by dry scabs. On the 17th the redness and induration had almost entirely disappeared from the belly, the scabs had fallen from the inserts, and deep holes marked their location. On the 19th the end of the monkey's tail was seen to be much injured, as though crushed or bitten (probably bitten by an old male, No. 5, tied near), and all variola lesions were scabbed and dry. By December 23 the animal was pronounced well.

This case we considered variola inoculata in the monkey, characterized by fever and signs of local inflammation on the fifth day, by primary and secondary eruptions (the latter appearing on the ninth day), and by continued fever for about seventeen days.

Monkey 20.—A small unvaccinated male was inoculated at 4 points on the belly with fresh vesicle contents on December 4. The temperature chart (chart 3) is attached, and attention invited to its resemblance to charts 1 and 2.

Summarizing the above experiments with fresh vesicle contents, we may say that inoculation with it, by way of scarifications of the skin: (1) caused variola inoculata in two unvaccinated monkeys, the primary lesions, secondary lesions, and temperature curves being alike in the two instances and probably characteristic; (2) caused in a vaccinated monkey a fever very similar to that produced in variola inoculata, but gave rise to
CHART 2.—Temperature chart of monkey 19.
Chart 3—Temperature chart of monkey 29.
neither primary nor secondary skin lesions. This fever might well be the manifestation of *variola sine eruptione*. Placed free on the mucous membranes of the conjunctivæ, nares, and mouth, the virus caused no disturbance, or, if any, so little as to be insufficient for interpretation as an evidence of infection.

Of the fresh vesicle contents tubed and not used on the above monkeys, the greater part, probably 20 tubes, was used for the inoculation, by scarifications and intravenously, of 2 horses. Neither animal showed symptoms or signs that could be interpreted as smallpox. The remainder, which was partly clear vesicle contents and partly contents drawn on December 7 and showing slight turbidity, was kept in an ordinary ice chest for twenty-four days and was then used to inoculate 2 monkeys.

**Monkey 30.**—A medium-sized unvaccinated male was inoculated at several sites on the abdomen with 24-day-old vesicle contents on December 31. On January 7 an enlargement of a right inguinal lymphatic gland was noted, and on the 8th there was a transient rise of temperature as shown by chart 4.

**Monkey 23.**—A large unvaccinated male monkey was inoculated December 31 at several points on the abdomen with 24-day-old vesicle contents. On January 6 five points and lines of induration, swelling, and slight redness were noted about inserts, and the temperature was elevated as shown by chart 5.
By the 8th the induration, redness, and swelling were all beginning to diminish. Dry scabs covered the points of insertion. No secondary lesions developed. On January 7 some of these scabs were raised and the beds on which they rested scraped. These scrapings and the triturated scabs were used to inoculate monkeys 8 and 16.

Summarizing the above we may say that vesicle contents, capable when fresh of causing variola inoculata in monkeys, so loses its virulence by being kept for twenty-four days in the ice chest as to be no longer capable of producing the typical disease with prolonged fever and primary and secondary lesions.

It did produce an ephemeral rise in temperature in both instances after an incubation period prolonged beyond the ordinary length, and in one of the two instances it gave rise to abortive and atypical primary lesions. In neither instance did secondary lesions or severe disturbance result.

The above finding would indicate that a working and satisfactory smallpox prophylactic might be secured by storage and attenuation of virulent vesicle contents, but prophylaxis by vaccination as practiced is so safe, satisfactory, and efficient that the pursuit of the clue appears at present unnecessary.
II. EXPERIMENTS WITH SCABS OR "DISKS" FROM THE ABOVE CASE OF SMALLPOX IN MAN

As the lesions on the person of the Dutch traveler matured and the scabs fell off or were picked off, they were all collected and saved; one-half of them were placed in glycerin and one-half were placed dry in a sterile test tube.

On December 19, the patient's sixteenth day in the hospital and about the twenty-third day of his sickness, some of each lot of scabs were triturated in saline solution and some with the serum of monkey 6 (a vaccinated monkey), so as to make thick suspensions. With these suspensions monkeys 28, 22, 26, 27, and 29 were inoculated, 5 or 6 insertions being made on the belly of each.

Monkey 28.—This monkey received scabs preserved in glycerin and triturated with vaccinated monkey's serum. No local lesions developed. On the eighth and tenth days the monkey showed sharp rises of temperature, as indicated by chart 6. He thereafter appeared well.

The sites of inoculation were first reddened on December 8; on the 10th the redness and induration were very marked, as in monkey 19. On the 11th small vesicles or pustules marked the insertions, and two of them were ruptured. The next day the swelling and redness had begun to subside and the lesions were scabbed. On the 13th small secondary lesions, papules, were seen on the legs and about the anus. On December 15 a profuse eruption of small vesicles and pustules, more numerous than in monkey 19, was present on the palms, arms, legs, face, and scalp. The abdominal lesions were subsiding, and the inflammatory process in the abdominal wall was almost gone. On the 17th the belly wall was more inflamed and indurated and the swollen ridges were black on top; apparently secondary infection had occurred. All the secondary lesions were either pustules or scabs. On December 18 the tops of the swollen ridges on the belly sloughed, leaving extensive ulcers, and it may here be stated that these ulcers were not completely healed until the end of the month. Numerous pustules of the secondary lesions were yet unscabbed, but by December 21 all had become so, and desquamation was completed by the 26th, the completion being delayed on the palms, where the disks were held down by thickened epidermis, and on the legs, where entanglement of hairs in the scabs doubtless delayed it.

This case we also regard as one of variola inoculata in the monkey, characterized by fever and signs of local inflammation
on the fifth day; by primary and secondary eruptions, the latter appearing on, or escaping notice until, the tenth day; and continued fever until the fifteenth and possibly the nineteenth day.

*Monkey 12.*—A medium-sized unvaccinated monkey was given a drop of fresh vesicle contents in each eye, each nostril, and each side of the mouth on the morning of December 5. The virus was placed free on the mucous surfaces. No local lesions resulted, and no systemic disturbance other than a trifling rise
of temperature on the sixth, seventh, and eighth days, and we are unable to affirm that any infection occurred.

On January 16 he was again found to be sick and to have a high temperature, and on January 19 he died. Autopsy showed streptococcus septicaemia as the cause of death. That the sharp rises in temperature on December 26 and 28 were related to the septicemia that caused death three weeks later, notwithstanding the interval of apparent health and normal temperature, is possible.

*Monkey 22.*—Inoculated with dry scabs triturated in 0.85 per cent saline solution; this monkey showed no reaction, either local or general.

*Monkey 26.*—Inoculated with glycerinated scabs; this monkey showed no reaction.

*Monkey 27.*—Inoculated with glycerinated scabs; showed no reaction.

*Monkey 29.*—Inoculated with glycerinated scabs; showed no reaction.

On December 24 monkeys 12 and 17 were inoculated at 6 points on the belly with dried scabs, and monkey 33 with both dry and glycerinated scabs. None of them showed general or local disturbance.

III. EXPERIMENTS WITH SCABS FROM A CASE OF RECOVERED VARIOLOID ON THE SIXTEENTH DAY

In addition to the case of smallpox above mentioned, the United States Army transport *Sherman* arrived in port on December 1, 1912, with a naval recruit in his sixteenth day of modified small-pox, which he had contracted in San Francisco and developed after leaving Honolulu. The attack had been mild, the lesions abortive, and at the time of his arrival here the man showed only a few small, dry, brown scabs. These were all collected, and on December 2 were triturated in sterile 0.8 per cent salt solution and used to inoculate 3 monkeys, Nos. 6, 17, and 18.

*Monkey 6.*—This animal had been successfully vaccinated in October. No lesions followed inoculation with the scabs. The animal had an irregular temperature from the first and was sickly. On December 18 it was killed, in order to get vaccine immune serum.

*Monkey 17.*—A medium-sized female, unvaccinated, showed no disturbance and no lesions as a result of the inoculation.

*Monkey 18.*—A small unvaccinated male showed neither lesions nor systemic disturbance as a result of the inoculation. He was later (December 24) successfully vaccinated.
IV. EXPERIMENTS WITH SCABS FROM VARIOLOUS MONKEYS

While monkeys 19 and 20 were suffering from their variola, attempts were made to obtain vesicle contents from them, but the vesicles were so small and so soon ruptured by the animals that it was found impracticable. Scabs were collected, however, as the lesions dried, and these were used to inoculate monkeys 24, 25, 30, and 31. The results in all of these animals were quite negative, with the exception of monkey 24.

Monkey 24.—A medium-sized female was inoculated December 19 with scabs from monkeys 19 and 20. No general or febrile disturbance resulted, but on December 26 there was swelling, redness, and marked induration of 3 points of insertion and their surroundings. The lesions formed dry scabs. The induration persisted about ten days, and the monkey remained well.

Whether or not the above monkey suffered from modified primary lesions of smallpox we cannot know positively, but it seems probable. At any rate, all of the above experiments with smallpox scabs or disks from man and monkeys indicate that such material has but feeble virulence and that such as it has is speedily lost.

On January 7, 1913, the scabs were lifted from the lesions on monkey 23 (see above), the underlying tissue curetted, and the pulp so obtained used to inoculate monkeys 8 and 16.

Monkey 8.—A monkey that had been vaccinated in October with the vaccine scab from a pig, atypical but supposedly successful "takes" having been obtained, showed redness and slight swelling at the points of inoculation with pulp from No. 23, but nothing at all characteristic or strongly suggestive of smallpox or vaccinia.

Monkey 16.—This monkey had been unsuccessfully inoculated in November with vaccine triturated in 1 per cent phenol in 0.85 per cent saline solution and so kept for two weeks, no "take" resulting. Inoculated with "pulp" from the lesions of monkey 23 on January 7, the animal had a rise of temperature beginning the sixth day thereafter as shown by chart 7.

Beginning on the seventh day after inoculation, the animal showed marked induration and some oedema of and about the sites of inoculation and thick dry scabs formed. The induration was deep. On January 16 (tenth day after inoculation) the scabs were lifted, the areas beneath curetted, and the pulp so obtained used to inoculate monkeys 25, 19, 28, and 3, the first three of which have been discussed, and the last being a monkey vaccinated in October. None of them showed general or local disturbance.
This experiment indicates that the virus in vesicle contents, although attenuated by storage and further attenuated by passage, was still recognizably active in this animal, but not sufficiently so to survive another passage.

SUMMARY

1. Fresh vesicle contents from a case of human variola is capable, when inoculated into abrasions or scarifications on non-vaccinated monkeys, of producing variola inoculata in those monkeys, the disease being marked by fever and by primary and secondary lesions.

2. Such vesicle contents kept at ice-chest temperature for twenty-three days loses most of its virulence, but may still, in a proportion of instances, produce a mild and atypical variola inoculata, which in turn and in further modified form may be passed to other monkeys.

3. Active and fresh vesicle contents inoculated on vaccinated monkeys may produce a fever closely resembling that of variola inoculata in the monkey and a condition permitting of interpretation as variola sine exanthemate in the monkey.

4. Smallpox scabs or disks from man or monkey possess but a low degree of virulence, or very quickly lose their virulence.

5. When inoculation of such scabs does result in the production of infection this may be manifested only locally at the site of inoculation (case 24). In other words, the "B" part of smallpox virus survives longest in scabs.
We admit that this small series of experiments affords but little proof of the correctness of our hypothesis as to the relationship of variola and vaccinia. On the contrary, we do not see that it affords any evidence in disproof. The case of monkey 5, although of little value standing alone, is certainly susceptible of being cited as an instance of variola sine exanthemate, as an instance of separation of the elements of smallpox virus (the pock-producing or "B" part having acted on the monkey in October; the toxæmia-producing, pyrogenic, or "A" element in December), and as proof that vaccination protects against the pock-forming element of smallpox rather, or to a greater degree, than against the whole disease. We feel justified in restating our hypothesis that smallpox is due to a dual and divisible virus, one part of which is the cause of vaccinia and the pock stage of smallpox, the other part being necessary for the production of the highly contagious, febrile, general disease with an initial stage and preliminary rashes.

2 This Journal, Sec. B (1913), 8, 17-28.
A BACTERIOLOGICAL EXAMINATION OF CERTAIN ARTESIAN WELLS IN RIZAL, CAVITE, AND BULACAN PROVINCES, P. I.

By Marshall A. Barber

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Since artesian wells have become one of the chief sources of potable water in Rizal, Cavite, and Bulacan Provinces, P. I., it was thought advisable to make a careful bacteriological test of a number of them for the presence of pollution. In addition, it seemed of scientific interest to compare the numbers of bacteria found in different waters from these sources. These wells may fairly represent similar wells in these and other provinces.

In all, 34 wells were examined, 22 of them flowing and 12 pumping. Some of the pumping wells were formerly flowing, and in all flowing wells, as a rule, there has been a diminution in the rate of flow since the wells were sunk. Among the 12 pumping wells are included 2—Malolos (Plaza Malolos) and Bocaue—which flow intermittently at the present time. In some of the wells the flow is said to be greater at high tide.

In practically every case nutrient media were taken to the well itself, and the water transferred to most of the tubes or plates directly from the source. Whenever practicable, the tip of the sterile pipette was brought directly into the flowing stream in taking samples. Usually an additional sample was packed in ice and transported to the laboratory for additional or confirmatory tests. It was early found that the water of the flowing wells was nearly sterile; so, in order to get some comparisons, a larger range of tests was made and larger quantities of water sown than an ordinary sanitary analysis would require.

In all agar tests the water was well mixed with liquified agar cooled to 42° C. Plate cultures were made only where stated in the table. Plates were kept at high room temperature (28° to 32° C.). All other cultures were incubated three days, then removed to room temperature. The rate of flow was estimated by noting the time required to fill a can of approximately 5 gallons’ (19 liters’) capacity.
The results obtained from the flowing wells were so uniform that it is not necessary to tabulate the results of all the 22 in the series. In order to give an idea of the method employed and the character of the results, the findings in two of them, the one showing the highest degree of purity and the one showing the lowest, are given in full. One well gave a slightly lower degree of purity than the one chosen, but that well gave brackish water and was little used for drinking; so the next to the poorest is tabulated. The well showing the highest degree of purity was at Malolos, barrio Caingin. Rate of flow, 49 liters per minute; temperature of water, 28°.4 C.; standing water near well from overflow; no houses near.

Table I.—Water from well in the barrio of Caingin, Malolos, Bulacan Province.

<table>
<thead>
<tr>
<th>Amount of water taken</th>
<th>Nutrient medium</th>
<th>20 hours at 36°</th>
<th>40 hours at 36°</th>
<th>3 days at 36°</th>
<th>6 days, 3 at room temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glucose agar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Plain agar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Litmus-lactose agar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Bile-lactose agar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar-plate culture</td>
<td>(c)</td>
<td>(c)</td>
<td>(c)</td>
<td>(c)</td>
</tr>
</tbody>
</table>

* Amoeba test negative.  
* Growth on surface only.  
* One colony near margin.

The well opposite the municipal building at Meycauayan gave the lowest degree of purity. This water was slightly brackish, and was reputed to have medicinal virtues. It is generally used for drinking purposes. Rate of flow, about 5.7 liters per minute; temperature, 29° C.
In Table III is given a list of all the flowing wells examined with a part of the data obtained from each. A complete examination was made of all of these, and some of them were examined twice; but, as stated above, the results were so uniform that it is not necessary to give them in detail. Under number of colonies per cubic centimeter the entries "one or less," "two or less," etc. mean that the maximum number found in any one cubic centimeter sample was one or two, respectively. Where "one or more" is entered, some very small number above one was found in some tube where an exact determination was difficult. In every water there was at least one of the 1 cubic centimeter samples which remained sterile after five days or more, and in most cases several samples of 5 cubic centimeters remained sterile.
Results such as are exhibited by Table I raise the questions as to whether or not the water as it comes from the source is absolutely sterile and whether or not the few growths which appeared in the media are contaminations. In practically every well some algæ were growing inside the mouth of the exit tube and under the stream of water, and it may be from colonies of bacteria among them that some of the growths came.

In a well (Malolos, barrio Mambong) flowing 23 liters per minute, samples were taken, and afterward the mouth of the exit tube was thoroughly scrubbed with stiff test-tube brushes. Four and one-half hours later a second lot of samples was taken. Both lots of samples gave a high degree of purity, but the second was not superior to the first. This experiment was repeated with another well (Paombong, church square) having a flow of 57 liters per minute. Here only a few minutes intervened between the first and second tests. There was little difference between the two tests; both were nearly, but not quite, sterile. Four cubic centimeters of ordinary broth were added to 10 cubic centimeters of this water and the mixture remained clear two days at high room temperature, becoming clouded only on the third day.

Even if all sources of contamination within reach could be eliminated, there would still be a possibility of contaminants growing on the casing deep in the ground. Further, before absolute sterility could be proved, it would be necessary to employ all sorts of media, nonnitrogenous and otherwise, in order to eliminate every possible kind of bacterium; and there would still remain the possibility of the presence of thermophiles of different grades and of forms unable to grow on any artificial medium. It would, therefore, be a difficult, if not impossible, task to prove absolutely the sterility of a water of this sort. The relative freedom of these waters from bacteria seems the more remarkable when we consider that they are at a temperature (28° to 32°) which is very favorable to the growth of most bacteria.

A number of kinds of bacteria were found in these wells, but the commonest type was an actively motile bacillus, readily forming zoöglaea.

2. PUMPING WELLS

While the flowing wells examined are located near the coast, most of the pumping wells are 8 kilometers or more inland; although two of them are near the coast and in the immediate neighborhood of flowing wells.
The results of these tests are given in detail in Tables IV to XVI, inclusive. Samples were for the most part placed in the media at the well, but some samples were taken to the laboratory for further examination, as was done in the case of the flowing wells. Enough water was pumped out to flush out the pipe before samples were taken, although the wells were in continuous use during the time of day when samples were collected. Two wells which flowed intermittently are included in this list.

In Tables IV to XVI the fractions under the heading “gas” refer to the proportion of gas formed in the closed arm of the fermentation tube.

**TABLE IV.—Alabang Agricultural Experiment Station, Rizal Province, Well 1.**

Water, fresh; depth of wells, 167 meters; in use since July, 1908. Water of several wells pumped to collecting tank. Samples (taken April 11, 1913) from pipe leading from collecting tank.

<table>
<thead>
<tr>
<th>Nutrient medium</th>
<th>24 hours at 36°C</th>
<th>48 hours at from 36-37°C</th>
<th>3 days at from 36-37°C</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Glucose-broth fermentation tube</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 do</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 Glucose agar</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 do</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 Plain agar</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 do</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 Litmus-lactose fermentation tube</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 do</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 Litmus-lactose agar</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 do</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* + about 3.
+ + + about 4.
+ + + about 5 or more.
+ + + about 6.
+ + + about 7.

Many colonies present.
**Philippine Journal of Science**

**Table V.—Alabang Experiment Station, Rizal Province. Well 2.**

Samples taken (April 11, 1918) from pipe leading directly from well—not through collecting tank; water, sweet; depth, 231 meters; in use since October, 1908.

<table>
<thead>
<tr>
<th>Amount of water sown</th>
<th>Nutrient medium</th>
<th>24 hours at 36°</th>
<th>43 hours at from 36-37°</th>
<th>3 days at from 36-37°</th>
<th>6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>Glucose-broth fermentation</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
</tr>
<tr>
<td>1 do</td>
<td>12 - +</td>
<td>12 - +</td>
<td>12 - +</td>
<td>12 - +</td>
<td></td>
</tr>
<tr>
<td>5 do</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>Glucose agar</td>
<td>0 - +</td>
<td>0 - +</td>
<td>(e) 0 - +</td>
<td>0 - +</td>
</tr>
<tr>
<td>1 do</td>
<td>0 - +</td>
<td>0 - +</td>
<td>(b) 0 - +</td>
<td>0 - +</td>
<td></td>
</tr>
<tr>
<td>5 do</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td></td>
</tr>
<tr>
<td>1 Plain agar</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>Litmus-lactose agar</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
</tr>
<tr>
<td>1 do</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td></td>
</tr>
<tr>
<td>5 do</td>
<td>0 0 - +</td>
<td>(e) 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>Litmus-lactose broth fermentation tube</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
</tr>
<tr>
<td>1 do</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td></td>
</tr>
<tr>
<td>5 do</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td>0 0 - +</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>Bile-lactose-litmus fermentation tube</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td>(?) - +</td>
</tr>
<tr>
<td>1 do</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td>(?) - +</td>
<td></td>
</tr>
<tr>
<td>5 do</td>
<td>0 - +</td>
<td>0 - +</td>
<td>0 - +</td>
<td>(?) - +</td>
<td></td>
</tr>
<tr>
<td>1 Plain agar plate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 6 or more.
* Many colonies present.
* From 100 to 200 colonies.
* Neutral.
* Slight.
* Three days at room temperature, 236 colonies.
Barber: Examination of Artesian Wells

**Table VI.—First examination of water from well in the church square, Bocaue, Bulacan Province.**

First examination, March 8, 1913. Now flowing 3.8 liters per minute; said to flow most at high tide; water, sweet; temperature, 29° C.; ditch with standing water near.

<table>
<thead>
<tr>
<th>Amount of water sown</th>
<th>Nutrient medium</th>
<th>Result. *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 hours at 36°</td>
</tr>
<tr>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Glucose-broth fermentation</td>
<td>a 78</td>
<td>+</td>
</tr>
<tr>
<td>5 do.</td>
<td>a 78</td>
<td>+</td>
</tr>
<tr>
<td>1 Glucose agar</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 do.</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>1 Plain agar</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 do.</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>5 Litmus-lactose agar</td>
<td>+</td>
<td>(b)</td>
</tr>
<tr>
<td>5 Bile-lactose agar</td>
<td>+</td>
<td>(b)</td>
</tr>
</tbody>
</table>

* Amoeba test negative.

**Table VII.—Second examination of water from well in the church square, Bocaue, Bulacan Province.**

Second examination, March 27, 1913. Water now being raised by a pump.

<table>
<thead>
<tr>
<th>Amount of water sown</th>
<th>Nutrient medium</th>
<th>Result. *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>48 hours at 36°</td>
</tr>
<tr>
<td>cc.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 Glucose-broth fermentation tube</td>
<td>15</td>
<td>+</td>
</tr>
<tr>
<td>1 do.</td>
<td>a 78</td>
<td>+</td>
</tr>
<tr>
<td>5 do.</td>
<td>a 78</td>
<td>+</td>
</tr>
<tr>
<td>0.1 Glucose agar</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1 do.</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>5 do.</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>1 Plain agar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 do.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 Litmus-lactose agar</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>1 Bile-lactose agar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 do.</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>1 Plain agar-plate culture</td>
<td>15</td>
<td>+</td>
</tr>
</tbody>
</table>

* Amoeba test negative.
This water is in all probability polluted. From the 0.1 cc. sample in the glucose-broth fermentation tube a bacillus was isolated with coli-like morphology, motility doubtful. It produced acid and gas in lactose-, dulcite-, saccharose-, and mannite-litmus agars, and gas in bile-lactose agar. Nine-tenths of the closed arm of a glucose-broth fermentation tube was filled with gas, about half of which was absorbed by NaOH. See Table XVII.

**Table VIII.**—Water from wells at Fort William McKinley, Rizal Province.

Samples taken April 9, 1913; water, sweet; temperature, 30°C.; near Pasig River; depth, from 225 to 274 meters; water collected from several wells, about 2,270,000 liters used daily; samples were taken from the main leading from the collecting tank to the post.

<table>
<thead>
<tr>
<th>Amount of water used</th>
<th>Nutrient medium</th>
<th>24 hours at 36°C.</th>
<th>48 hours at 36°C.</th>
<th>3 days at 36°C.</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td>Glucose-broth fermentation tube</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.1</td>
<td>Glucose agar</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Litmus-lactose fermentation tube</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.1</td>
<td>Litmus-lactose agar</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>Bile-lactose fermentation tube</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>Litmus-lactose agar plate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar plate</td>
<td>(e)</td>
<td>(e)</td>
<td>(e)</td>
<td>(e)</td>
</tr>
</tbody>
</table>

* See Table XVII.
* Slight.
* ++ about 10.
* + + about 100.
* A Alkaline.
TABLE IX.—Water from well in Plaza Malolos, Malolos, Bulacan Province.

Sample taken March 18, 1913; water, sweet to taste; temperature, 29° C.; located on public street; not flowing spontaneously, but water could be siphoned from the pipe; depth, 64 meters; in use since 1908.

<table>
<thead>
<tr>
<th>Amount of water sown</th>
<th>Nutrient medium</th>
<th>Result. a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 hours at 30°</td>
</tr>
<tr>
<td>1</td>
<td>Glucose-broth fermentation</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>do.</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Glucose agar</td>
<td>0</td>
</tr>
<tr>
<td>4.6</td>
<td>do.</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>do.</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Litmus-lactose agar</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Bile-lactose agar</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar plate</td>
<td>0</td>
</tr>
</tbody>
</table>

a Amoeba test negative.  
b Many colonies present.

TABLE X.—Water from well on Aglipay Street, Marikina, Rizal Province.

Samples taken March 23, 1913; water with slight mineral taste; temperature, 28° C.; very little standing water near; depth, 70 meters; in use three years.

<table>
<thead>
<tr>
<th>Amount of water sown</th>
<th>Nutrient medium</th>
<th>Result. a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>45 hours at 30°</td>
</tr>
<tr>
<td>0.1</td>
<td>Glucose-broth fermentation</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>do.</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>do.</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Glucose-agar</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>do.</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>do.</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Litmus-lactose agar</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Bile-lactose agar</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>do.</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar plate</td>
<td>0</td>
</tr>
</tbody>
</table>

a Amoeba test negative.  
b Many colonies present.  
c +3 or 4.  
d +4 or more.
TABLE XI.—Water from well near the municipal building, Mariquina, Rizal Province.

Samples taken March 28, 1913; water with slight mineral taste; temperature, 28° C.; shallow ditch near; depth, 29 meters; in use since August, 1909.

<table>
<thead>
<tr>
<th>Amount of water sown</th>
<th>Nutrient medium</th>
<th>Result. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td></td>
<td>45 hours at 36°</td>
</tr>
<tr>
<td>0.1</td>
<td>Glucose-broth fermentation tube</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>Glucose agar</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Plain agar</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Litmus-lactose agar</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Bile-litmus agar</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar plate</td>
<td>0</td>
</tr>
</tbody>
</table>

a Amoeba test negative.

TABLE XII.—Water from well on Montalban Street, Montalban, Rizal Province.

Samples taken March 28, 1913; water, sweet; temperature, 27° C.; standing water 2 meters away; depth, 19 meters; in use since May, 1909.

<table>
<thead>
<tr>
<th>Amount of water sown</th>
<th>Nutrient medium</th>
<th>Result. a</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td></td>
<td>45 hours at 36°</td>
</tr>
<tr>
<td>0.1</td>
<td>Glucose-broth fermentation tube</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Glucose agar</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Plain agar</td>
<td>16</td>
</tr>
<tr>
<td>1</td>
<td>Litmus-lactose agar</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Bile-lactose agar</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar plate</td>
<td>0</td>
</tr>
</tbody>
</table>

a Amoeba test negative.
TABLE XIII.—Water from well near schoolhouse, Montalban, Rizal Province.

Sample taken March 28, 1913; water, sweet to taste; temperature, 27° C.; standing water in a ditch about 6 meters away; depth, 23 meters; in use since May, 1909.

<table>
<thead>
<tr>
<th>Amount of water drawn</th>
<th>Nutrient medium</th>
<th>Result.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td></td>
<td>45 hours at 36°</td>
</tr>
<tr>
<td>0.1</td>
<td>Glucose-broth fermentation...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>Glucose agar...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Litmus-lactose agar...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Bile-lactose agar...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar plate...</td>
<td>17</td>
</tr>
</tbody>
</table>

* Amoeba test negative.  
* Many colonies present.

TABLE XIV.—Water from well on Arangu Street, San Mateo, Rizal Province.

Samples taken March 18, 1913; water, brackish; temperature, 27.7° C.; standing water from a drain at edge of well; depth, 46 meters; in use since April, 1909.

<table>
<thead>
<tr>
<th>Amount of water drawn</th>
<th>Nutrient medium</th>
<th>Result.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cc.</td>
<td></td>
<td>45 hours at 36°</td>
</tr>
<tr>
<td>0.1</td>
<td>Glucose-broth fermentation tube...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>12</td>
</tr>
<tr>
<td>1</td>
<td>Glucose agar...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Litmus-lactose agar...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Bile-lactose agar...</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>...do...</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Plain agar plate...</td>
<td>1</td>
</tr>
</tbody>
</table>

* Amoeba test negative.  
* b Many colonies present.  
* + 6 or more.  
* + 10 or more.
Table XV.—Water from well in the church square, San Mateo, Rizal Province.

Samples taken March 28, 1913; water has a slight mineral taste; temperature 27.8° C.; no standing water near; depth, 46 meters; in use since April, 1909.

<table>
<thead>
<tr>
<th>Amount of water used</th>
<th>Nutrient medium</th>
<th>Result. *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose-broth fermentation tube</td>
<td>45 hours at 36°</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>(b)</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>(b)</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Amoeba test negative.  ** Many colonies present.  *** Overgrown.
TABLE XVI.—Water from well in Taytay, Rizal Province.

Samples taken April 9, 1913; water, slightly brackish; temperature, 28.6°C.; standing water only from overflow; depth, 156 meters; in use since June, 1911.

<table>
<thead>
<tr>
<th>Amount of water sown.</th>
<th>Nutrient medium.</th>
<th>24 hours at 36°.</th>
<th>48 hours at 36°.</th>
<th>3 days at 36°.</th>
<th>8 days at 36°.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gas.</td>
<td>Acid.</td>
<td>Colonies or</td>
<td>Gas.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>clearness.</td>
<td></td>
</tr>
</tbody>
</table>

| cc. | Glucose-broth fermentation tube | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + |
| 0.1 | do | 1 | + | + | + | 0 | + | + | 0 | + | + |
| 1   | do | 5 | + | + | + | + | + | + | + | + | + |
| 1   | do | 0 | + | + | + | + | + | + | + | + | + |
| 0.1 | Lactose-broth fermentation tube | 0 | + | + | + | + | + | + | + | + | + |
| 1   | do | 0 | + | + | + | + | + | + | + | + | + |
| 5   | do | 0 | + | + | + | + | + | + | + | + | + |
| 1   | do | 1 | + | + | + | + | + | + | + | + | + |
| 6   | do | 0 | + | + | + | + | + | + | + | + | + |
| 5   | do | 0 | + | + | + | + | + | + | + | + | + |
| 6   | Lactose-bile fermentation tube | 0 | + | + | + | + | + | + | + | + | + |
| 1   | do | 0 | + | + | + | + | + | + | + | + | + |
| 5   | do | 0 | + | + | + | + | + | + | + | + | + |
| 5   | Lactose-bile agar | 445 | + | + | + | + | + | + | + | + | + |
| 1   | Lactose-bile agar plate | 254 | + | + | + | + | + | + | + | + | + |
| 1   | Plain agar plate | 254 | + | + | + | + | + | + | + | + | + |

*See Table XVII.*  
*Alkaline.*  
*Slight.*  
*14 Acid.*

Further tests were made of a number of samples which showed gas and acid in litmus-lactose agar or in bile media, in order to determine if Bacterium coli was present. Such samples from 6 different wells were transferred to litmus-lactose agar. No acid or gas appeared in stab cultures, although transfers were made directly from the original bile or litmus-lactose samples which had shown gas or gas and acid. In 3 wells colonies were isolated which showed gas and acid in litmus-lactose agar. These colonies were tested on various media, and the results are given in Table XVII.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Boccau:</td>
<td>1 cc. sample in glucose-broth fermentation tube.</td>
<td>Colon-like</td>
<td>None after 4.5 hours glucose broth.</td>
<td>16</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(b)</td>
</tr>
<tr>
<td></td>
<td>0.1 cc. sample in glucose-broth fermentation tube.</td>
<td>do</td>
<td>do</td>
<td>16</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(?)</td>
<td>+</td>
</tr>
<tr>
<td>Taytay</td>
<td>1 cc. sample in bile-lactose fermentation tube.</td>
<td>do</td>
<td>do</td>
<td>15</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Fort William McKinley:</td>
<td>1 cc. sample in litmus-lactose agar plate. Red colony.</td>
<td>Very fine bacillus not colon-like.</td>
<td>Active</td>
<td>15</td>
<td>12</td>
<td>0</td>
<td>(a)</td>
<td>(b)</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.1 cc. sample in litmus-lactose fermentation tube.</td>
<td>do</td>
<td>do</td>
<td>15</td>
<td>12</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
</tbody>
</table>

a Slight, or 1/10 less.  
b Slight.  
c Slight or 0.  
d About 3/10.
Although some of these colonies resemble colon bacteria in some respects, it is evident that they vary somewhat from typical *Bacterium coli*.

From a comparison of the results obtained from the flowing and the pumping wells, it is evident that the pumping wells show a somewhat lower grade of bacterial purity. The best pumping well shows a greater degree of bacterial contamination than the poorest flowing well. It does not seem likely that the majority of the bacteria found in the pumping wells come from the deep water-bearing strata into which the wells are sunk, since flowing wells, in some cases only a few hundred yards away, show a much higher degree of purity; and it does not seem probable that there is seepage of surface water into these deep strata, since, in the two intermittently flowing wells, at least, there is pressure enough to bring the water near the surface. Neither is it probable that the source of contamination is in the pump. The Fort William McKinley pump, operated by machinery, raises about 600,000 gallons daily, and it is unlikely that such a volume of water would be much contaminated in passing through a pump with the ordinary protection from contamination. The waters of Alabang No. 1 and Alabang No. 2 are also raised by machinery, and the sample from No. 1 was taken from the pipe before it reached the collecting reservoir. These three wells showed about the same degree of contamination as those in which a hand pump was used.

All things considered, it seems probable that the source of contamination is water entering the wells above the deeper strata. In a flowing well, pressure is outward, and, obviously, there could be no inflow of water above the source, unless from strata in which the water is also under pressure. When the water in the well falls somewhat below the surface of the ground, as is the case in the pumping wells, the direction of the pressure in the upper part of the well, at least, is reversed and water may enter at any permeable point. The comparatively low degree of contamination in the pumping wells would indicate that such contaminating water enters in small quantities or is partially filtered before gaining access to the well.

It is improbable that any of the bacteria occurring in the pumping wells at the time of examination are dangerous to health. Fort William McKinley water is used unboiled by several thousand people, and there is no evidence that water-borne diseases come from its use. It is, of course, possible that some of these wells might become sources of disease under conditions other than those prevailing at the time of examination.
In summary, the waters from the flowing wells show a remarkable high degree of bacterial purity and may be regarded as safe from pollution by pathogenic bacteria. The pumping wells show a much lower degree of bacterial purity, although it is unlikely that any of them were polluted to a dangerous degree at the time of examination. These wells should be examined occasionally—especially during the prevalence of water-borne diseases—since they cannot be regarded as absolutely safe from pollution.
<table>
<thead>
<tr>
<th>Imus-cose agar (5 cc. samples)</th>
<th>Bile-lactose agar (5 cc. samples)</th>
<th>Acid in litmus-lactose agar (5 cc. sample)</th>
<th>Colonies per cc.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 or less</td>
<td></td>
</tr>
<tr>
<td>(?)?</td>
<td>(?)?</td>
<td>(?)?</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 or more</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 or less</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 or less</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>do</td>
<td></td>
</tr>
</tbody>
</table>

*Not known.*
<table>
<thead>
<tr>
<th>Town</th>
<th>Province</th>
<th>Location</th>
<th>Date of taking sample</th>
<th>Depth</th>
<th>In use since</th>
<th>Percent rate of flow per minute</th>
<th>Taste of water</th>
<th>Temperature of water</th>
<th>Surounding of well</th>
<th>Gas in—</th>
<th>Cylinders per cc</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloocan</td>
<td>Rizal</td>
<td>Church square</td>
<td>Mar. 29</td>
<td>88.5</td>
<td>do</td>
<td>15 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>No houses near</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hagonz</td>
<td>Bulacan</td>
<td>Plaza Burgos</td>
<td>Mar. 29</td>
<td>53</td>
<td>Aug. 20</td>
<td>15 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>No houses near</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Las Pinas</td>
<td>Cavite</td>
<td>Church square</td>
<td>Apr. 9</td>
<td>31</td>
<td>Apr. 21, 12</td>
<td>5 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>No houses near</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malolos</td>
<td>Bulacan</td>
<td>Barrio Abac</td>
<td>Mar. 29</td>
<td>31</td>
<td>Jun. 20</td>
<td>5 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>About 6 meters from road</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Barrio Bajoc</td>
<td>Mar. 29</td>
<td>46</td>
<td>Apr. 27, 12</td>
<td>5 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>No houses near</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Barrio Barrio</td>
<td>Mar. 29</td>
<td>9</td>
<td>Barrio Barrio</td>
<td>5 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>About 6 meters from road</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Barrio Barrio</td>
<td>Mar. 29</td>
<td>41</td>
<td>Nov. 21</td>
<td>5 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>No houses near</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Barrio Barrio</td>
<td>Mar. 29</td>
<td>41</td>
<td>Nov. 21</td>
<td>5 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>About 6 meters from road</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Barrio Santa Rosa</td>
<td>Mar. 29</td>
<td>63</td>
<td>Jun. 21</td>
<td>5 gallons</td>
<td>Slow</td>
<td>20.5</td>
<td>Houses near</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Malabon</td>
<td>do</td>
<td>Brackish</td>
<td>Mar. 29</td>
<td>122</td>
<td>Jan. 90</td>
<td>Very large flow</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mayamoc</td>
<td>do</td>
<td>Opposite mutual building</td>
<td>Mar. 29</td>
<td>74</td>
<td>Nov. 29</td>
<td>1.5 gallons</td>
<td>do</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>No. 3</td>
<td>Mar. 29</td>
<td>82</td>
<td>Nov. 29</td>
<td>Very large flow</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Navotas</td>
<td>Cavite</td>
<td>Railway station</td>
<td>Mar. 29</td>
<td>100</td>
<td>Dec. 29, 12</td>
<td>Very large flow</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pampanga</td>
<td>Bulacan</td>
<td>Church square</td>
<td>Mar. 29</td>
<td>98</td>
<td>Dec. 29, 12</td>
<td>15 gallons</td>
<td>Brackish</td>
<td>20.5</td>
<td>Houses within 30 meters</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Near schoolhouse</td>
<td>Mar. 29</td>
<td>83</td>
<td>Mar. 27</td>
<td>About 3 liters</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Church square</td>
<td>Mar. 29</td>
<td>330</td>
<td>Nov. 29, 12</td>
<td>6 gallons</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Talon</td>
<td>Mar. 29</td>
<td>107</td>
<td>Feb. 29, 12</td>
<td>5 gallons</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>San Nicolas</td>
<td>Mar. 29</td>
<td>106</td>
<td>Feb. 29, 12</td>
<td>Very large flow</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>San Nicolas</td>
<td>Mar. 29</td>
<td>72</td>
<td>Mar. 29</td>
<td>5 gallons</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Do</td>
<td>do</td>
<td>Santa Cruz</td>
<td>Mar. 29</td>
<td>52</td>
<td>May 19, 12</td>
<td>Medium</td>
<td>Brackish</td>
<td>20.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

---

**Table III.—List of all the flowing wells examined.**

<table>
<thead>
<tr>
<th>Glucose broth fermentation (in 1 cc. sample)</th>
<th>Lactose broth fermentation (in 1 cc. sample)</th>
<th>Bile lactose agar samples)</th>
<th>Acid in litmus-lactose agar samples</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Positive in 5 cc., but there was possibility of contamination of sample en route to laboratory.*

*Not known.*
INFANT MORTALITY IN THE PHILIPPINE ISLANDS

By W. E. Musgrave

(From the Department of Medicine, College of Medicine and Surgery, University of the Philippines)

Infant mortality in Manila is greater than it is in any other city from which we have records. This excessive mortality is not due to a single cause, and it is not due to natural conditions of the country. It is due to a multiplicity of artificial causes that may be classified into: Predisposing causes, prenatal and postnatal, and immediate or active causes.

A thorough study of the predisposing causes of infant mortality necessitates careful investigation of the mentality, financial responsibility, social and political economy of the people, the sanitary conditions—including character and quality of medical attendance—conditions of childbirth, general hygiene, personal hygiene, habits, vices, and customs of the race. In this connection, also, must be considered the influence of heredity, with particular reference to tuberculosis, syphilis, and other diseases transmitted directly or indirectly through generations—in other words, the eugenic estimate of the race.

Of the more direct influences bearing upon the prospects of the child after birth, there must be considered the environment, the character and method of feeding, and the influence of disease.

The committee for the investigation of infant mortality has been proceeding with its work along lines as indicated in the above outline, and a preliminary report of its findings has been submitted to the Philippine Legislature.

In the study of so complex a subject as is that of infant mortality, one must constantly bear in mind: First, careful attention to all details in order to secure facts, and, secondly, the necessity for constant attention in order to keep cause and effect in their proper relation to the question under discussion. A great many of the mistakes made in reports of students of this subject are in mistaking cause for effect and making recommendations in accordance therewith.

I shall only discuss, in the briefest possible way, a few of the most important questions involved in this great problem, and only the food situation for adults and children will be considered in this report.
FOOD SITUATION

The under-developed and under-nourished condition of the great masses of the Filipino people is due to a number of causes, the principal one being insufficient quantity and injudicious variety of foodstuffs employed. The cause of the enormous influence of the faulty nutrition of the mothers upon infant mortality, directly and indirectly, is one of the most important subjects within the scope of any investigation of this character. The small amount of data accumulated to date does not warrant definite conclusions, but a brief consideration of some of the facts is indicated.

Without going into the question of the much-discussed influence of special varieties of rice upon health and mortality, we may discuss the much larger question of the influence of metabolism disturbances, due to nutritional errors, upon infant mortality. As will be seen in another place in this report, the mortality in breast-fed children is higher than it is among children artificially fed. This condition, so far as we know, is peculiar to the Philippine Islands. The logical, and we believe the correct, explanation of this is the deficiency in quantity and quality of mothers' milk. So far as ordinary analysis shows the breast milk of Filipina mothers is of satisfactory quality for nutritional purposes. However, certain diseases (particularly infantile beriberi) are generally believed to be caused by some abnormality of mothers' milk. In a considerable number of cases studied from the clinics of the Philippine General Hospital, deficient quantity has been a rather constant finding. When these facts are considered, together with the under-nourished condition of the majority of the mothers due to the ravages of disease, we must conclude that faulty nutrition of the mothers is one of the principal factors in the enormous mortality of breast-fed children. The correction of this condition resolves itself into a discussion of methods for the improvement of the quantity and quality of mothers' milk and of the artificial feeding of babies.

In individual cases and to meet the immediate demands, satisfactory artificial feeding offers the obvious solution of the question. However, such a policy applied to the whole country would, eventually, lead to conditions more unwholesome than are those of the present time, and the ultimate solution of the problem, therefore, must depend upon improvement in the nutrition of the race. There are not in history more pathetic examples of unavailing self-sacrifice than are daily seen in our large clinics, of poor, half-starved, under-nourished mothers attempting to supply
from their breasts food for one or more children, when their own metabolisms are in a starved condition. When asked the direct question as to the supply of foodstuffs, these mothers almost invariably state that they have plenty to eat, and the pathetic part of the story is that they believe that they are stating facts. These abnormal premises are the result of a peculiar unexplainable psychology that is of very wide application in this country that the administration of food is more to satisfy hunger than to produce flesh and blood, and that the cheapest way in which hunger may be satisfied produces a satisfactory form of existence. It has been stated repeatedly that Filipinos do not care for foods other than fish and rice, with a few condiments and vegetables, but investigation tends to show that this is not a fact, and that these people have the same appetites and desire for fat and heat-producing foods as have people of other countries.

**INFANT FEEDING**

*Good milk is the only satisfactory food during infancy.* Mothers' milk, under normal conditions, is the ideal food, and next, because of its physiological adaptability and because it is the only class of milk it is possible to produce in quantities sufficient to meet the world's needs, is cows' milk. With the conditions discussed above, showing the causes for deficiency in the quantity of mothers' milk, together with the well-known fact that fresh, clean, raw, cows' milk is not obtainable in large quantities in the Philippine Islands, and that the prospect for a sufficient local production seems very remote, there is shown a new problem in infant feeding.

In considering the physiological requirements for the production of satisfactory baby food, it must be remembered that milk is just as essential an article of diet for the nursing mother in cases of breast feeding as it is for the baby in cases of artificial feeding, and recommendations for the solution of our local problem must bear this point in mind. The milk production of the Philippine Islands is practically nil when considered in relation to the requirements of the country. The principal supply consists of carabaos' milk and goats' milk, with a few dairies located in the larger cities, making a business of supplying cows' milk. We have gone rather carefully into the question of the quality of these milks, it being impossible in the time allowed to do anything regarding the correct estimation of the quantity produced. Nor is this necessary, because investigation of the quality leads to but one conclusion, and that is that practically all fresh milk produced in this country is dangerous to health, in whatever man-
ner used, and the marketing of these products should be inter-
dicted by law. Carabaos' milk and goats' milk, when obtained
from healthy clean animals, properly fed, and under proper san-
tary surroundings, are excellent milks, but the requisite condi-
tions do not obtain in the Philippine Islands, and with possibly
one or two exceptions the conditions regarding the local supply
of cows' milk are equally unsatisfactory. Nor is this all, for
by no method of reasoning can we foresee a time when it will
be practicable to produce satisfactory surroundings consistent
with an ample supply of fresh milk at a reasonable price. The
present custom of collecting, transporting, and using the local
milk supply is unbelievably filthy, unsanitary, and consequently
dangerous, and a continuance of the present practice with the
facts before us should fix criminal responsibility for the loss of
life. A general idea of the methods in vogue in collecting and
marketing carabaos' milk is shown in a report by Doctors Abella
and Gabriel. Briefly, the milk sold on the streets of Manila—
and presumably in other cities as well—is from twenty-six to
thirty hours old; has been diluted with tap water, or worse;
has been collected and transported in dirty receptacles; has been
milked by unclean persons from unclean animals; and both chem-
ical and bacteriological examination, of course, shows this milk
to be just about as bad as it is possible to make it. We have
not seen a single sample that would even approach the margin
of safety for its use by human beings, and in many instances evi-
dences of sewage contamination and the presence of extremely
dangerous bacteria are found in samples of milk bought in the
open market. The same is true, to a less degree, of so-called
fresh cows' milk sold in Manila. Under special conditions, which
are obtained only at the expense of a very high cost of produc-
tion, surroundings have been produced by which clean milk
could be marketed. Notable in this respect is the very excellent
work of La Gota de Leche which by careful supervision of model
dairies has been able to produce good milk; but even under these
circumstances, which raise the cost of milk to 50 centavos ¹ a liter,
the distinguished officials controlling the policy of this insti-
tution have felt it necessary to sterilize the milk before allowing
its consumption by the babies under their care. If sterilization
still is necessary after the precautions and expenses incident
to the production of milk by La Gota de Leche, the problem of
furnishing raw, fresh milk in quantities sufficient to influence

¹ One centavo equals $0.005, United States currency.
infant mortality in this Archipelago would appear to be one surrounded by impossible difficulties.

Taking all the evidence into consideration, a raw, fresh milk supply, sufficient to meet the absolute requirements of the country, does not seem to be within the bounds of possibility—at least within a reasonable length of time. All authorities acknowledge that raw milk contains elements of nutritional value not found in any sterilized milk, and so far as we are informed the only differences to be found between sterilized milk are differences in chemical composition. Therefore, in all probability, sterilized milk of local production has no advantage over imported sterilized milk. The question, then, resolves itself purely into one of financial consideration. Other things being equal, the cheapest milk should be the one adopted for our general use.

**Pasteurization**

So much has been written recently regarding the methods of Pasteurization of milk in tropical countries that a very brief consideration of this subject seems pertinent. Formerly, Pasteurization was considered an efficient method of preparing milk for human consumption, because of the destruction by this method of dangerous disease-producing bacteria. We now know that the so-called pathogenic organisms are not the only, even if they are the most dangerous, bacteria in milk. Pasteurization, of course, does not destroy spore-bearing bacteria, and, therefore, any milk not kept below a temperature of from 20 to 22° C. after Pasteurization acts as a culture medium for these germs not destroyed by the low degree of heat used in the method of Pasteurization. Intrinsically, most of the bacteria of this class are not considered pathogenic, but as a result of their multiplication the chemical composition of the milk is altered, and as by-products of this alteration there are produced dangerous chemical poisons which are very important factors in the morbidity results produced by the ingestion of milk. Conditions for the growth of bacteria in the Philippine Islands are ideal, and with a very limited ice supply and without much prospect of improving this condition the after care of either fresh or Pasteurized milk becomes impossible for the vast majority of people. Actual experimentation has shown that the multiplication of bacteria in Pasteurized milk is so rapid that within a few hours after Pasteurization such milk is almost as dangerous as if this process had not been employed.

We come, then, to completely sterilized milk as being the only
variety of this life-giving food practicable of extensive employment in this country, at least at the present time.

Fortunately, conditions are not so bad as they would appear at first sight. Sterilized milk when used under proper conditions is a very satisfactory food for infants, and is just as satisfactory for all other purposes as is raw milk, and another fortunate circumstance is that the Philippine Islands enjoys a splendid market of imported sterilized, natural, and condensed milks of excellent quality at very reasonable prices; so that, as pointed out by me in an article presented to the Congress of Filipino Physicians, the milk supply of the Philippine Islands compares very favorably with that of many other countries and cities. It is a fact that sterilized milks are a little more indigestible than are raw milks, and there are certain metabolism conditions, for example, scurvy, that may be incurred as a result of the use of sterilized food. However, both the indigestibility and the metabolism-disturbing qualities of such milk are easily and satisfactorily controlled by simple methods well-known to the medical profession. These methods are so successful that in one series of records of more than 1,000 babies born in the Philippine Islands, and fed entirely on these sterilized foods, there has not been a single case of metabolism disturbance nor a death from disease of importance that could be justly attributed to the use of such food.

It may be of interest to note that there was imported into the Philippine Islands during the fiscal year 1912, an equivalent of between 18 and 20 million kilograms of milk, at an approximate valuation of 15,000,000 pesos.\footnote{In United States currency, 7,500,000 dollars.}

METHODS OF ARTIFICIAL FEEDING

The methods employed in the artificial feeding of infants among the poor people of Manila are faulty in many particulars. In the first place, notwithstanding the accessibility of a very good milk supply, the foods supplied to children in a majority of cases are those of condensed, sweetened, skimmed milk of the cheapest varieties, and consequently poor in quality. In another place I have discussed this subject at length.\footnote{Proceedings of the Congress of Filipino Physicians, held in Manila this year.} In this report it was shown that the apparent economy in the use of this food, figured from a financial basis alone, is not a true economy, because milk compounds of this class contain from 50 to 65 per cent of ordinary sugar. When the caloric value of
the actual milk contained in these tins is figured at the current prices, and this price subtracted from the total price of a tin of one of these mixtures, it is found that the people pay an average of from 50 to 75 centavos a kilogram for ordinary sugar, which they can buy in a tienda for 11 centavos a kilogram.

It is, of course, unnecessary to dwell upon the undesirability of the use of this class of foods, and it only remains to point out that it is bad in principle, and what apparently, heretofore, has not been recognized that it is a more expensive method of feeding than would be necessary by the employment of good qualities of milk.

It should be stated that there is one favorable feature in the use of sugar-preserved milk compounds, and that is that the excessive amount of sugar preserves the food from the time of the opening of the tin until the food is entirely consumed. This is, of course, an important problem with poor people who cannot afford the ice necessary for the preservation of any pure milk, whether sterilized or not, after the tin is opened. However, this should not be a serious obstacle in the adoption of the use of a better grade of milk, because the method that is used to a greater or less extent among the poorer people of the United States, in which a number of neighbors who have nursing children alternate in the opening of the tins of food, so that each tin when opened is consumed by a number of babies in a few hours, might well be adopted here. Another solution of this problem that already is being employed by some manufacturers consists in marketing milk in much smaller tins.

The next most important faulty custom consists in the dilution of milk compounds with unsafe water. In our investigation of the causes of death of 300 babies, it is found that tap water, either with or without boiling, is used as a diluent in most instances. As a majority of the houses of these people are at considerable distances from the nearest faucet, the water is carted by water carriers and kept in earthenware jars or other vessels, under the most unsanitary conditions; in many instances whatever safety might be secured by boiling the water is destroyed by the subsequent manipulations and care of the water and by the methods employed in making the dilutions of the milk mixtures. The proof that these mixtures are dangerous to the health of the baby, on account of the introduction of bacteria, is shown by the analyses of the contents of a number of nursing bottles already prepared for consumption by the

* Small, native shop.
baby. These analyses of the finished product of food just before administration show dangerous contamination in practically every instance, and this has been found particularly true in cases of children dying from gastro-intestinal disturbances.

Other faulty methods which need not be discussed at length here are the almost universal custom of feeding babies with the greatest irregularity in time, quantity, and strength of food administered.

The remedy for these conditions, obviously, is education, both by theoretical instruction and, best of all, by practical demonstration as may be seen in the wards and clinics of the hospitals and La Gota de Leche, and, as has been recommended by the Committee for the investigation of Infant Mortality, by the establishment of nursery maids training schools and day nurseries.

The remedy controlling the character and quality of foodstuffs employed, however, lies in the hands of the legislative body, and in the opinion of the committee above referred to the question should be treated by discriminating high import duty on unsatisfactory milk compounds and by allowing free entry to the better qualities.

In order to solve the infant mortality question in this or any other country, the first essential is to secure the influence of a favorable and interested public opinion.

The attitude of public opinion in health matters is a very popular one, and even in older countries with more advanced civilization it is only within recent years that conservation of health has been of much interest to the general public.

Public opinion is vitally active regarding the pecuniary interests of a country, as exemplified in commercial activities and improvements, and even in the health and protection of draft animals and in the comfort and well-being and protection from cruelty to domestic animals.

However, with regard to the great vital question of the conservation of the health of its citizens and the saving and protection of the lives of infants, there exists a curious indifference that only springs, periodically, into activity as the result of some spectacular catastrophe, and dies down again with the restoration of the usual equilibrium.

The great Taal Volcano eruption destroyed some two thousand lives and a great deal of property, and its results sent waves of horror throughout the world. There are more lives uselessly sacrificed to tuberculosis in the Philippine Islands every month
than were destroyed by the Taal eruption; and the economic loss to the country by decrease in potential energy and earning capacity of its citizens, to say nothing of the actual loss of life, costs the country daily many times the value of property destroyed by Taal.

The recent catastrophe in Cebu and other southern islands sent a wave of horror over the country and called out Government and private reserves to meet the requirements of the situation. The loss of life and health is greater from criminal obstetrical practices in the Philippine Islands every day of the year than was the loss of life at Cebu. The financial drain upon the resources of the country as a result of these preventable and criminal practices is a greater daily drain than the total value of the property destroyed by this unavoidable calamity.

In older and more experienced countries there is at last an awakened public opinion regarding the economic consideration of health problems, and one of the most important questions for us is to secure the support of this valuable weapon in our campaign for the conservation of the lives of the potential citizens of this country.
THE PROTECTIVE POWER OF NORMAL HUMAN MILK AGAINST POLYNEURITIS GALLINARUM (BERIBERI)

By R. B. Gibson

(From the Laboratory of Physiology, College of Medicine and Surgery, University of the Philippines)

Andrews has pointed out in a recent paper that the cause of infantile beriberi, to which the high death rate of infants in Manila is in part attributable, is primarily due to the quality of the mothers' milk. The disease is not an infection or toxæmia of either the mother or child. Presumably the etiology is associated with a deficiency of the protective substances or "vitamines" of the milk, induced by the too exclusive consumption of milled rice by the mother. The symptoms of the beriberi may not be apparent in the mother on the first examination, but usually appear later if the child continues nursing.

Probably with a deficiency (of the protective substances) in her diet the mother draws on her own storehouse for this substance for her child, thus diminishing her own supply and producing the disease in herself.

Doctor Andrews and I had planned to study the protective power of the milk of the mothers of infantile beriberi cases, the diagnosis of which could be verified by autopsy. However, I have not yet been able to obtain a case in which autopsy was permitted and which, at the same time, would supply sufficient milk for experimentation. The milk obtained was scant, and the secretion ceased in the course of a few days in the two instances where the autopsy was allowed and the diagnosis verified. Some control observations on the protective power of normal human milk have been made, and I have thought it worth while to present these in a short paper.

In view of the conclusive evidence that rice polyneuritis is a nutritional disease caused by dietetic deficiency, it seems hardly necessary to accept the presence of a toxic substance in the milk as suggested by Guerrero from experiments on the frog heart. Analyses of the milk of women with beriberi infants, reported by Andrews, show that some of the samples are quite normal,

1 This Journal, Sec. B (1912), 7, 67.
so far as proteid, fat, and carbohydrate are concerned; however, the amounts of phosphate and calcium are increased, the latter from three to four times the normal. The increased calcium, in itself, ought to be the cause of the early cessation of the heart’s action in Guerrero’s experiments.

Vedder and Clark have reported experiments with normal cows’ milk. Fowls fed on polished rice and receiving in addition 5 cubic centimeters of canned milk received little or no protection against polyneuritis gallinarum. With 5 cubic centimeters of fresh cows’ milk, they received partial protection as indicated by the prolongation of the “incubation” period. It is to be expected that sterilized milk would not be as efficient as fresh cow’s milk, in as much as Fraser and Stanton have found that the protective vitamine may be destroyed by autoclaving.

The milk used in the present experiments was obtained from the obstetrical ward of the Philippine General Hospital. It was not obtained until three days after parturition. The women usually enter the ward previous to labor. They were, therefore, for several days on the diet given below before the milk was collected. The breasts were milked by hand into a sterile flask, mixed samples being obtained daily.

The diet was as follows:

Breakfast. Two eggs, 2 slices of bread, coffee, milk, and sugar.
Lunch and dinner. Fish or stewed beef, unpolished rice, cooked vegetables, pudding or banana, tea, and milk.

It was planned in the present experiments to feed 4 groups of 3 fowls each on (1) 50 grams of polished rice and 5 cubic centimeters of human milk, (2) 50 grams of polished rice and 10 cubic centimeters of human milk, (3) 50 grams of polished rice and 20 cubic centimeters of human milk, and (4) 50 grams of polished rice alone, per day. On some days it was impossible to get any milk because of the lack of suitable patients in the ward, and on other days it was necessary to cut down or omit the milk ration for one or more of the fowls receiving 20 cubic centimeters per day. Fortunately, the milk was obtained in abundance from the twenty-seventh to the fortieth day, inclusive, of the experiment. On the fortieth day 1 fowl (8) receiving 20 cubic centimeters of milk came down with mild though typical neuritis, the experiment indicating conclusively that the administration of 20 cubic centimeters of human milk with the polished

*This Journal, Sec. B (1912), 7, 423.
*Studies from Institute for Medical Research. Federated Malay States (1911), No. 12.
rice is insufficient to prevent the onset of neuritis. The experiments are given in the accompanying table.

It is evident from the results obtained that the almost continuous daily administration of 5 cubic centimeters (fowl 3) and 10 cubic centimeters (fowl 5) of human milk with the polished rice did not prevent polyneuritis. Furthermore, 20 cubic centimeters of the milk is insufficient as stated above. Clark has shown that degeneration of the nerves of fowls may be observed as early as the seventh day of continuous rice feeding; it would seem, then, that histological examination of the nerve on about the twentieth day of the experiment would be a more exact method of determining whether or not a certain substance is protective. For example, fowls 1, 7, and 10 (the control) have not developed neuritis in fifty-seven days. However, fowls 2, 6, 9, and 11 showed distinct degeneration when killed on the twentieth day. Finally it would seem, if Vedder's statement that fowls kept on 5 cubic centimeters of fresh cows' milk with milled rice are partially protected be accepted, that normal human milk must contain not more than one-fourth of the amount of the vitamine of the former.
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Killed; sciatic nerve showed moderate degeneration.
| Week | 27  | 28  | 29  | 30  | 31  | 32  | 33  | 34  | 35  | 36  | 37  | 38  | 39  | 40  | 41  | 42  | 43  | 44  | 45  | 46  | 47  | 48  | 49  | 50  | 51  | 52  | 53  | 54  | 55  | 56  | 57  |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|      | 1340| 1354| 1337| 1302| 1325| 1300| 1275| 1247| 1297| 1265| 1262| 1244| 1240| 1270| 1295| 1267| 1250| 1250| 1247| 1289| 1277| 1279| 1270| 1267| 1256| 1254| 1254| 1279|
|      | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  | 10  |
|       | 1,422| 1,417| 1,412| 1,404| 1,420| 1,377| 1,367| 1,367| 1,369| 1,347| 1,345| 1,334| 1,335| 1,373| 1,373| 1,379| 1,379| 1,379| 1,379| 1,379| 1,379| 1,379| 1,379| 1,293| 1,293| 1,293| 1,293|
|       | 1,252| 1,267| 1,299| 1,277| 1,280| 1,267| 1,255| 1,254| 1,289| 1,295| 1,294| 1,327| 1,289| 1,275| 1,270| 1,275| 1,267| 1,260| 1,260| 1,293| 1,293| 1,293| 1,293| 1,293| 1,293| 1,293| 1,293|
|       |      |      |      |      |      |      |      |      |     |      |      |      |      |      |      |      |      |      |     |      |      |      |      |      |      |      |      |      |

* Fed by hand from this day on, so that the fowls always received 50 grams of rice daily.
Bordet \(^1\) says:

Von Pirquet and especially Friedberger, have developed the theory that the phenomena of anaphylaxis play an essential part in the production of the symptoms observed in the course of contagious diseases. According to these scientists, the symptoms declare themselves when the antibodies, elaborated by the organism, begin to react with the microbes. This theory was based especially on the fact * * * that the simple contact of microbes with fresh serum may produce anaphylatoxin; this contact naturally takes place constantly in the infected organism.

Evidence of the relation of fever in infections to anaphylaxis is given in the work of Friedberger and Mita.\(^2\) The injection of appropriate minute amounts of foreign protein into guinea pigs, sensitized to that protein, produces a marked transient rise in body temperature following the characteristic fall. The same results are observable with anaphylatoxin prepared in vitro and, also, with normal guinea pigs (although relatively greatly increased amounts of the protein must be employed in the latter case).\(^3\) Successive injections yield temperature curves corresponding in type to those of infectious fevers.

In anaphylactic shock, the symptoms resemble the picture obtained for the intoxication resulting from the intravenous injection of proteoses (page 479). This similarity has been repeatedly pointed out.\(^4\)

Anaphylactic-like shock in the normal organism is claimed

\(^1\) *Journ. State Med.* (1913), 21, 459.
\(^2\) *Zeitschr. f. Immunitätsforsch.*, Orig. (1911), 10, 216.
\(^3\) Friedberger and Mita believe that anaphylaxis is an intensification of the ordinary reaction of the normal organism to foreign proteins. The shock following the injection of toxic normal sera has been similarly interpreted [Doerr and Moldovan, *Zeitschr. f. Immunitätsforsch.*, Orig. (1910), 7, 223].
\(^4\) Cf. E. Zunz, *ibid.* (1913), 16, 581.
for tissue extracts, for methyl guanidine, for β-imidazolethylamine, and for globin, histone, and protamine. Anaphylactic symptoms have also been obtained with acetic acid, saponin, potassium cyanide, hirudin, metasilicic acid, colloid iron hydroxide, nucleic acid, and snake venoms; with sodium oleate, oleic acid, morphine, codeine, and strophanthine; and with salts of the heavy metals and tannin and phosphomolybdic acid.

There is evidence, also, that the formation of anaphylatoxin is associated with the hydrolytic cleavage of the protein antigen. Thus Pfeiffer says:


As already stated above, Friedberger and Mita have associated fever production with the anaphylactic symptom-complex. The close relationship of "peptone" intoxication to anaphylaxis is further suggested by the pyrexial action formerly ascribed to the proteoses. A summary of the literature on proteose fever may be given briefly.

Endo- and extra-cellular substances have been separated from bacteria. These substances withstand boiling, and are precipitated with alcohol or ammonium sulphate, their behavior in these respects resembling that of proteoses. When injected into healthy animals, these extracted substances incite fever,

5 Popielski. See page 480.
6 Heyde, Zentralbl. f. Physiol. (1911), 25, 441.
7 Dale, Journ. Physiol. (1906), 34, 163; ibid. (1910), 41, 318; ibid. (1912), 43, 182.
8 Schittenhelm und Weichardt, Zeitschr. f. Immunitätsforsch., Orig. (1912), 14, 609.
9 Doerr und Russ, Zentralbl. f. Bakt. etc., Orig. (1912), 63, 243; Doerr und Moldovan, loc. cit.
10 Friedberger und Moreschi, Berl. klin. Wochenschr. (1912), 49, 741.
11 Szymanowski, Zeitschr. f. Immunitätsforsch., Orig. (1912), 16, 1.
and, in tubercular animals, show effects somewhat similar to tuberculin. 13

Ott and Collmar 14 first noted that the introduction of proteoses intravenously produces a considerable and immediate rise in body temperature in rabbits. Matthes 15 found that the subcutaneous injections of proteoses (dentero-albumose of Neumeister) are more pyrogenic for tubercular than for sound animals. Krehl, 16 Krehl and Matthes, 17 and Rolly 18 report experiments in which pyrexial effects from proteoses were obtained. When prepared in the laboratory from protein material, however, the proteoses are neither so uniformly pyrogenic nor so pronounced in their effects as are the products of bacterial origin. Von Behring, 19 on the contrary, failed to observe any augmentation of temperature as the result of injecting sterile proteose solutions. Klemperer 20 does not consider the causative factor of fever to be proteose, but ascribes such effect to adherent impurities.

Considerable emphasis was laid by Krehl and Matthes 21 on the occurrence of proteoses in the urine in both infections and aseptic febrile conditions. Schultess 22 reached the same conclusion. Morawicz and Dietschy 23 have shown that the presence of proteoses in the urine is by no means constant in fever. Subsequently, Krehl retracted his ideas in regard to proteose fever. 24

Other substances, injected or formed in the course of intermediate metabolism, have been reported as possessing more or less pyrogenic action. These include boiled or unheated enzymes; 25 various proteins, amines, amino-acids, and sodium salts; 26 pu-

14 Journ. Physiol. (1887), 8, 218.
15 Deutsches Arch. f. klin. Med. (1894), 54, 391.
16 Arch. f. exp. Path. u. Pharm. (1895), 35, 222.
17 ibid. (1896), 36, 437; ibid. (1898), 40, 434.
18 Deutsches Arch. f. klin. Med. (1902), 78, 250.
19 Lehrbuch der allgemeinen Therapie, von Eulenberg und Samuel (1898-99), 3, 991.
20 Naturforscherversammlung, Kassel (1903), 2, 67.
21 Arch. f. exp. Path. u. Pharm. (1898), 40, 430.
22 Deutsches Arch. f. klin. Med. (1897), 58, 325; ibid. (1898), 60, 55.
23 Arch. f. exp. Path. u. Pharm. (1905), 54, 88.
25 Ott and Collmar, loc. cit.; Krehl, loc. cit.; Edelberg, Arch. f. exp. Path. u. Pharm. (1880), 12, 283.
26 Krehl, loc. cit.
rines and their precursors; ammonia and organic acids; and sodium-halogen salts.

Friedberger and Mita seize upon the possibility of the formation of pyrogenic proteoses to explain the temperature rise in sensitized guinea pigs referred to above. Thus they say:

Wir müssen annehmen, dass die gewöhnliche Anaphylaxis dadurch zustande kommt, dass aus dem parenteral eingeführten Eiweiss Spaltprodukte entstehen, die tödlich wirken, und ebenso ist es bei der Darstellung des Anaphylatoxins in vitro.

Genau wie wir nun hier durch Verringerung der eingeführten Menge zuerst statt Tod Temperatursturz, dann nach einer Dosis, die die Temperatur scheinbar unbbeeinflusst lässt, Temperatursteigerung haben, bis wir schliesslich an einen unteren Schwellenwert kommen, genau so verhält es sich wenn in den präparierten Organismus nicht fertiges Anaphylatoxin, sondern Eiweiss in untödlicher Dosis eingeführt wird.

It is inconceivable to me that 0.0000005 cubic centimeter of sheep’s serum, as used in some of Friedberger and Mita’s experiments, would yield sufficient proteose to produce pyrogenic effects, even though the animals be highly sensitized.

Further evidence, however, for a febrile reaction in anaphylaxis of causal relation to proteoses is given in a recent paper by E. Zunz. This investigator studied the active and passive anaphylaxis for peptic proteoses, prepared from fibrin according to the methods of Adler, of Haslam, and of Pick, and for the Siegfried peptone from the same material. He finds that, if a third intravenous injection of heteroalbumose or protalbumose is given eight, twenty-five, or thirty days subsequent to the production of the anaphylactic shock, a rise of from 1° to 2° in the rectal temperature may be observed (although no effects were obtained for longer periods than thirty days).

The identity of “peptone” intoxication with anaphylactic shock has recently been questioned. Bordet believes that any such conclusion “is so far very unprecise.” And, in as much as he has shown that anaphylatoxin may be developed in vitro from

37 Loc cit.
38 Loc cit.
normal serum by the addition of agar, he concludes that "the theory of the production of poison by digestion and disintegration of the antigen does not therefore appear to be justified." Friedberger \(^8\) claims that Bordet's results with agar are to be ascribed to admixed proteins, although the formation of anaphylatoxin with kaolin suspensions seems to have been earlier demonstrated.

Loewit \(^4\) believes that there is insufficient evidence to show that "peptone" shock and anaphylactic shock are identical.


Additional evidence that the symptoms of anaphylactic shock are not due to proteoses is given by Auer and Van Slyke.\(^5\) These investigators examined, with the recent refined analytical methods, the amino-acid, peptone, and proteose content of the lungs of guinea pigs which showed undoubted anaphylactic shock. The analytical figures are essentially identical for the "shock" and the control animals.

A question of extreme importance to "peptone" intoxication is whether the symptoms are actually due to the proteoses injected or to the admixture of some substance physiologically very active. Continuing the work of earlier investigators, Chittenden and Mendel and their associates had established the fact that the rapid introduction of proteoses directly into the circulation would produce a marked fall in the blood pressure of the dog; other changes were an increased lymph flow and inhibition of the clotting powers of the blood, deep narcosis, and anuria. Pick and Spiro \(^6\) concluded that these effects may be due to a contaminating substance, "peptozyme," of animal origin and soluble in alcohol. Underhill \(^7\) took up the subject anew; employing native proteoses or those made from isolated plant proteins with vegetable enzymes, heat, or acid hydration, he obtained the "peptone" shock. Furthermore, he duplicated Pick and Spiro's inactive preparations, except for a more extended hydrolysis, and still observed the characteristic effects.

\(^8\) Zeitschr. f. Immunitätsforsch., Orig. (1913), 17, 323.
\(^4\) Arch. f. exp. Path. u. Pharm. (1913), 73, 1.
\(^6\) Am. Journ. Physiol. (1903), 9, 345.
Underhill's work, however, does not seem to have been considered by subsequent writers. Popielski and his associates ascribe the active physiological principle of intestinal extracts and of Witte "peptone" to an alcohol-soluble substance "vasodilatine." In a recent paper, Popielski says:

Vasodilatin ist ein chemisch einheitliches Körper; es ist weder Cholin, noch β-Imidazoläthylamin, noch entsteht es durch Zerfall von Cholin.

The experiments, to be reported here, were carried out several years ago because of the suggestion by Krehl of the possibility of a contaminating substance being responsible for the pyrexial effects ascribed to the proteoses. The association of proteoses and of fever with the anaphylactic reaction has made them timely, and I have thought it worth while to present them in the present paper. The original problem was simply an inquiry as to whether pyrexial effects result from the injection of proteoses, prepared by gentle hydration from pure proteins without subsequent drastic treatment. Now, however, the results must be considered also in their relation to the subject of anaphylaxis.

**TECHNIQUE OF THE EXPERIMENTS**

A description of the proteoses used in the present experiments has been given in an earlier paper on the pharmacological action of these products on the heart. The proteoses were made from four-times reprecipitated caseinogen, from recrystallized edestin, and from thoroughly washed pig fibrin, by digestion with a very active scale pepsin prepared by me. The proteoses were salted out with ammonium sulphate, redissolved, and again salted out. The salt was removed by dialysis for two weeks, with thymol as the preservative. The dialyzate filtrate was concentrated in vacuo as 50° to a sirup, precipitated with 95 per cent alcohol, and dehydrated with hot absolute alcohol. The mixed proteoses were obtained as a fine white powder which was easily soluble in water. The filtrates from the alcohol-precipitated proteoses were concentrated, precipitated with hot absolute alcohol, and, on drying, yielded a fine white powder, evidently proteose.

These alcohol-soluble proteoses may be prepared from Witte peptone or other mixed proteose preparations by extracting with

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38 *Arch. f. d. ges. Physiol. (Pflüger) (1907), 120, 451; ibid. (1908), 121, 239; ibid. (1909), 126, 483; also Czubalski, ibid. (1908), 121, 395; Gizelt, ibid. (1908), 123, 540.
40 **Gibson and Shultz, Journ. Pharm. & Exp. Therap. (1909-10) 1.
hot 95 per cent alcohol. On standing in the cold the proteoses separate out as semicrystalline bodies, resembling the spherules which are intermediate stages in the change of the amorphous ovalbumin or serum albumin to the crystalline form. An ovalbumin alcohol-soluble proteose, so prepared, produced the typical fall in blood pressure, etc., when injected intravenously into a puppy; there was, however, no inhibition of blood clotting. (The experiment was not repeated for lack of sufficient material.)

Rabbits and guinea pigs were used as experimental animals. The experiments were so conducted that the animals should receive the least possible handling during the series of observations. They were amply fed. Temperature readings (rectal) on a series of animals were always taken in the same order; in this way the time of the observation could be noted in the protocols under a single hour. In the guinea pig experiments, the proteoses (always dissolved in about 4 to 5 cubic centimeters of physiological saline) were sterilized at 100°; while for the subsequent observations the proteose solutions (injected dissolved in 10 cubic centimeters of saline) were passed through a Berkefeld filter. The subcutaneous injections were made with aseptic precautions.

Control observations were made on all the animals. The temperature regulatory mechanism in the guinea pig and rabbit is somewhat uncertain and unstable. The animals are heavily coated with fur; sweat glands are lacking or are few or rudimentary; and the cutaneous circulation, in the rabbit, at least so far as temperature regulation is concerned, is practically nil. Their small size offers relatively a greater body surface for loss of heat by radiation and conduction than obtains for the larger animals. Furthermore, the animals are timid and easily excitable, and environmental influences or handling may be reflected in the temperature readings. Individual variations are considerable, and diurnal changes are much greater and less constant than for man.

The experiments on guinea pigs were carried on in the Sheffield Laboratory of Physiological Chemistry, Yale University, in 1904. The observations on rabbits given here were made in 1906 in the Research Laboratory of the Department of Health of the City of New York with proteoses prepared at the Sheffield Laboratory.

## EXPERIMENTS ON GUINEA PIGS

**Table I.**—Experiment 10. *Pepsin caseoses (alcohol soluble).*

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td></td>
</tr>
<tr>
<td>Mar. 3</td>
<td>38.3</td>
<td>38.4 to 39.6</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>39.0</td>
<td>38.5 to 39.5</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>39.1</td>
<td>38.7 to 39.5</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>38.8</td>
<td>38.8 to 39.0</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>39.3</td>
<td>39.2 to 39.6</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>39.1</td>
<td>38.8 to 39.5</td>
<td>6</td>
</tr>
</tbody>
</table>

There are, apparently, no pyrexial effects to be noted in the above experiment. The temperature of the animal is at no observation higher after the injection than was observed at times in the controls.

**Table II.**—Experiment 14. *Caseoses.*

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td></td>
</tr>
<tr>
<td>Mar. 23</td>
<td>38.2</td>
<td>38.7 to 39.4</td>
<td>6</td>
</tr>
<tr>
<td>24</td>
<td>38.9</td>
<td>38.6 to 39.0</td>
<td>5</td>
</tr>
<tr>
<td>25</td>
<td>38.5</td>
<td>38.2 to 38.7</td>
<td>3</td>
</tr>
<tr>
<td>25</td>
<td>38.8</td>
<td>38.2 to 39.2</td>
<td>11</td>
</tr>
<tr>
<td>26</td>
<td>39.3</td>
<td>38.8 to 39.6</td>
<td>5</td>
</tr>
</tbody>
</table>

In this experiment careful examination of the temperature variation shows that the proteose injection was without effect on the day of administration.
**Table III.—Experiment 22. Edestinoses (alcohol soluble).**

Guinea pig; female; weight, 600 grams. Received a subcutaneous injection of 4.5 cubic centimeters of physiological salt solution at 10 a.m. on April 27 and on April 28, and of 0.4 gram of edestinoses at 12:30 p.m. on April 29.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td></td>
</tr>
<tr>
<td>Apr. 25</td>
<td>Average</td>
<td>Range of variation</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>39.2</td>
<td>38.8 to 39.4</td>
<td>7</td>
</tr>
<tr>
<td>27</td>
<td>39.1</td>
<td>38.9 to 39.3</td>
<td>7</td>
</tr>
<tr>
<td>27</td>
<td>38.9</td>
<td>38.9 to 39.0</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>38.9</td>
<td>38.6 to 39.2</td>
<td>7</td>
</tr>
<tr>
<td>28</td>
<td>38.5</td>
<td>38.5 to 38.6</td>
<td>2</td>
</tr>
<tr>
<td>29</td>
<td>38.4</td>
<td>38.1 to 38.8</td>
<td>7</td>
</tr>
<tr>
<td>29</td>
<td>38.1</td>
<td>37.7 to 38.4</td>
<td>4</td>
</tr>
<tr>
<td>30</td>
<td>38.9</td>
<td>38.5 to 39.2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>39.0</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

When compared with the saline injection on April 27 and with the observations on April 25 and 26, it does not appear that the proteoses are pyrexial.

**Table IV.—Experiment 23. Caseoses (alcohol soluble).**

Guinea pig; male; weight, 440 grams. Injections of 4.5 cubic centimeters of physiological salt solution at 10 a.m. on April 27 and on April 28, and of 0.46 gram of caseoses at 12.30 p.m. on April 29.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td></td>
</tr>
<tr>
<td>Apr. 25</td>
<td>Average</td>
<td>Range of variation</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>39.4</td>
<td>38.7 to 39.8</td>
<td>7</td>
</tr>
<tr>
<td>27</td>
<td>39.1</td>
<td>38.6 to 39.8</td>
<td>7</td>
</tr>
<tr>
<td>27</td>
<td>38.6</td>
<td>38.6 to 39.6</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>39.1</td>
<td>38.2 to 40.0</td>
<td>6</td>
</tr>
<tr>
<td>28</td>
<td>37.9</td>
<td>37.8 to 38.0</td>
<td>2</td>
</tr>
<tr>
<td>29</td>
<td>38.6</td>
<td>37.9 to 39.0</td>
<td>6</td>
</tr>
<tr>
<td>29</td>
<td>38.4</td>
<td>37.6 to 39.0</td>
<td>4</td>
</tr>
<tr>
<td>30</td>
<td>38.8</td>
<td>38.2 to 39.7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>39.1</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

The temperature changes in this guinea pig are very abrupt and erratic. A temperature rise (April 27) following the injection of the physiological salt solution is more marked than that noted subsequent to the administration of the proteose; similarly, higher normals were obtained for April 25 and 26, when no injections were made.
### Table V.—Experiment 24. Caseoses.

Guinea pig; male; weight, 570 grams. Subcutaneous injection of 4.5 cubic centimeters of physiological salt solution at 10 a.m. on April 27 and at 12 a.m. on April 28, and of 0.45 gram of caseoses in 4.5 cubic centimeters of salt solution at 12:30 p.m. on April 29.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range of variation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td></td>
</tr>
<tr>
<td>Apr. 25</td>
<td>38.2</td>
<td>38.0 to 39.6</td>
<td>7 No injection.</td>
</tr>
<tr>
<td>26</td>
<td>38.9</td>
<td>38.6 to 39.5</td>
<td>7 Do.</td>
</tr>
<tr>
<td>27</td>
<td>38.7</td>
<td>38.6 to 39.8</td>
<td>2 Before injection.</td>
</tr>
<tr>
<td>27</td>
<td>38.9</td>
<td>38.4 to 39.4</td>
<td>7 Saline, 4.5 cc.</td>
</tr>
<tr>
<td>28</td>
<td>38.5</td>
<td>38.3 to 38.8</td>
<td>2 Before injection.</td>
</tr>
<tr>
<td>28</td>
<td>38.9</td>
<td>38.3 to 39.6</td>
<td>7 Saline, 4.5 cc.</td>
</tr>
<tr>
<td>29</td>
<td>38.6</td>
<td>38.0 to 39.2</td>
<td>4 Before injection.</td>
</tr>
<tr>
<td>29</td>
<td>38.9</td>
<td>38.0 to 40.0</td>
<td>7 Proteose, 0.45 gm.</td>
</tr>
<tr>
<td>30</td>
<td>38.4</td>
<td></td>
<td>1 No injection.</td>
</tr>
</tbody>
</table>

While in this experiment the caseoses produce a transient temperature rise to a figure slightly higher than any of the control observations, the pyrexial effects are not especially convincing. The control saline injections would possibly be called somewhat pyrogenic if the normal observations on the two days preceding were not in existence. As it is, the form of the curve of the variations on the two saline injection days is strikingly similar to the proteose day. The experiment demonstrates in a striking degree the danger of the misinterpretation of temperature observations in which the initial readings are taken as the normal for the guinea pig.

### Table VI.—Experiment 25. Edestinoses.

Guinea pig; male; weight, 440 grams. Injections of 4.5 cubic centimeters of physiological salt solution at 10 a.m. on April 27 and 28, and of 0.4 gram of edestinoses (in 4 cubic centimeters of salt solution at 12:30 p.m. on April 29.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
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<tr>
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<td>Average</td>
<td>Range of variation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>°C.</td>
<td>°C.</td>
<td></td>
</tr>
<tr>
<td>Apr. 25</td>
<td>38.4</td>
<td>39.0 to 40.0</td>
<td>7 No injection.</td>
</tr>
<tr>
<td>26</td>
<td>38.9</td>
<td>38.4 to 39.3</td>
<td>7 Do.</td>
</tr>
<tr>
<td>27</td>
<td>38.9</td>
<td>38.9</td>
<td>2 Before injection.</td>
</tr>
<tr>
<td>27</td>
<td>38.7</td>
<td>38.3 to 39.0</td>
<td>7 Saline, 4.5 cc.</td>
</tr>
<tr>
<td>28</td>
<td>38.1</td>
<td>37.9 to 38.4</td>
<td>2 Before injection.</td>
</tr>
<tr>
<td>28</td>
<td>38.6</td>
<td>38.1 to 38.9</td>
<td>7 Saline, 4.5 cc.</td>
</tr>
<tr>
<td>29</td>
<td>38.5</td>
<td>38.4 to 38.6</td>
<td>2 Before injection,</td>
</tr>
<tr>
<td>29</td>
<td>38.0</td>
<td>38.0 to 39.7</td>
<td>7 Proteoses, 0.45 grams.</td>
</tr>
<tr>
<td>30</td>
<td>38.4</td>
<td></td>
<td>1 No injection.</td>
</tr>
</tbody>
</table>

The control injection records in this experiment show less variation than do the series of readings on the two previous normal days. The sudden jump of the record to 39°.7 within two hours of the proteose injection is

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of interest. Higher figures, however, were obtained on the first normal control day, although not subsequently. Any pyrogenic effect, if manifested at all, in this experiment, must be interpreted as such from a consideration of the control saline injection figures alone.

**Table VII.**—*Experiment 26. Edestinoses (alcohol soluble).*

Guinea pig; male; weight, 650 grams. Injection of 4 cubic centimeters of physiological salt solution at 10 a. m. on April 27 and 28, and of 0.4 gram of edestinoses (in 4 cubic centimeters of saline) at 12:30 p. m. on April 29.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range of variation</td>
<td></td>
</tr>
<tr>
<td><strong>°C.</strong></td>
<td><strong>°C.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr. 25</td>
<td>39.3</td>
<td>38.9 to 39.6</td>
<td>7 No injection.</td>
</tr>
<tr>
<td>25</td>
<td>39.8</td>
<td>38.7 to 39.0</td>
<td>7 Do.</td>
</tr>
<tr>
<td>27</td>
<td>38.8</td>
<td>38.7 to 38.9</td>
<td>2 Before injection.</td>
</tr>
<tr>
<td>27</td>
<td>38.8</td>
<td>38.4 to 39.0</td>
<td>7 Saline, 4 cc.</td>
</tr>
<tr>
<td>28</td>
<td>38.2</td>
<td>38.2 to 38.2</td>
<td>2 Before injection.</td>
</tr>
<tr>
<td>29</td>
<td>38.6</td>
<td>38.2 to 39.2</td>
<td>7 Saline, 5 cc.</td>
</tr>
<tr>
<td>29</td>
<td>38.7</td>
<td>38.5 to 38.8</td>
<td>4 Before injection.</td>
</tr>
<tr>
<td>30</td>
<td>39.5</td>
<td>38.5 to 40.2</td>
<td>7 Proteoses, 0.5 gm.</td>
</tr>
</tbody>
</table>

The readings on the proteose day undoubtedly show a febrile reaction.

**Table VIII.**—*Experiment 31. Edestinoses.*

Guinea pig; female; weight, 690 grams; used previously in experiment 22. Injections of 5 cubic centimeters of physiological salt solution at 11 a. m. on May 4, and of 0.5 gram of proteoses in 5 cubic centimeters of salt solution at 11 a. m. on May 5.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range of variation</td>
<td></td>
</tr>
<tr>
<td><strong>°C.</strong></td>
<td><strong>°C.</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 2</td>
<td>38.3</td>
<td>38.1 to 38.8</td>
<td>7 No injection.</td>
</tr>
<tr>
<td>3</td>
<td>38.0</td>
<td>37.7 to 38.4</td>
<td>7 Do.</td>
</tr>
<tr>
<td>4</td>
<td>38.1</td>
<td>37.9 to 38.3</td>
<td>3 Before injection.</td>
</tr>
<tr>
<td>4</td>
<td>38.5</td>
<td>38.3 to 38.8</td>
<td>6 Saline, 5 cc.</td>
</tr>
<tr>
<td>5</td>
<td>38.6</td>
<td>37.8 to 38.2</td>
<td>3 Before injection.</td>
</tr>
<tr>
<td>5</td>
<td>*38.7</td>
<td>36.1 to 39.2</td>
<td>9 Proteoses, 0.5 gm.</td>
</tr>
<tr>
<td>6</td>
<td>38.5</td>
<td>38.5</td>
<td>1 No injection.</td>
</tr>
</tbody>
</table>

*Omitting the one subnormal reading.

A rise to 39°.2 following the characteristic fall after the edestinose injection suggests some slight influence exerted by the proteoses; this rise, however, is so little marked that any pyrogenic effect is very doubtful. It may be due to the extra handling of the animal, for a larger number of readings were taken on this day than during the control periods.
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TABLE IX.—Experiment 32. Alcohol-soluble edestinoses and mixed edestinoses.

Guinea pig: male; weight, 600 grams; used previously in experiment 24 on April 29. Injection of 5 cubic centimeters of physiological salt solution, 0.6 gram of alcohol-soluble edestinose, and 1 gram of mixed edestinoses at 11 a. m. on May 4, 5, and 6, respectively.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range of variation</td>
<td></td>
</tr>
<tr>
<td>°C.</td>
<td>°C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 2</td>
<td>38.2</td>
<td>37.7 to 38.8</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>38.2</td>
<td>37.8 to 38.4</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>38.6</td>
<td>38.3 to 38.8</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>38.3</td>
<td>38.0 to 38.7</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>38.2</td>
<td>38.3 to 38.6</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>38.6</td>
<td>38.2 to 39.2</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>38.0</td>
<td>37.8 to 38.3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>35.0</td>
<td>34.9 to 39.0</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>37.9</td>
<td>37.5 to 38.2</td>
<td>3</td>
</tr>
</tbody>
</table>

The temperature readings are much lower than in experiment 24. The subnormal temperature on May 6 is of interest. Pyrogenic effects are slight and transient if the effects can be interpreted as pyrexial.

TABLE X.—Experiment 33. Alcohol-soluble caseoses and edestinoses.

Guinea pig; female; weight, 500 grams. Injection of 5 cubic centimeters of physiological salt solution, 0.5 gram of alcohol-soluble caseoses, and 0.75 gram of edestinoses at 11 a. m. on May 4, 5, and 6, respectively.

<table>
<thead>
<tr>
<th>Date</th>
<th>Temperature</th>
<th>Observations</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range of variation</td>
<td></td>
</tr>
<tr>
<td>°C.</td>
<td>°C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr. 2</td>
<td>38.4</td>
<td>37.8 to 38.9</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>38.2</td>
<td>37.8 to 38.5</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>38.7</td>
<td>38.0 to 39.1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>38.6</td>
<td>38.2 to 38.8</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>38.4</td>
<td>38.4 to 38.8</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>39.0</td>
<td>36.9 to 39.2</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>39.0</td>
<td>38.9 to 39.0</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>39.0</td>
<td>38.7 to 39.2</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>38.3</td>
<td>38.1 to 38.6</td>
<td>3</td>
</tr>
</tbody>
</table>

* Omitting the subnormal reading.

A questionable febrile rise of a fraction of a degree is noted as the result of the injection of the alcohol-soluble caseoses on April 5.
TABLE XI.—Summary of experiments on guinea pigs.

<table>
<thead>
<tr>
<th>Proteoses</th>
<th>Fever; experiment No.</th>
<th>Questionable; experiment No.</th>
<th>No fever; experiment No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edestinoses</td>
<td>26</td>
<td>25, 32, 33</td>
<td>31</td>
</tr>
<tr>
<td>Edestinoses (alcohol soluble)</td>
<td>25</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>Caseoses</td>
<td>24</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td>Caseoses (alcohol soluble)</td>
<td></td>
<td>22, 23</td>
<td>10</td>
</tr>
<tr>
<td>Total (12)</td>
<td>2</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

Only 2, out of 12 experiments, have given a definite pyrogenic reaction. It would seem, then, that the above preparations are not consistently pyrexial for guinea pigs.

EXPERIMENTS ON RABBITS

In the following experiments, there is no evidence of fever as the result of the proteose injections. The protocols are given, therefore, without individual discussion.

TABLE XII.—Experiment 46. Control saline injection.

Rabbit; weight, 1,175 grams. Injected with 10 cubic centimeters of physiological salt solution at 10.15 a.m. on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>33.5</td>
<td>Feb. 26</td>
<td>12.00</td>
<td>39.1</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>40.1</td>
<td>26</td>
<td>27</td>
<td>39.9</td>
</tr>
<tr>
<td>26</td>
<td>9.00</td>
<td>39.5</td>
<td>27</td>
<td>12.30</td>
<td>39.5</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>39.9</td>
<td>26</td>
<td>27</td>
<td>39.9</td>
</tr>
<tr>
<td>27</td>
<td>9.15</td>
<td>39.4</td>
<td>27</td>
<td>9.00</td>
<td>39.4</td>
</tr>
<tr>
<td>27</td>
<td>10.15</td>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Injection.

TABLE XIII.—Experiment 47. Control.

Rabbit; weight, 1,290 grams. Rabbit was handled exactly like the others, but its skin was merely punctured with the syringe needle on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>40.0</td>
<td>Feb. 26</td>
<td>12.00</td>
<td>39.4</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>40.0</td>
<td>27</td>
<td>27</td>
<td>39.5</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.4</td>
<td>27</td>
<td>12.30</td>
<td>39.5</td>
</tr>
<tr>
<td>26</td>
<td>7.30</td>
<td>40.2</td>
<td>27</td>
<td>27</td>
<td>39.8</td>
</tr>
<tr>
<td>27</td>
<td>9.15</td>
<td>39.1</td>
<td>28</td>
<td>9.00</td>
<td>39.4</td>
</tr>
<tr>
<td>27</td>
<td>10.15</td>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Skin pricked.
Table XIV.—Experiment 48. Edestinoses.

Rabbit; weight, 1,100 grams. Injection of 0.6 gram of edestinose in 10 cubic centimeters of physiological salt solution at 10.15 a.m. on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature °C</th>
<th>Date</th>
<th>Time</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>39.5</td>
<td>Feb. 27</td>
<td>12.00</td>
<td>39.4</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>40.2</td>
<td>27</td>
<td>2.30</td>
<td>40.0</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.4</td>
<td>27</td>
<td>6.00</td>
<td>39.9</td>
</tr>
<tr>
<td>26</td>
<td>12.30</td>
<td>39.6</td>
<td>27</td>
<td>9.00</td>
<td>39.8</td>
</tr>
<tr>
<td>26</td>
<td>7.00</td>
<td>40.0</td>
<td>27</td>
<td>11.45</td>
<td>39.7</td>
</tr>
<tr>
<td>27</td>
<td>9.15</td>
<td>39.3</td>
<td>28</td>
<td>9.00</td>
<td>39.4</td>
</tr>
<tr>
<td>27</td>
<td>10.15</td>
<td>(*)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Injection.

Table XV.—Experiment 49. Control injection.

Rabbit; weight, 1,425 grams. Injection of 10 cubic centimeters of physiological salt solution at 10.15 a.m. on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature °C</th>
<th>Date</th>
<th>Time</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>39.5</td>
<td>Feb. 27</td>
<td>12.00</td>
<td>38.4</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>40.0</td>
<td>27</td>
<td>2.30</td>
<td>40.2</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.0</td>
<td>27</td>
<td>6.00</td>
<td>39.6</td>
</tr>
<tr>
<td>26</td>
<td>12.30</td>
<td>39.6</td>
<td>27</td>
<td>9.00</td>
<td>39.5</td>
</tr>
<tr>
<td>26</td>
<td>7.00</td>
<td>39.6</td>
<td>27</td>
<td>11.45</td>
<td>39.5</td>
</tr>
<tr>
<td>27</td>
<td>9.15</td>
<td>39.3</td>
<td>28</td>
<td>9.00</td>
<td>39.3</td>
</tr>
<tr>
<td>27</td>
<td>10.15</td>
<td>(*)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Injection.

Table XVI.—Experiment 50. Edestinoses.

Rabbit; weight, 1,450 grams. Injection of 1 gram of edestinose at 10.15 p.m. on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature °C</th>
<th>Date</th>
<th>Time</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>40.0</td>
<td>Feb. 27</td>
<td>10.15</td>
<td>(+)</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>39.9</td>
<td>27</td>
<td>12.00</td>
<td>38.7</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.5</td>
<td>27</td>
<td>2.30</td>
<td>40.2</td>
</tr>
<tr>
<td>26</td>
<td>12.30</td>
<td>39.5</td>
<td>27</td>
<td>6.00</td>
<td>38.8</td>
</tr>
<tr>
<td>26</td>
<td>7.00</td>
<td>40.0</td>
<td>27</td>
<td>11.45</td>
<td>40.2</td>
</tr>
<tr>
<td>27</td>
<td>9.15</td>
<td>39.4</td>
<td>28</td>
<td>9.00</td>
<td>39.4</td>
</tr>
</tbody>
</table>

* Injection.
### Table XVII. — Experiment 51. Edestinoses (alcohol soluble).

Rabbit; weight, 1,175 grams. Injection of 1 gram of estedinoses at 10.15 a.m. on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time, a.m. p.m.</th>
<th>Temperature, °C.</th>
<th>Date</th>
<th>Time, a.m. p.m.</th>
<th>Temperature, °C.</th>
</tr>
</thead>
<tbody>
<tr>
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<td>40.2</td>
<td>Feb. 27</td>
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<td>38.0</td>
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<td>7.00</td>
<td>40.0</td>
<td>27</td>
<td>2.30</td>
<td>38.3</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.5</td>
<td>27</td>
<td>6.00</td>
<td>37.9</td>
</tr>
<tr>
<td>26</td>
<td>12.30</td>
<td>39.7</td>
<td>27</td>
<td>9.00</td>
<td>37.9</td>
</tr>
<tr>
<td>27</td>
<td>7.00</td>
<td>39.3</td>
<td>27</td>
<td>11.45</td>
<td>37.6</td>
</tr>
<tr>
<td>27</td>
<td>10.15</td>
<td>(s)</td>
<td>28</td>
<td>9.00</td>
<td>37.7</td>
</tr>
</tbody>
</table>

* Injection.

### Table XVIII. — Experiment 52. Edestinoses.

Rabbit; weight, 1,100 grams. Received 0.66 gram of estedinoses at 10.15 a.m. on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time, a.m. p.m.</th>
<th>Temperature, °C.</th>
<th>Date</th>
<th>Time, a.m. p.m.</th>
<th>Temperature, °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>40.0</td>
<td>Feb. 27</td>
<td>12.00</td>
<td>39.3</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>40.0</td>
<td>27</td>
<td>2.30</td>
<td>39.5</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.5</td>
<td>27</td>
<td>6.00</td>
<td>39.8</td>
</tr>
<tr>
<td>26</td>
<td>12.30</td>
<td>39.4</td>
<td>27</td>
<td>9.00</td>
<td>39.8</td>
</tr>
<tr>
<td>27</td>
<td>7.00</td>
<td>39.1</td>
<td>27</td>
<td>11.45</td>
<td>39.4</td>
</tr>
<tr>
<td>27</td>
<td>9.15</td>
<td>39.0</td>
<td>28</td>
<td>9.00</td>
<td>39.2</td>
</tr>
</tbody>
</table>

* Injection.

### Table XIX. — Experiment 53. Caseoses.

Rabbit; weight, 1,150 grams. Injection of 0.5 gram of caseoses at 10.15 a.m. on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time, a.m. p.m.</th>
<th>Temperature, °C.</th>
<th>Date</th>
<th>Time, a.m. p.m.</th>
<th>Temperature, °C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>39.6</td>
<td>Feb. 27</td>
<td>12.00</td>
<td>38.9</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>39.7</td>
<td>27</td>
<td>2.30</td>
<td>38.9</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.6</td>
<td>27</td>
<td>6.00</td>
<td>39.9</td>
</tr>
<tr>
<td>26</td>
<td>12.30</td>
<td>39.4</td>
<td>27</td>
<td>9.00</td>
<td>40.0</td>
</tr>
<tr>
<td>26</td>
<td>7.00</td>
<td>39.9</td>
<td>27</td>
<td>11.45</td>
<td>39.8</td>
</tr>
<tr>
<td>27</td>
<td>9.15</td>
<td>39.4</td>
<td>28</td>
<td>9.00</td>
<td>39.3</td>
</tr>
</tbody>
</table>

* Injection.
<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>40.2</td>
<td>Feb. 27</td>
<td>12.00</td>
<td>40.2</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>40.3</td>
<td>27</td>
<td>2.30</td>
<td>40.0</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.6</td>
<td>27</td>
<td>6.00</td>
<td>40.1</td>
</tr>
<tr>
<td>26</td>
<td>12.30</td>
<td>39.6</td>
<td>27</td>
<td>9.00</td>
<td>40.1</td>
</tr>
<tr>
<td>27</td>
<td>7.00</td>
<td>40.2</td>
<td>28</td>
<td>11.45</td>
<td>40.0</td>
</tr>
<tr>
<td>27</td>
<td>3.15</td>
<td>39.3</td>
<td></td>
<td>9.00</td>
<td>39.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Injection.

TABLE XXI.—Experiment 55. Fibrinoses.

Rabbit; weight, 1,400 grams. Received an injection of 0.95 gram of fibrinose preparation at 10.15 a.m. on February 27. The animal gradually collapsed, dying about noon.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>39.4</td>
<td>Feb. 26</td>
<td>7.00</td>
<td>39.5</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>39.8</td>
<td>27</td>
<td>9.15</td>
<td>39.2</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.4</td>
<td>27</td>
<td>10.15</td>
<td>(o)</td>
</tr>
<tr>
<td>26</td>
<td>12.30</td>
<td>39.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Injection.

TABLE XXII.—Experiment 56. Control saline injection.

Rabbit; weight, 1,600 grams. Injected with 10 cubic centimeters of physiological salt solution at 10.15 a.m. on February 27.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
<th>Date</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 25</td>
<td>3.00</td>
<td>40.0</td>
<td>Feb. 27</td>
<td>12.00</td>
<td>39.8</td>
</tr>
<tr>
<td>25</td>
<td>7.00</td>
<td>40.2</td>
<td>27</td>
<td>2.30</td>
<td>39.7</td>
</tr>
<tr>
<td>26</td>
<td>9.15</td>
<td>39.5</td>
<td>27</td>
<td>6.00</td>
<td>39.6</td>
</tr>
<tr>
<td>26</td>
<td>12.00</td>
<td>39.4</td>
<td>27</td>
<td>8.45</td>
<td>39.5</td>
</tr>
<tr>
<td>26</td>
<td>7.00</td>
<td>40.1</td>
<td>27</td>
<td>11.45</td>
<td>39.7</td>
</tr>
<tr>
<td>27</td>
<td>9.15</td>
<td>39.8</td>
<td>28</td>
<td>9.15</td>
<td>39.2</td>
</tr>
<tr>
<td>27</td>
<td>10.15</td>
<td>(o)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Injection.
TABLE XXIII.—Summary of experiments 46 to 56 inclusive.

<table>
<thead>
<tr>
<th>Proteose preparation</th>
<th>Experiments</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control physiological saline</td>
<td>3</td>
<td>No fever.</td>
</tr>
<tr>
<td>Control—no injection</td>
<td>1</td>
<td>Do.</td>
</tr>
<tr>
<td>Edestinoses</td>
<td>3</td>
<td>Do.</td>
</tr>
<tr>
<td>Edestinoses (alcohol soluble)</td>
<td>1</td>
<td>No fever. Temperature dropped below normal, and remained low.</td>
</tr>
<tr>
<td>Caseoses</td>
<td>1</td>
<td>No fever.</td>
</tr>
<tr>
<td>Caseoses (alcohol soluble)</td>
<td>1</td>
<td>Do.</td>
</tr>
<tr>
<td>Fibrinoses</td>
<td>1</td>
<td>Animal died as result of injection.</td>
</tr>
</tbody>
</table>

In the experiments with rabbits, the slight rise in temperature with some of the proteoses is insignificant and is amply accounted for by the control days and the saline-injected rabbits. In the earlier rabbit experiments, conducted at the Sheffield Laboratory, the results were not so uniformly nonpyrogenic. I believe that facility in handling the rabbits, gained from experience in the previous work, has been a considerable factor in promoting the uniformity of the experimental results in this last series.

From the evidence presented, then, it would seem that the primary cleavage products of pepsin-hydrochloric acid digestion, when prepared without drastic treatment, from purified and well-characterized proteins, never have more than a slight pyrogenic effect when injected subcutaneously into rabbits and guinea pigs. Any temperature rise, if present, is insufficiently pronounced to permit a direct inciting rôle to be ascribed to such proteoses in the production of the severe naturally occurring fevers.
ALBINISM IN THE PHILIPPINE ISLANDS

By Victor G. Heiser and Rafael Villafranca

(From the Bureau of Health, Manila, P. I.)

One plate

So far as known, the first investigation of albinism in the Philippine Islands is that which was undertaken by the Bureau of Health. Later Villafranca, while district health officer for Bohol Province, undertook the study of this condition among Filipinos. These investigations are embodied in a statistical report of 198 new cases.

It is evident that all people who appear to be partial albinos are not necessarily albinos, although there are many incomplete and imperfect cases among individuals who have red, yellow, yellowish, or auburn hair. The majority of such cases are probably modified albinism. But as all of them are included in most of the estimates, it is evident why South America, the Philippines, and other places where dark complexions predominate are said to furnish more cases of albinism than other regions.

In the early report mention was made of some of the peculiar views entertained by Filipinos as to the cause of albinism. Scientific men are just as far apart in their views as to the cause of this condition. Some state that the albinos once constituted a separate race and that the cases that are characteristic are atavistic in character. Blumenbach, Winterbottom, Sprengel, and Otto considered albinism to be a disease or the result of disease. Buffon explained its existence on the theory of ship-wreck or the abandonment of Europeans, whose offspring resulting from union with the local inhabitants retained some of the original characteristics of their white ancestors. Later this
theory was renounced by Buffon.\textsuperscript{1} Lecat\textsuperscript{1} attributes it to the influence of heat; Mansfield\textsuperscript{4} and others to maternal impressions. The latter theory is very common in the Philippines, as is also the theory that it is due to the morbid imagination of the mother during the period of pregnancy.

The consensus of opinion now seems to be that the condition is due to faulty development of the pigment-producing apparatus.

According to Geoffroy Saint-Hilaire,\textsuperscript{1} three varieties of albinism exist: the complete form, in which the pigmentary matter is entirely lacking; the partial form, in which the pigment exists in some places and is absent in others; and the incomplete form, in which pigment exists in all parts, but in quantity below the normal.

In nearly all cases albinism is congenital. However, partial albinism may be accidental; but no case has been recorded of complete albinism being other than congenital.

Doctor Montinola, district health officer for the Province of Occidental Negros, who has given the subject considerable study, believes that the disease is of neuropathic origin and that in all cases the condition can be referred to defects in the nervous constitution of the ancestors.

Doctor Hurley, district health officer for Iloilo Province, collected 10 cases in his district, in which he believed that consanguinity was an important factor. In some cases the relationship was so close as to include the parents in the same family.

In complexion albinos are white, yellowish, red, or reddish. Their eyes are blue, and in complete albinos the globe of the eye is entirely deprived of pigment. In Bohol, albinos are usually shortsighted and fear the light, and in many cases there is some bodily deformity. If the eyelids of these people are opened, rapid oscillations of the eyeball take place. Such cases exist elsewhere, but more particularly in this island.

There is a tradition among these people to the effect that many centuries ago a white race lived in the mountains, and that the present albinos are the atavistic descendants of some members of this race who intermarried with the dark-skinned natives.

It is a common belief that albinos are mentally subnormal. This is true only in so far as degeneration has influenced their physical condition. In albinos with healthy bodies no intellectual inferiority has been noticed, and cases of weak mentality which have been observed could be accounted for by some organic defect.

\textsuperscript{1} Ciencias Médicas, etc., see footnote 3.
Livingstone, states that in certain parts of Africa albinos were destroyed because they were looked upon as the omens of evil. In other countries they are worshiped as favored beings. Neither of these beliefs is entertained in the Philippines.

Doctor Llora, district health officer for Leyte Province, records a superstition that albinism is caused by an evil spirit called cahoy-non, who resides in the field and exerts his evil influence on persons who incur his displeasure. This belief is confined to the lower class. Albinos are neither hated nor admired; they are simply looked upon as having incurred the displeasure of this spirit for which there is no remedy.

Defects of vision are not constant. Eighteen cases of photophobia, 13 cases of nystagmus, and 3 cases of myopia are recorded in the report submitted. These are the cases that are usually called moon-eyed. Properly adjusted glasses will remedy the cases of myopia as it will in other people.

According to many observers and travelers, albinism is to be found among all the races and in every zone of the earth. It is known by different names in different countries; for instance, albinia, acromia congénita, and leucopatia. In the Philippines, also, albinism has different names according to the dialects of the provinces in which it is found. In the Visayan dialect of Leyte Province it is called ila, a word which signifies “marked;” others call it pamusag, which signifies “whitened” or “painted white.” In Misamis it is called vulgarly kabang, when it is incomplete, and linakaran sa buan when it is complete. In Albay Province it is called akos sin adlao, which signifies “son of the sun;” in Zambales, anac avaló ó labang; in Ilocos Sur, ampurao.

Albinism is found in the lower animals and even in vegetables. Villafranca has seen carabaos, pigs, and rats that were complete albinos. Doctor Garcia, district health officer for Zambales, describes, in his report on albinism, an American albino sow which gave birth to 9 pigs, 5 almost complete and 4 partial albinos. In the almost complete albino pigs the skin was transparent and of a pinkish white color, the hair was black, and the eyes blue. On the other hand, the partial albino pigs suffered from lack of pigment in certain parts of the skin and none was found in the hair of the body.

The first report on this subject by the Bureau of Health is as follows:

5 Ciencias Médicas, etc., see footnote 3.
ALBINISM IN THE PHILIPPINE ISLANDS. 5

At the instance of Dr. H. Fraser of the Institute for Medical Research, Kuala Lumpur [Kuala Lumpur], Federated Malay States, Dr. C. H. Usher, of Aberdeen, Scotland, and Prof. Frederick Starr, of Chicago University, this office issued on April 28, 1908, the following circular, addressed to the medical inspectors and district health officers of this Bureau:

In view of the general interest in the question of albinism, information is respectfully requested as to whether albinos have come under your observation, and if so, you are respectfully requested to furnish this office without delay answers to the following questions:

1. The pedigrees of families in which one or more cases of albinism have occurred. The more extensive such pedigrees are the better.

2. All information is desired bearing on whether albinism is or is not the expression of a prevalence of scanty pigmentation in a particular stock. Hence particulars are desired as to color of hair and eyes, fecundity, general physical and mental vigor, and the occurrence in albinotic families of any other defects than albinism.

3. The influence of cousin marriages is of great importance to be carefully followed up.

4. Incomplete family records and particulars of single cases of albinism will also be useful and welcome.

5. Photographs of albinos will be valued, especially albinos of dark races.

6. Incomplete or partial albinism; instances of pied albinism are desired.

The investigators venture to ask whether you will kindly aid the research by sending particulars of any cases. Whilst the information itself will be treated as confidential, full acknowledgment of its source will be made when the subject comes to publication.

Incomplete notes often contain useful information and will be welcome when full records can not be obtained.

* * * * * * *

With the [above] circular there was sent a leaflet prepared by Dr. C. H. Usher, containing information as to the prevalence of albinism and a form for making reports, as follows:

ALBINISM.

Albinism occurs among all races, even the darkest. It appears to be frequent among Malayan peoples. I desire to secure specific information regarding all possible cases. The following will help to render observation definite. When impossible to make a full report, give what you can. The first three items are indispensable.

Report on case of albinism.

Name of subject.
Residence.
Race or tribe.
Hair; color; quality; secure a sample if possible.
Skin; color; quality; blushing? sunburn?
Eyes; color; movement; squinting? myopie?
Carefully draw the iris and color to show pigment distribution, etc.

Disposition and character. Ability in different directions; deficiency in
different directions.

Occurrence. Is the case sporadic? If not, give all possible information
regarding similar occurrences in the family. Are the parents related?
Name all the children in the family in order, marking the cases.

What is the native word for an albino? What is its literal meaning?
What, if any, popular ideas regarding albinos? What do “the people
say” about them?

Secure photograph of the subject; where possible, two views—one square
front, the other exact profile. [End leaflet.]

As a result of these circulars, forty-five cases of albinos were reported
from seven provinces; Albay, 2; Bohol, 11; Ambos Camarines, 5; Ilocos
Sur, 5; Manila, 1; Pampanga, 16; Tarlac, 5.

It is not claimed that the figures presented are correct or approximately
correct. It is not reasonable to suppose that on the island Province
of Bohol, with a population of 269,223, there are 11 albinos; while in the
near-by island Province of Cebu with a population of 653,729 there is not
a single albino, though it is probably true that albinism is more prevalent
in Bohol than in other provinces, as there is more “folk-lore” concerning
the condition. The Bohol term for albino is “bulao” from the Visayan
word “bulauan” which means gold. Albinos with blond hair and dark skin
are called “bugao” (yellow) and those who are entirely white are known
as “uguis” (decolorized). In this province there is a tradition of a white
people known as Taguibanua (cave dwellers) who once lived in the mountain
caves of the island, and the popular belief is that albinos are the result
of the mingling of these cave dwellers with the natives.

By some of the inhabitants it is believed that a few of the Taguibanua
still exist, and, that whenever one is seen by a pregnant woman, an albino
child is the result. This latter theory is accepted in the Province of Albay
where there also exists a tradition of an ancient white race.

Another theory that prevails in both of these provinces, and more or less
in all other provinces, is that albinism is due to some peculiar phase of
the moon at the moment of conception.

In the provinces around Manila an albino is known as “anak arao,”
“child of the sun,” from the belief that the mothers of albino children
during pregnancy develop a “fancy” for gazing at the sun. This theory
is also prevalent to some extent in all parts of the Philippines.

The accompanying table of “Albinism in the Philippine Islands” is pre-
sented as evidence of good faith and as a token that this office will continue
the investigation of this interesting subject until it can publish a reliable
table of albinism in the Philippine Islands.
ILLUSTRATIONS

PLATE I

Fig. 1. Male complete albino of Dimiao, Bohol.
2. Female complete albino of Manila, Luzon.
Fig. 1. Male complete albino of Dimiao, Bohol.

Fig. 2. Female complete albino of Manila, Luzon.

PLATE I.
<table>
<thead>
<tr>
<th>No.</th>
<th>Mentality.</th>
<th>Other members of the family albinos.</th>
<th>Other members of the family not albinos.</th>
<th>Remarks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>V.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Lor</td>
<td>His mother's mother, great grandparents, 3 brothers, a sister, and other relatives.</td>
<td>3 brothers</td>
<td>Daylight disagreeable, sight best after dark.</td>
</tr>
<tr>
<td>4</td>
<td>D. do</td>
<td>Grandmother, great grandparents, 3 brothers, a sister, and other relatives.</td>
<td>do</td>
<td>Not shortsighted, feminine voice, eyes normal, marked double strabismus.</td>
</tr>
<tr>
<td>5</td>
<td>Peerage</td>
<td>Father, great grandparents, and 2 children.</td>
<td>2 daughters</td>
<td>Eyes normal, strabismus, not shortsighted.</td>
</tr>
<tr>
<td>6</td>
<td>Sot</td>
<td>1 sister and a grandfather.</td>
<td></td>
<td>Eyes normal.</td>
</tr>
<tr>
<td>7</td>
<td>Jar</td>
<td>All grandparents, 1 sister, and 1 daughter.</td>
<td>7 children</td>
<td>Do.</td>
</tr>
<tr>
<td>8</td>
<td>Pur</td>
<td></td>
<td></td>
<td>Do.</td>
</tr>
<tr>
<td>9</td>
<td>J. do</td>
<td>Father, father's parents, great grandparents, and 7 brothers.</td>
<td>9 brothers</td>
<td>Eyes normal.</td>
</tr>
<tr>
<td>10</td>
<td>Jo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Pe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>A.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>M.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Fr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Leelligent</td>
<td>No history of albinism in family.</td>
<td>All relations</td>
<td>No relationship between parents.</td>
</tr>
<tr>
<td>16</td>
<td>Vido</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Nil and apathetic.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Nw.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Gido</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
</tbody>
</table>

The Serrano family, residence Maradodon, Cabugao, Ilocos Sur, when all were living consisted of mother, father, 2 boys, and 3 girls, who were not albinos, and 1 boy and 2 girls who were albinos. Of the 2 boys who are not albinos, both are living, while the 3 nonalbino girls are dead. Of the 3 albino children, the boy is dead. Of the albino children, 33\(\frac{1}{3}\) per cent are dead, while of the nonalbinos 60 per cent are dead, from which it may be assumed that albinism did not affect their physical condition. The 2 albino sisters are marked about equally; hair is of extremely light straw color, almost white. No history of albinism on either side. Parents not related. Mother is said to have been very fond of white flowers during pregnancy. Both albinos rendered uncomfortable by light.
### Table 1: Albinoism among Filipinos in the Philippine Islands

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>Condition</th>
<th>Occupation</th>
<th>Degree of Relation</th>
<th>Residence</th>
<th>Skin</th>
<th>Eyes</th>
<th>Character and Constitution</th>
<th>Marriage</th>
<th>Other members of the family</th>
<th>Other members of the family</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pimentel Sario</td>
<td>Male</td>
<td>40</td>
<td></td>
<td></td>
<td>Unclear</td>
<td></td>
<td>White</td>
<td>Dark yellow</td>
<td>Fine, Fine, Fine</td>
<td></td>
<td>Father, Mother, Grandmother</td>
<td>Dead, Dead, Dead, Dead</td>
<td>Barefoot</td>
</tr>
<tr>
<td>2</td>
<td>Zafra Balala</td>
<td>Female</td>
<td>20</td>
<td></td>
<td></td>
<td>Unclear</td>
<td></td>
<td>White</td>
<td>White</td>
<td>Fine, Fine, Fine</td>
<td></td>
<td>Father, Mother, Grandfather</td>
<td>Dead, Dead, Dead, Dead</td>
<td>Barefoot</td>
</tr>
<tr>
<td>3</td>
<td>Luis Maltes</td>
<td>Male</td>
<td>30</td>
<td></td>
<td></td>
<td>Unclear</td>
<td></td>
<td>White</td>
<td>Yellow</td>
<td>Green, Green, Green</td>
<td></td>
<td>Father, Mother, Grandmother</td>
<td>Dead, Dead, Dead, Dead</td>
<td>Barefoot</td>
</tr>
<tr>
<td>4</td>
<td>Baldocar Maltes</td>
<td>Male</td>
<td>30</td>
<td></td>
<td></td>
<td>Unclear</td>
<td></td>
<td>White</td>
<td>Black</td>
<td>Brown, Brown, Brown</td>
<td></td>
<td>Father, Mother, Grandfather</td>
<td>Dead, Dead, Dead, Dead</td>
<td>Barefoot</td>
</tr>
<tr>
<td>5</td>
<td>Francisco Narisit</td>
<td>Male</td>
<td>30</td>
<td></td>
<td></td>
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*From the Annual Report of the Director of Health for the fiscal year ending June 30, 1910. This table has been slightly changed for the sake of uniformity. (Editor).*
<table>
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<th>No.</th>
<th>Mentality.</th>
<th>Other members of the family albinos.</th>
<th>Other member of the family not albinos.</th>
<th>Remarks.</th>
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</thead>
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<td>1 brother and 1 sister</td>
<td>Parents and 1 brother.</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>2 brothers</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Brous</td>
<td></td>
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<td>Father and grandfather</td>
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<td>of mother, mother's sister, also 4</td>
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<tr>
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<td></td>
<td>brothers.</td>
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<tr>
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<td>7 children</td>
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<td>3 sisters and 1 aunt.</td>
<td>7 brothers</td>
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</tr>
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<td>1 brother, 2 sisters, and 1 aunt.</td>
<td>5 brothers</td>
<td></td>
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</tr>
<tr>
<td>31</td>
<td></td>
<td>1 sister, 1 brother, and 1 aunt.</td>
<td></td>
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<td>32</td>
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<td>2 brothers and 1 aunt</td>
<td>2 children</td>
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<td>42</td>
<td>½</td>
<td>than average</td>
<td>Yrs</td>
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<td>45</td>
<td>½</td>
<td>1 brother</td>
<td>1 daughter</td>
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</table>

The Cabasug family of the barrio of Pila, Cabazuao, Ilocos Sur, consists of father, mother, and 1 son not albinos, and 3 sons who are albinos, all living. These albino children resemble European children. Parents are fourth cousins. Mother twice married and had albino child (now dead) by first husband. Father has unpigmented birthmark, 1.5 square cm. in extent, just below left outer malleolus. Mother has jet black hair with exception of brown strands in left temporal region. These albinos seem uncomfortable in the light. Parents dark and not related. From Iloilo. Observed by Doctor Catanjal in 1894. Observed by Doctor Catanjal in 1902. Has photophobia, "nictalopia," and nystagmus. White semicircle surrounding upper part of both cornes. Toenails blackish.

Chinese mestizos.

Children of Maura Toñgio; 1 albino sister dead; great grandmother of father albino. Mother is cousin of Maura Toñgio, not albino; father not an albino. One of grandparents English mestizo.

Orbits continually moving laterally. Born very small with unperforated anus; photophobia. Great grandfather English mestizo. Brothers both myops. Had an albino brother who is dead. No consanguinity between ancestors.

Have been albinos for 3 generations, originally came from Badoc, Ilocos Norte. Parents were cousins.
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<thead>
<tr>
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<th>Sex</th>
<th>Age</th>
<th>Condition</th>
<th>Occupation</th>
<th>Degree of</th>
<th>Residence</th>
<th>Skin</th>
<th>Hair</th>
<th>Eyes</th>
<th>Character and conclusion</th>
<th>Monotony</th>
<th>Other relations of the family members</th>
<th>Other number of the family members</th>
<th>Remarks</th>
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<td>Grayish brown</td>
<td>Dark</td>
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<td>do</td>
<td>do</td>
<td>do</td>
<td>do</td>
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<td>Light brown</td>
<td>Grayish brown</td>
<td>Dark</td>
<td>1 brother and 1 sister</td>
<td>Parents</td>
<td>Father and mother</td>
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<td>1 brother</td>
<td>1 sister</td>
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</tbody>
</table>

The Colburn family of the barrio of Fka. Cabasug, Bataugan, Batau, consists of father, mother, and 1 son who are albinos, and 1 son who are not albinos. These children resemble Caucasians children.

Parents are fourth cousins. Mother having marital and had albino child (now dead) by first husband. Father has semicircular fundus, 1.5 square cm, in extent. Not before left same condition. Mother has jet black hair with exception of brown streaks in left temporal region. These children seem unhealthful in the light.

Parents dead and not related. From Both. Observed by Doctor Catanjala in 1902.

Has phobophobia, 'nictalopia,' and dyschromasia.

White semicircles surrounding inner part of both eyes.

Terra firma.

Chinese marriage.

Children of Mateo Tabanos, 1 albino brother.

Mother is cousin of Mateo Tabanos, not albino.

One of grandparents English mestizo.

Orbits continually moving laterally.

Born very small with unperforated anus; phothophobia. Great specificity English mestizo.

Father's brother was albino. Had an albino brother who is dead. No consanguinity between ancestors.

Have been albinos for 3 generations, originally from Balay, Herrera. Parental consanguinities.

1 son and 1 daughter.
<table>
<thead>
<tr>
<th>No.</th>
<th>Mentality.</th>
<th>Other members of the family albinos.</th>
<th>Other members of the family not albinos.</th>
<th>Remarks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>eeble</td>
<td>Maternal grandfather's son.</td>
<td>6 brothers</td>
<td>Maria Fortón is a sister of Magdalena Fortón.</td>
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<tr>
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<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>do</td>
<td>None</td>
<td>do</td>
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</tr>
<tr>
<td>4</td>
<td>do</td>
<td>Sister of maternal uncle.</td>
<td>3 brothers</td>
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<tr>
<td>5</td>
<td>formal</td>
<td>None</td>
<td>2 brothers</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>do</td>
<td>do</td>
<td>9 brothers</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>do</td>
<td>do</td>
<td>1 daughter</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td>She shades her eyes when exposed to strong sunlight, and lids and lashes moved rapidly (photophobia and nystagmus).</td>
</tr>
<tr>
<td>10</td>
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<td>do</td>
<td>do</td>
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<td>do</td>
<td>do</td>
<td>do</td>
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<tr>
<td>12</td>
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She constitutes, refers also to having seen various cases of complete albinos in some villages of Cebu Province: Sr. Mendoza, third member of the provincial board of Bohol, also states that he has seen entire families of albinos. He, Constabulary, refers also to having seen various cases of complete albinos in some villages of Cebu Province; Sr. Mendoza, third member of the provincial board of Bohol, also states that he has seen entire families of albinos.

Feet and hands too feeble to walk or to support anything; can scarcely see in the daytime, but can see at night.
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**TABLE II.**—Albinism among Filipinos in the Philippine Islands—Continued.
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Note: Table A lists the findings of the Philippine Islands. Continued.
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<td>Male</td>
<td>25</td>
<td>Single</td>
</tr>
<tr>
<td>156</td>
<td>Maria Bayop</td>
<td>Female</td>
<td>25</td>
<td>Single</td>
</tr>
<tr>
<td>157</td>
<td>Laconia Juvencio</td>
<td>Male</td>
<td>25</td>
<td>Single</td>
</tr>
<tr>
<td>158</td>
<td>Bibiana Vada</td>
<td>Female</td>
<td>25</td>
<td>Single</td>
</tr>
<tr>
<td>159</td>
<td>Juan Bautista</td>
<td>Male</td>
<td>25</td>
<td>Single</td>
</tr>
<tr>
<td>160</td>
<td>Maria Bayop</td>
<td>Female</td>
<td>25</td>
<td>Single</td>
</tr>
<tr>
<td>161</td>
<td>Laconia Juvencio</td>
<td>Male</td>
<td>25</td>
<td>Single</td>
</tr>
<tr>
<td>162</td>
<td>Bibiana Vada</td>
<td>Female</td>
<td>25</td>
<td>Single</td>
</tr>
<tr>
<td>163</td>
<td>Juan Bautista</td>
<td>Male</td>
<td>25</td>
<td>Single</td>
</tr>
</tbody>
</table>

**Table II.—Albinism among Filipinos in the Philippine Islands—Continued.**
<table>
<thead>
<tr>
<th>No.</th>
<th>Mentality</th>
<th>Other members of the family albinos</th>
<th>Other members of the family not albinos</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>164</td>
<td>Good</td>
<td>1 brother</td>
<td>2 brothers</td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>do</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>166</td>
<td>do</td>
<td>1 brother</td>
<td>2 brothers</td>
<td></td>
</tr>
<tr>
<td>167</td>
<td>Feeble</td>
<td>1 brother</td>
<td>2 brothers</td>
<td></td>
</tr>
<tr>
<td>168</td>
<td>do</td>
<td>1 brother</td>
<td>2 brothers</td>
<td></td>
</tr>
<tr>
<td>169</td>
<td>do</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170</td>
<td>do</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>171</td>
<td>Normal</td>
<td>None</td>
<td>2 brothers</td>
<td></td>
</tr>
<tr>
<td>172</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>173</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>174</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>175</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>176</td>
<td>Feeble</td>
<td>Grandfather and sister</td>
<td>3 brothers</td>
<td></td>
</tr>
<tr>
<td>177</td>
<td>Good</td>
<td>2 brothers</td>
<td>1 brother</td>
<td></td>
</tr>
<tr>
<td>178</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>179</td>
<td>do</td>
<td>1 sister</td>
<td>1 sister</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>181</td>
<td>do</td>
<td>2 sisters</td>
<td>2 sisters</td>
<td></td>
</tr>
<tr>
<td>182</td>
<td>Precocious</td>
<td>1 cousin, mother’s side</td>
<td>2 brothers</td>
<td></td>
</tr>
<tr>
<td>183</td>
<td>According to age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>184</td>
<td>Normal</td>
<td>1 sister</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>185</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td></td>
</tr>
<tr>
<td>186</td>
<td>do</td>
<td>3 sisters</td>
<td>3 children</td>
<td></td>
</tr>
<tr>
<td>187</td>
<td>do</td>
<td>do</td>
<td>Children</td>
<td></td>
</tr>
<tr>
<td>188</td>
<td>do</td>
<td>do</td>
<td>1 sister</td>
<td></td>
</tr>
<tr>
<td>189</td>
<td>do</td>
<td>5 brothers and relatives</td>
<td>7 brothers</td>
<td></td>
</tr>
<tr>
<td>190</td>
<td>do</td>
<td>5 brothers (dead)</td>
<td>2 children</td>
<td></td>
</tr>
<tr>
<td>191</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>192</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>193</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>194</td>
<td></td>
<td>Maternal grandfather</td>
<td></td>
<td></td>
</tr>
<tr>
<td>195</td>
<td></td>
<td>Grandfather and 3 brothers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>196</td>
<td></td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>197</td>
<td>Normal</td>
<td>1 paternal aunt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>198</td>
<td>Better than other girls</td>
<td>Father and 1 brother</td>
<td>4 sisters</td>
<td></td>
</tr>
</tbody>
</table>

Remarks:
- Father, died aged 52, mother 60, not albinos.
- Foot wide, hands short and thick, ears long and wide.
- Somewhat myopic and appears photophobic.
- Photophobia. The father of Joaquin Villanueva is nephew of the father of Victorino de León. The father of Victorino de León is second cousin of his mother. Albinism of these congenital.
- Seems to have been much whiter than at present.
Table II.—Albinism among Filipinos in the Philippine Islands—Continued.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Sex</th>
<th>Age</th>
<th>Race</th>
<th>Condition</th>
<th>Occupation</th>
<th>Degree of civilization</th>
<th>Residence</th>
<th>Side</th>
<th>Eye</th>
<th>Color</th>
<th>Refraction</th>
<th>Iris</th>
<th>Character and constitution</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>Maria Naval</td>
<td>Female</td>
<td>31</td>
<td>Married</td>
<td>House servant</td>
<td>Totoo, Cebu</td>
<td>White, narrow</td>
<td>White</td>
<td>Pink</td>
<td>Pink</td>
<td>Pink</td>
<td>White</td>
<td>Happy and stout</td>
<td>Good</td>
<td>1 brother</td>
</tr>
<tr>
<td>155</td>
<td>Valentina Sevilla</td>
<td>Male</td>
<td>20</td>
<td>Single</td>
<td>House servant</td>
<td>Totoo, Cebu</td>
<td>White, narrow</td>
<td>White</td>
<td>Pink</td>
<td>Pink</td>
<td>Pink</td>
<td>White</td>
<td>Happy and stout</td>
<td>Good</td>
<td>1 brother</td>
</tr>
<tr>
<td>156</td>
<td>Carlos Naval</td>
<td>Male</td>
<td>49</td>
<td>Single</td>
<td>Weaver</td>
<td>Parit</td>
<td>White, narrow</td>
<td>White</td>
<td>Pink</td>
<td>Pink</td>
<td>Pink</td>
<td>White</td>
<td>Happy and stout</td>
<td>Good</td>
<td>1 brother</td>
</tr>
<tr>
<td>157</td>
<td>Severino Pascua</td>
<td>Male</td>
<td>32</td>
<td>Married</td>
<td>Weaver</td>
<td>Cagayan, Ilocos Sur</td>
<td>White, narrow</td>
<td>White</td>
<td>Pink</td>
<td>Pink</td>
<td>Pink</td>
<td>White</td>
<td>Happy and stout</td>
<td>Good</td>
<td>1 brother</td>
</tr>
<tr>
<td>158</td>
<td>Filomena Pascua</td>
<td>Female</td>
<td>35</td>
<td>Married</td>
<td>Weaver</td>
<td>Cagayan, Ilocos Sur</td>
<td>White, narrow</td>
<td>White</td>
<td>Pink</td>
<td>Pink</td>
<td>Pink</td>
<td>White</td>
<td>Happy and stout</td>
<td>Good</td>
<td>1 brother</td>
</tr>
<tr>
<td>159</td>
<td>Catalina Pascua</td>
<td>Male</td>
<td>32</td>
<td>Married</td>
<td>Weaver</td>
<td>Cagayan, Ilocos Sur</td>
<td>White, narrow</td>
<td>White</td>
<td>Pink</td>
<td>Pink</td>
<td>Pink</td>
<td>White</td>
<td>Happy and stout</td>
<td>Good</td>
<td>1 brother</td>
</tr>
<tr>
<td>160</td>
<td>Santiago Pascua</td>
<td>Male</td>
<td>35</td>
<td>Married</td>
<td>Weaver</td>
<td>Cagayan, Ilocos Sur</td>
<td>White, narrow</td>
<td>White</td>
<td>Pink</td>
<td>Pink</td>
<td>Pink</td>
<td>White</td>
<td>Happy and stout</td>
<td>Good</td>
<td>1 brother</td>
</tr>
<tr>
<td>161</td>
<td>Gregorio Toda</td>
<td>Male</td>
<td>50</td>
<td>Married</td>
<td>Weaver</td>
<td>Sierra Nevada</td>
<td>White, narrow</td>
<td>White</td>
<td>Pink</td>
<td>Pink</td>
<td>Pink</td>
<td>White</td>
<td>Happy and stout</td>
<td>Good</td>
<td>1 brother</td>
</tr>
</tbody>
</table>

Note: This table continues with information on various individuals, including their names, sex, age, race, condition, occupation, degree of civilization, residence, side, eye color, refraction, iris color, character and constitution, and remarks. The table provides additional details such as the relationship to family members, eye conditions, and other notes.
THE LIFE HISTORY OF CESOPHAGOSTOMUM APIOSTOMUM:  
I. DEVELOPMENT OUTSIDE OF THE HOST

By Ernest Linwood Walker

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

Four plates

In 1905 Brumpt reported a case of oesophagostomiasis in a Negro from the Omo River near Lake Randolph in East Africa. The worm in this case was described by Railliet and Henry (1905) under the name Cesophagostomum brumpti.

Thomas, in 1910, found a fatal case in a native of Manaos, Brazil, and described the pathological anatomy and histology of the case very completely. The worm in this case was likewise referred to Railliet and Henry for identification. These authors described the parasite under the name Cesophagostomum stephanostomum var. thomasi Railliet and Henry, 1910.

Leiper, of the London School of Tropical Medicine, in 1911, found among the hookworms collected by Doctor Foy, of the West African Medical Staff, 6 oesophagostoma, which had been passed in the stools of a native at Ibi, North Nigeria. Leiper identified the worms in this case as Cesophagostomum apiostomum Willach, 1891, a species common in the intestine of apes. Furthermore, this author is of the opinion that O. brumpti Railliet and Henry, 1905, is identical with O. apiostomum Willach.

These are the only cases of the infection of man with this worm that have so far been reported, but Weinberg (1908) believes that the few cases in man so far reported in Africa are due to the fact that necropsies on Negroes are rare in the African colonies and that attention has been directed especially to parasites of the blood.

Beside man and apes, cattle, sheep, goats, pig, and Dasypus are subject to oesophagostomiasis, but in these latter animals the infection is due to other species of Cesophagostomum.

Cesophagostomiasis is characterized by hæmorrhagic cysts or tumors in the submucosa or muscularis of the large intestine—rarely of the small intestine—which project usually both inside and outside of the gut, and which contain the immature adult oesophagostomum. At maturity the cyst ruptures and the adult
worm escapes into the lumen of the intestine. The ruptured cysts are liable to become invaded by intestinal bacteria, which may give rise to ulcerations, perforation of the intestine, and peritonitis.

Of the 3 human cases described, in Foy's (Leiper) case the parasites were passed in the stools during treatment for hookworm, and there is no record of clinical symptoms or pathological conditions; in Brumpt's case, in which the infection was discovered post mortem in a patient dead from other causes, only 6 oesophagostomum nodules were found; in Thomas's case there was an intense infection, and death of the patient was from septic peritonitis due to lesions caused by these worms.

Our knowledge of the life history of *Oesophagostomum* is extremely meager. Weinberg (1908) and Thomas (1910) infer from their observations that the larva of the worm reaches the wall of the intestine by the way of the blood where it ruptures a small blood vessel and becomes encysted in the submucosa, in the internal or external muscularis, or in the subperitoneal space. Here the larva grows, molts, and becomes an immature adult oesophagostomum. The cyst is then ruptured, and the worm escapes into the lumen of the intestine. Weinberg found free immature adults in the intestinal contents of a few of his cases of infection in monkeys. However, no one has described a mature adult, and the ova of the worm have never been found in the lumen of the intestine. Moreover, no author has observed or even speculated on how the larvae get into the blood or what is the early larval life of the worm. Are the eggs laid in the intestine of the host or after the worm has been passed in the faeces? If laid in the intestine, do the eggs develop there or outside of the host? If development takes place outside of the host, is it only *intra ovum* or is there a free larval stage? And if a free larval stage exists is it in the open or is an alternative host required?

Among the monkeys used for experimental purposes in the biological laboratory of this Bureau, infection with oesophagostomum occurs in a large percentage of the animals. Therefore, unusual opportunity has been afforded for investigating the life history of this worm. These monkeys are collected from different parts of the Philippine Islands, and include at least two

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1 Weinberg records in one case the presence of an adult female worm and spherical eggs measuring 52 microns in a cyst from the caecum of a chimpanzee; but in view of my own observations it is doubtful whether the worm ever becomes mature in the cyst.
species, names undetermined. The oesophagostomum found infecting these monkeys has been compared with the descriptions, and appears to be *Oesophagostomum apiostomum* Willach, the species common in apes and, according to Leiper, found in man. Since the infections are usually not heavy and the immature adults escape into the lumen of the intestine only at intervals, mature females and free ova are difficult to find. Careful search will, however, reveal a few ova from time to time in the faeces or in the contents of the large intestine at necropsy.

The ovum at the time of its passage in the fresh faeces is oval, gray, thin shelled, and in an advanced stage of segmentation. Earlier stages of segmentation can be found in eggs from the intestine, especially the cæcum. In general appearance the ovum resembles closely that of *Ankylostoma*. The ovum of *Oesophagostomum* is, however, larger than that of *Ankylostoma, Necator, or Strongyloides*, all of which it somewhat resembles. The ovum of *Oesophagostomum* measures from 0.044 to 0.057 millimeter in breadth by 0.073 to 0.084 millimeter in length. Its general characters and its resemblance to the ovum of the hookworm are well illustrated in Plate I, fig. 1.

That the ovum described from the faeces is that of oesophagostomum was proved by comparison with the ova found in a mature female worm. If pressure be exerted on such a worm under the cover glass, the whole viscera is evacuated through the oral orifice, the uteri filled with ova remaining intact. Comparison and measurements of such nearly mature ova *intra uterum* show that they are identical with the ova found in the faeces and described as those of oesophagostomum. Moreover, none of the monkeys contained intestinal worms, the ova of which could be mistaken for those of oesophagostomum.

Development of the ova of oesophagostomum takes place readily in cultures made in the same manner as for the development of the ova of the hookworm. Powdered charcoal is added to the faeces and, if necessary, a little sterile water, and the whole is thoroughly mixed and spread in a thin layer in a sterile Petri dish. The development takes place less readily, but to some extent, in the undiluted faeces.

The ova hatch in such cultures in from twelve to twenty-four hours, nearly all that will develop doing so by the end of the first day. The ovum gives rise to a rhabditiform larva measuring about 0.34 millimeter in length by 0.016 millimeter in breadth. This larva (Plate I, fig. 2) possesses certain peculiarities which plainly distinguish it from the rhabditiform larvae of the hook-
worm or of *Strongyloides*, the latter of which are frequently numerous in the same culture of monkey's faeces. The newly hatched *Oesophagostomum* larva is characterized: first, by an extremely long filiform tail and, secondly, by the zigzag course of the intestine which is plainly visible in the living worm. These two characteristics are well represented in Plate I, fig. 3, and Plate II, fig. 2.

The small rhabditiform larva grows rapidly, and under favorable conditions of culture and temperature attains its maturity in from three to four days. In the process of growth it molts twice. At the last molt the old skin is not shed, but remains as a sheath inclosing the larva. Within the sheath the larva contracts somewhat in breadth and more in length, so that it is separated from the old larval skin by a considerable space. The larva no longer possesses the long filiform tail, which was present up to the last molt, as is seen from the inclosing larval skin (Plate III, fig. 1). The character of the *Oesophagus* has also changed during the last molt from the rhabditiform to the strongyliform (compare Plate II, fig. 1, with Plate IV, fig. 1).

This larva inclosed in the skin of the last molt remains alive and active, but undergoes no further development in the culture. It is the mature larva ready to infect a new host. It differs from the mature larva of the hookworms and *strongyloides* in size and shape, and especially in the long filiform tail of the old larval skin inclosing the worm. The mature larva is about 0.9 millimeter long and 0.03 millimeter thick.

The larval development of *Oesophagostomum apiostomum*, therefore, is strikingly similar and wholly comparable with the development of *Ankylostoma duodenale*, which is perhaps to be expected from the somewhat near relationship of the two worms. From this similarity in the development of the larva, one would expect by analogy that the method of entrance of the larva into the body of the host would also be similar. It has been demonstrated by Looss (1911), and substantiated by other investigators, that infection with *ankylostoma* larvae may take place not only by ingestion, but also by passage through the moistened skin. This is supposed to take place especially in case of persons going about barefooted. The larvae enter the follicles and attain the blood vessels, by which they are carried to the lungs. Here they leave the blood and enter the air vesicles and then travel by the way of the bronchi, trachea, *oesophagus*, and stomach to the intestine. The method by which the larva of *oesophagostomum* enters the body of its host and attains its position in the tissues of the large intestine is now being experimentally investigated.
LITERATURE CITED


Weinberg, M. *Esophagostomose des anthropoides et des singes inférieurs.* *Arch. parasit.* (1908–10), 13, 161.
ILLUSTRATIONS

Plate I, fig. 3, and Plate IV, figs. 1 and 2, are reproductions of camera-lucida drawings by Teodosio S. Espinosa, the remaining figures are reproductions of photomicrographs by Charles Martin. Plate I, fig. 2, and Plate II, fig. 2, are from stained preparations; the others from unstained preparations killed in 70 per cent alcohol and mounted in glycerine.

PLATE I

Fig. 1. Ovum of Oesophagostomum apiostomum. × 350.
2. Young rhabditiform larva. × 84.
3. Young rhabditiform larva at higher magnification, showing the filiform tail, intestinal tract, and anlage of reproductive organs. × 390.

PLATE II

Fig. 1. Cephalic end of young rhabditiform larva, showing shape of oesophagus. × 350.
2. Body of young rhabditiform larva, showing undulating course of intestinal tract. × 350.
3. Young rhabditiform larva, showing posterior end of intestinal tract and anus. × 350.

PLATE III

Fig. 1. Mature larva inclosed in skin of last molt. × 84.
2. Cephalic end of mature larva inclosed in skin of last molt. × 350.
3. Posterior part of body of mature larva inclosed in skin of last molt × 350.

PLATE IV

Fig. 1. Cephalic end of mature larva, showing strongyliform oesophagus. × 390.
2. Caudal end of mature larva. Note the long filiform tail of old larval skin. × 390.
PLATE I. OVUM AND YOUNG Rhabditiform Larva of GESOPHAGOSTOMUM APIOSTOMUM WILLACH.
Fig. 1. Cephalic end of young rhabditiform larva, showing shape of oesophagus. × 350.

Fig. 2. Body of a young rhabditiform larva, showing undulating course of intestinal tract. × 350.

Fig. 3. Young rhabditiform larva, showing posterior end of intestinal tract and anus. × 350.

PLATE II. YOUNG RHABDITIFORM LARVA OF OESOPHAGOSTOMUM APIOSTOMUM WILLACH.
PLATE III. MATURE LARVA OF CESOPHAGOSTOMUM APIOSTOMUM WILLACH.

Fig. 1. Mature larva inclosed in skin of last molt. × 84.

Fig. 2. Cephalic end of mature larva inclosed in skin of last molt. × 350.

Fig. 3. Posterior part of body of mature larva inclosed in skin of last molt. × 350.
Walker: *Œsophagostomum apiostomum: L.*

[Phil. Journ Sci., VIII, B, No. 6.]

**Fig. 1.** Cephalic end of mature larva, showing strongyliform oesophagus. \( \times 390. \)

**Fig. 2.** Caudal end of mature larva. Note the long filiform tail of old larval skin. \( \times 390. \)

*Plate IV. Œsophagostomum apiostomum Willach.*
DURATION OF THE INFECTIVENESS OF VIRULENT RINDERPEST BLOOD IN THE WATER LEECH, HIRUDO BOYNTONI WHARTON

By William Hutchins Boynton

(From the Veterinary Division, Bureau of Agriculture, Manila, P. I.)

This investigation was suggested by the fact that in the campaign against rinderpest in the Philippines particular difficulty is experienced in ridding low swampy districts of the disease. On numerous occasions such localities have been apparently free from rinderpest, but in four or five weeks the disease has reappeared. In most instances the movement of animals was apparently controlled, and it did not seem possible that the disease was introduced from other districts.

Consideration of these apparently spontaneous outbreaks with reference to the localities in which they appeared to be most frequent led me to examine leeches, to determine if they could maintain the virus of rinderpest alive for any length of time. Leeches subsist on blood, and consume large quantities at one feeding. Cattle sick with rinderpest are apt to seek cool places and water holes during the febrile stage of the disease, while carabaos do so normally. This gives the leeches ample opportunity to feed upon them. Persons engaged in field work have repeatedly observed leeches attached to carabaos immediately after they emerged from carabao wallows. These facts indicate that leeches may be a factor in the rinderpest problem.

On examining the literature on leeches, I have found that Bass and Johns cite the statement of Sakharov, Rosenbach, Blumer, Hamburger, and Mitchel that they kept malaria plasmodia alive for several days in leeches that had been allowed to draw the blood of malaria patients.

Laveran and Mesnil state:

Various trypanosomes which were found by Brumpt in fresh-water fishes, can be divided into several groups according to their mode of evolution in the bodies of leeches (Hemiclipsis).

1 To be published as Bulletin No. 29, Bureau of Agriculture of the Government of the Philippine Islands.

2 Archibald R. Ward, Chief.

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Elsewhere they state, in discussing a trypanosome disease of horses in Annam, that Vassal (3) found that—

The blood of leeches which had fed on infected animals was infective, on injection into rats, immediately after the meal of blood, but not four hours later. The trypanosomes are killed off very readily in the stomach of the leech.

Daniels and Alcock state (4):

Many parasites maintain their virulence for a considerable period in the stomach of leeches, but leeches are not known to act as carriers of disease.

Nencki, Sieber, and Wijnikewitch (5) allowed leeches to feed upon animals infected with rinderpest. Later they examined the blood in these leeches for the presence of the organism regarded by them as the causative agent of rinderpest, but without success.

The leech employed in these experiments is a new species, *Hirudo boyntoni.*

The leeches used in the first of the following experiments were procured from La Carlota, Occidental Negros, a locality which for several years has not been known to be infected with rinderpest. It was thought best to select leeches from such an uninfected region when beginning the study.

On July 13, 1912, ten leeches were allowed to feed on bull 3397, in the second day of febrile temperature of an attack of rinderpest. As soon as the leeches had become engorged, they were placed in water and kept in a cool place. The virulence of the blood in these leeches was tested upon cattle after various intervals, as described in experiments 1 and 2.

Experiment 1.—At 10.00 a. m., July 14, twenty-four hours after the leeches had fed on the sick animal, 2 leeches were placed in 50 cubic centimeters of physiological salt solution, which caused them to disgorge the blood. The mixture of disgorged blood and salt solution was injected subcutaneously into bull 3396, which was placed in a screened stall. This animal did not contract the disease, but at a later date proved to be susceptible.

Experiment 2.—On July 15, two of the leeches which had fed on July 13, were placed in 50 cubic centimeters of physiological salt solution which caused them to disgorge the blood which they had held for forty-eight hours. This mixture of blood and salt solution was injected subcutaneously into bull 3390. The

animal showed a rise in temperature on the morning of July 19, diarrhoea with inappetence on July 22, and died during the forenoon of July 24. The symptoms and lesions gave conclusive evidence that it had contracted rinderpest and died of that disease.

On July 16, 1912, at 11.00 a.m., several leeches were allowed to feed on bull 3402 in the second day of temperature of an attack of rinderpest. As soon as the leeches had become engorged, they were placed in water and kept in a cool place. The infectiveness of the blood contained in these leeches was determined at various intervals by testing upon cattle as shown in experiments 3 to 6.

Experiment 3.—On July 17, 1912, at 11.00 a.m., 2 leeches which had fed on July 16 were placed in 100 cubic centimeters of physiological salt solution which caused them to disgorge the blood which they had held for twenty-four hours. This mixture of blood and salt solution was injected subcutaneously into bull 3405. The animal had a rise in temperature on July 22, developed diarrhoea and inappetence on July 26, and died on July 27. From the symptoms and lesions it was concluded that the animal had contracted rinderpest and died of that disease.

Experiment 4.—On July 21, 1912, several of the leeches which had fed on July 16 died and disgorge blood into the water in which they were being kept. The mixture of water and blood was given as a drench to bull 3404. This animal showed a febrile temperature on July 31, which was ten days after receiving the drench. Diarrhoea with inappetence appeared on August 6 and continued until August 10, after which the animal gradually recovered. The case presented all the symptoms of a severe attack of rinderpest. At a later date the animal received virulent blood and was proved to be immune, thus showing that it had passed through the disease.

Experiment 5.—On July 21, 1912, the dead leeches which were mentioned in experiment 4, and which had fed on an infected animal on July 16, were thoroughly disintegrated in a mortar containing physiological salt solution, and the fluid was injected subcutaneously into bull 3400. This animal showed a high temperature on the evening of July 27, diarrhoea with inappetence on July 31, and died during the forenoon of August 7. From the symptoms and lesions it was concluded that this animal had contracted rinderpest and had died of that disease.

Experiment 6.—On August 2, 1912, two leeches which had fed on July 16, seventeen days previously, were placed in physiolog-
ical salt solution which caused them to disgorge blood. This mixture of blood and salt solution was injected subcutaneously into bull 3478. The animal showed a rise in temperature on August 5, but on August 9 was found positive for surra, which at the time was thought to be the possible cause of the rise in temperature. However, the animal developed diarrhoea and ate but little on August 11, showed inappetence on August 12, and died on August 14. From the symptoms and autopsy findings it was evident that this animal had an attack of rinderpest as well as of surra.

On July 29, 1912, sixteen leeches were allowed to feed on bull 3400, in the second day of febrile temperature of an attack of rinderpest, and were placed in water in a cool place. These were employed at different periods, shown in experiments 7 to 10, to test upon cattle the infectiveness of the blood that they contained.

Experiment 7.—On August 4, six days after having fed, 2 leeches were placed in 50 cubic centimeters of physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected into bull 3477. This animal displayed the first rise in temperature on the evening of August 7, was found to be infected with surra on August 9, and died on August 13. This animal developed nervous symptoms which are characteristic of a somewhat rare type of rinderpest. It also showed a subnormal temperature of 36°.2 C., which is frequently present in rinderpest just prior to death. Autopsy revealed slight lesions of that disease. It was evident that this animal had died from the combined effects of rinderpest and surra.

Experiment 8.—On August 10, 1912, twelve days after feeding, 2 leeches which had fed on July 29 were placed in 50 cubic centimeters of physiological salt solution, which caused them to disgorge. The blood and salt solution was injected into bull 3492. The animal showed a rise of temperature on August 14, developed diarrhoea with partial inappetence on August 18, and died on August 19. From the various symptoms and from the autopsy findings, it was concluded that this animal had contracted rinderpest and died of that disease.

Experiment 9.—On August 16, 1912, eight small leeches, twelve days after having fed on July 29, were placed in physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3491. This animal showed no ill effects from the injection, but at a later date was proved to be susceptible to rinderpest.
Experiment 10.—On August 16, 1912, the water in which the leeches of experiment 9 had been kept since July 29—a period of twelve days—was given as a drench to bull 3488. This animal suffered no ill effects from the drench. It was later infected with rinderpest and died, showing that it had been susceptible to the disease at the time it had received the drench.

On August 17, 1912, twenty leeches were allowed to feed upon bull 3492 during the third day of temperature of an attack of rinderpest, and were placed in water in three different containers and kept in a cool place. The infectiveness of the blood contained in these leeches was tested on cattle at various periods in experiments 11 to 14.

Experiment 11.—On August 24, 1912, two leeches which had fed on August 17, seven days previously, were placed in 50 cubic centimeters of physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected into animal 3494. This animal showed a rise in temperature on September 3, ten days after receiving the injection. Diarrhoea developed on September 4, and inappetence on September 5. The animal showed a subnormal temperature of 36°.2 C. on the afternoon of September 6, and died that evening. Autopsy showed typical lesions of rinderpest. It was concluded that this animal had contracted a fatal attack of rinderpest.

Experiment 12.—On August 24, 1912, the water in which 8 leeches had been kept since August 17, an interval of seven days, was given by drench to bull 3495. The animal suffered no ill effects from the material. This animal was used in a subsequent experiment in which it was shown to be susceptible.

Experiment 13.—On August 27, 1912, three leeches which had fed on August 17, ten days previously, were placed in 75 cubic centimeters of physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected into bull 3488. This animal suffered no ill effects from the injection, and at a later date was proved to be susceptible to rinderpest.

Experiment 14.—On August 27, 1912, the water in which several leeches had been kept since August 17—a period of ten days—was given by drench to bull 3491. This animal suffered no ill effects from the material, but at a later date was proved to be susceptible to rinderpest.

On November 8, 1912, five leeches were allowed to feed on cow 3516, in the second day of febrile temperature of an attack of rinderpest, and were placed in water in a cool place. The
ineffectiveness of the rinderpest virus in the blood contained in these leeches was tested upon cattle in experiments 15 to 17.

Experiment 15.—On November 18, 1912, one leech, which had fed on November 8, ten days previously, was placed in 50 cubic centimeters of physiological salt solution which caused it to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3514. This animal showed an initial rise of temperature on November 23, developed diarrhoea on November 24, and inappetence on November 26. It displayed a subnormal temperature of 36°.7 C. on the morning of November 29, and died during the day. Autopsy showed typical lesions of rinderpest. It was thus proved positively that this animal had suffered a fatal attack of rinderpest.

Experiment 16.—On November 20, 1912, one leech, which had fed on November 8, twelve days previously on an infected animal, was placed in 50 cubic centimeters of physiological salt solution, which caused it to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3518. This animal showed the initial rise of temperature on November 26, which continued for several days, gradually subsiding to normal. The animal showed no diarrhoea nor inappetence. Blood was drawn from it and injected into a susceptible bull which developed a severe case of rinderpest and died. This proved that bull 3518 had been infected with a mild type of rinderpest, but had been able to transfer a severe type of the disease to another animal.

Experiment 17.—On November 23, 1912, one leech, which had fed on November 8, fifteen days previously, was placed in 50 cubic centimeters of physiological salt solution, which caused it to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3524. This animal showed an initial rise of temperature on November 28, developed diarrhoea with partial inappetence on December 2, and died during the daytime of December 6. The autopsy revealed marked lesions of rinderpest. Therefore, this animal had a fatal attack of rinderpest.

On November 30, 1912, four leeches were allowed to feed on cow 3524, in the second day of febrile temperature of an attack of rinderpest, and were placed in water in a cool place. The infectiveness of the blood retained in these leeches was tested upon cattle at various intervals as shown in experiments 18 and 19.

Experiment 18.—On December 10, 1912, one leech which had fed November 30, ten days previously, was placed on some green
feed which had been sprinkled with a small amount of sodium chloride. A few minutes after the leech came in contact with the fodder it disgorged a considerable amount of blood. This fodder was fed to bull 3535, and was eaten readily. This feeding had apparently no ill effect upon the animal. At a later date the same animal was proved to be susceptible to rinderpest.

Experiment 19.—On December 18, 1912, two leeches, which had fed on November 30, eighteen days previously, were placed in 100 cubic centimeters of physiological salt solution, which caused them to disgorge. The mixture of blood and salt solution was injected subcutaneously into bull 3538. This animal showed the initial rise in temperature on December 23, developed diarrhoea and inappetence on December 27, which symptoms continued until January 4, 1913, when the animal died. Autopsy revealed typical lesions of rinderpest. From these observations it was proved that this animal had contracted a fatal attack of rinderpest.

During the early part of January, 1913, I visited the Province of Ambos Camarines to investigate a disease affecting cattle and carabaos. A few animals located in Magarao, a barrio of Nueva Caceres, exhibited symptoms and blood changes which are characteristic of rinderpest, although in a mild form. A large number of leeches were collected in this locality.

On January 15, 1913, four leeches which had been collected in the vicinity were allowed to feed on a young carabao in Magarao which presented some symptoms of rinderpest. It had a rather high temperature, it refused food, the ears drooped, and a somewhat characteristic diarrhoea was present. When the leeches had engorged themselves, they were placed in a bottle partly filled with water, and were brought to the veterinary research laboratory at Alabang, where the blood that they contained was tested upon cattle at various intervals, as shown in experiments 20 and 21.

Experiment 20.—On January 18, 1913, one of the leeches which had fed on the sick carabao in Magarao three days previously was placed in 50 cubic centimeters of physiological salt solution to cause it to disgorge the blood which it contained. This mixture was injected into bull 3543. The animal presented a rise of temperature on January 24, displayed inappetence January 29, diarrhoea on January 30, and showed a bloody diarrhoea on January 31. This animal experienced a rather severe attack of the disease, but recovered.

From the results it was evident that the sick carabao in Magarao had been infected with rinderpest. This decided a matter
concerning which there had been considerable doubt, owing to
the absence of well-marked cases.

Experiment 21.—On February 24, 1913, two leeches which
had fed on the sick carabao at Magarao, Ambos Camarines, on
January 15, forty days previously, were disintegrated in a mortar
and the blood was injected into bull 3548. The animal appar-
etly suffered no ill effect from the injection but was proved
susceptible to rinderpest at a later date.

On January 22, 1913, twelve leeches were allowed to feed on
cow 3535, on the second day of febrile temperature of an attack
of rinderpest, and then placed in water in a cool place. The
blood contained in these leeches was tested upon cattle in ex-
periments 22 to 27.

Experiment 22.—On February 11, 1913, two leeches which
had fed on January 22, twenty days previously, were placed in
100 cubic centimeters of physiological salt solution, which caused
them to disgorge blood. This mixture of blood and salt solution
was injected into bull 3564. The animal suffered apparently no
ill effects from the injection. At a later date it was proved to
be susceptible to rinderpest.

Experiment 23.—On February 16, 1913, two leeches which
had fed on January 22, twenty-five days previously, were placed in
75 cubic centimeters of physiological salt solution, which caused
them to disgorge. This mixture was injected subcutaneously into bull 3566. The animal showed an initial rise of
temperature on February 22, developed inappetence on February
27, and diarrhoea on March 1. The inappetence continued to
March 4, when the animal again began to eat, but the diarrhoea
continued until March 7. This animal gradually recovered.
From the symptoms it was concluded that this animal experienced
an attack of rinderpest.

Experiment 24.—On February 22, 1913, one leech, which had
fed on January 22, thirty-one days previously, was caused to
disgorge by means of salt solution. The mixture of blood and
salt solution was injected into bull 3549. This injection had
apparently no ill effect upon the animal. Its susceptibility to
rinderpest was proved later.

Experiment 25.—On February 26, 1913, five leeches, which
had fed on cow 3535 on January 22, thirty-five days previously,
were allowed to feed on bull 3568. This animal suffered no ill
effects from the feeding. It was proved susceptible to rinderpest
at a later date.

Experiment 26.—On March 3, 1913, four leeches which had
fed on infected blood on January 22, forty days previously, were disintegrated in a mortar in salt solution. The mixture of blood and salt solution was injected subcutaneously into bull 3547. One of the leeches was dead, 1 was inactive, and 2 were in normal condition at the time they were put in the mortar. The animal apparently suffered no ill effects from the injection. It was proved by a subsequent inoculation to be susceptible to rinderpest.

Experiment 27.—On February 6, 1913, two liters of water were given by drench to bull 3547. Twelve leeches that had fed on infected blood fifteen days previously had been kept in this water. This animal suffered no ill effects from the drench. It was proved susceptible to rinderpest by a subsequent inoculation of virulent blood.

Experiment 28.—On February 8, 1913, three leeches were allowed to feed for fifteen minutes on cow 3546, during the third day of temperature of an attack of rinderpest, and were transferred to water for one hour. They were then placed on susceptible animal 3548, and allowed to become engorged. Cow 3548 suffered no ill effects from the biting of these leeches. She was proved susceptible to rinderpest by a subsequent inoculation of virulent blood.

Experiment 29.—On February 8, 1913, three leeches were allowed to feed for fifteen minutes on cow 3546, on the third day of temperature of an attack of rinderpest, and then transferred, without being placed in water, to susceptible cow 3549. The interval between the two feedings was twenty minutes. Animal 3549 suffered no ill effects from this feeding. It was proved susceptible to rinderpest at a later date by an inoculation of virulent blood.

On March 4, 1913, eleven leeches were allowed to feed for thirty minutes on cow 3564, on the fourth day of febrile temperature of an attack of rinderpest, after which they were transferred to water in a cool place. The infectiveness of the blood in these leeches was determined by testing upon cattle at various periods subsequently, as shown in experiment 30.

Experiment 30.—On March 10, 1913, the 11 leeches which had fed on cow 3564, on March 4, six days previously, were allowed to feed on cow 3570 until they become engorged. This animal apparently suffered no ill effects from the feeding. She was later proved to be susceptible to rinderpest.

Since the animals used in experiments 6 and 7 had been found to be infected with surra, it was thought best to test leeches for their ability to keep the trypanosome of surra alive in ingested
blood. In the following experiments guinea pigs were used since they are susceptible to surra.

On April 21, 1913, four leeches were fed for fifteen minutes on a guinea pig whose blood was heavily infected with the trypanosomes of surra, after which the leeches were placed in water and kept in a cool place. The infectivity of these leeches with regard to surra was tested in experiments 31 and 32.

Experiment 31.—On April 23, 1913, two days after the leeches had fed, 2 of them were allowed to feed on 2 healthy guinea pigs. These animals were kept under observation for one month, during which time they were not found to be infected.

Experiment 32.—On April 23, 1913, two days after the leeches had fed, 1 leech was placed in a small amount of physiological salt solution and thoroughly disintegrated in a mortar. The ingested blood was examined microscopically for trypanosomes, but none were found. The remaining blood was injected into a guinea pig, which was kept under observation for one month, but did not develop the disease.

Experiment 33.—On April 27, 1913, two leeches were allowed to feed for seven minutes on a guinea pig heavily infected with surra, after which time they were removed, kept out of water and placed on a healthy guinea pig. They commenced feeding on the healthy guinea pig in seven and in seven and one-half minutes, respectively, after having been taken from the infected animal, and were allowed to feed upon the healthy animal for ten minutes. This guinea pig was kept under observation one month, but remained negative for surra.

Experiment 34.—On April 27, 1913, two leeches were allowed to feed for ten minutes on a guinea pig heavily infected with surra, after which they were placed on a healthy guinea pig. They began feeding on the healthy animal in one hour and two minutes and one hour and five minutes, respectively, after being removed from the infected animal. This guinea pig was kept under observation for one month, but remained negative for surra.

During the early part of May, 1913, I visited the Province of Ilocos Sur to study some mild cases of rinderpest. On May 3, 1913, one leech was allowed to feed on a carabao which had shown no symptoms of rinderpest except ulcers in the mouth. The temperature of this animal had not been observed. It has been my experience to find no ulcers forming in the mouth in the virulent type of the disease until three or four days, and sometimes longer, after the initial rise in temperature. In a mild type of rinderpest, undoubtedly, a longer period than this would
elapse before ulcers would appear. Therefore, it was probable that this animal had nearly recovered and might not have been capable of spreading the disease.

Experiment 35.—On May 8, 1913, the leech, which on May 3 had fed on the carabao showing ulcers in its mouth, was caused to disgorge in physiological salt solution. This mixture of blood and salt solution was injected subcutaneously into bull 3573. The animal showed no ill effects from the injection. It was later proved susceptible to rinderpest.

On May 4, 1913, in Ilocos Sur, one leech was allowed to feed on a carabao which was recovering from an attack of rinderpest. The leech was placed in water and brought to the laboratory. This animal had presented some symptoms of rinderpest; as, for instance, a discharge from its eyes and nose and a mild diarrhoea. At the time the leech was allowed to feed, these symptoms had practically disappeared.

Experiment 36.—On May 8, 1913, the leech, which had been allowed to feed on the recovering animal on May 4, was caused to disgorge by placing it in salt solution. This mixture of blood and salt solution was injected subcutaneously into bull 3574. The animal showed no ill effects from his injection. It was proved to be susceptible to rinderpest at a later date.

CONCLUSIONS

1. From the results obtained in experiment 22 and others it is proved that the large water leech (Hirudo boyontoni Wharton) can retain the virus of rinderpest alive in its body for at least twenty-five days and in a virulent condition.

2. From experiment 4 it is shown that water in which leeches have disgorge blood by mechanical stimulation or other means, after holding it for a period of five days, will cause rinderpest when drunk by a susceptible animal.

3. The result obtained in experiment 5 proves that leeches, which have died from mechanical or other cause, after holding virulent blood for five days, are able to transmit the disease when the blood is ingested by a susceptible animal.

4. From experiments 4 and 11 it is shown that an animal may have an incubation period of ten days after being infected with material which has been held by a leech.

5. Experiment 16 shows that an animal may develop a very mild type of the disease when infected from blood that has been held in a leech for several days. Under such conditions an animal experiencing a mild attack may transmit a virulent and fatal
type of rinderpest. This suggests one of the possible causes for mild rinderpest frequently encountered in the field.

6. It was observed that the large leech held the virus alive considerably longer than small leeches.

7. It was noted that when leeches had been out of water for any length of time they disgorged the blood. Carabaos coming from wallows have frequently been observed to be covered with leeches. Carabaos are in the habit of eating grass around the edges of wallows, and there is a possibility of leeches getting out of the water and disgorging blood on the grass. If this were eaten by a susceptible animal within twenty-four hours, there would be a possibility of the animal contracting rinderpest, if the leech previously had fed, within the limits of time the virus is known to remain alive in the ingested blood, on an animal sick with rinderpest.

8. It has been observed that the virus will continue alive much longer in a leech if it be allowed to feed on an animal during the early stages of the disease. The immune bodies formed in the blood serum may have considerable effect on the virus in the later stages of the disease. Since the temperature of an animal suffering from rinderpest is usually highest during the first three or four days after the initial rise, it is possible that animals would seek cool places and water holes during the early stages of the disease rather than during the later period. This condition would give the leech ample opportunity to feed upon animals in the early stage of the disease.

9. Experiments 10, 12, 14, and 27 show that the water in which leeches have been kept is not infective to a susceptible animal, provided the leeches have not been injured in any way that would cause them to disgorge the blood which they contained.

10. The results of experiments 25, 28, 29, and 30 show that leeches cannot transmit the disease to a susceptible animal by feeding on it, after they have fed upon an animal suffering from rinderpest.

11. The results from experiments 31, 32, 33, and 34 indicate that the trypanosome of surra does not remain alive for any length of time in the ingested blood of a leech, and that a leech cannot transmit the disease by biting.

12. From the results obtained in the foregoing experiments, it appears that a leech may be responsible for the appearance of recognizable rinderpest forty days after imbibing virulent blood. Of this period, the leech could hold the blood twenty-five days, and to this may be added an incubation period of ten days, which was observed in one of the preceding experiments.
REFERENCES


THE PHYSICAL AND CHEMICAL PROPERTIES OF THE OLEORESIN OF ASPIDIUM * WITH RESPECT TO THE DETECTION OF ADULTERATIONS

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INTRODUCTION

The ethereal extract of male fern, the so-called "oleoresin of aspidium" of the United States pharmacopoeia, 1 since the time of its preparation by Peschier in 1825, 2 has been the most generally used of the numerous tæniafuges to be found in the materia medica; but of late it has been falling into disrepute. This fact is to be attributed directly to the variable results following its administration. That the extract as found upon the market varies to a great extent in physiological action, which is in a measure proportional to its tæniafuge properties, is manifest in the fact that it has caused severe symptoms of poisoning when given in doses of 4 grams; 3 while in other cases, 40 grams 4 have been administered without the appearance of evil symptoms. The lesser activity of the larger dose does not necessarily indicate an adulterated or deteriorated product as it is known that the activity of the extract is dependent upon a number of other factors as well—the locality in which the rhizomes are grown, the time of harvesting, et cetera. Thus, Matzdorff 5 reports a variation of from 0.815 to 4.145 per cent of crude filicin in rhizomes gathered in different parts of Germany and Russia, the highest filicin content being found in the Russian rhizomes. Berenger-Feraud 6 found that extracts prepared from rhizomes gathered in Normandy were much less active than the extracts prepared from rhizomes gathered in the Vosges or in the Jura Mountains. 7

* The species Aspidium filic mas Sw. and A. spinulosum Sw. do not properly belong in the genus Aspidium, but are now referred by botanists to the genus Dryopteris, as D. filic mas Schott and D. spinulosa O. Kuntze, respectively. As the name Aspidium is in general use in pharmacological literature to designate these plants, it is here retained.

1 The eighth revision of the United States Pharmacopoeia directs that the oleoresin of aspidium be prepared by using acetone as the exhausting menstrum, which is rarely if ever done by either American or foreign manufacturers, because of the production of an inferior article.

2 Repert. d. pharm. (1827), 27, 349.
5 Apoth. Zeitg. (1901), 16, 578.
7 Citation from Reuter, Pharm. Zeitg. (1891), 36, 245–246.
Kobert states that the Russian extract is about ten times as active as the German extract and twenty times as active as that obtained from France. Van der Marck and others have found the extract prepared from the rhizomes gathered in September to be the most active. In addition, it has been observed that individual idiosyncrasies or certain diseases predispose the patient to its toxic effects; likewise, the administration of an oily laxative—castor oil—with the extract is said to increase its toxic action, or the failure to administer any laxative at all may produce intoxication. Taking into due consideration all of the above factors, there is ample evidence both in the literature and the laboratory to show that the adulteration and deterioration of the extract is a large item in connection with the uncertainty of its therapeutic action. The proof for the latter statement will be presented as this paper progresses.

In accordance with our present knowledge, the ethereal extract of male fern owes its tæniafuge properties to the presence of a number of compounds; ketone-like combinations of phloroglucinol, mono-, di-, and trimethyl phloroglucinol with butyric acid and the condensation of 2 (flavaspidic acid, albaspidin, etc.), 3 (filix acid), or 4 (filmaron) such butanones. Poulsson attributes the action to the filix acid alone; Kobert is of the opinion that it is due to an intimate mixture of the filix acid with the fixed and volatile oils; Boehm states that filix acid, if at all active as a tæniafuge, is much less so than albaspidin; Jaquet, who has reported the latest work on the subject, concludes that the amorphous acid (filmaron) isolated by Kraft is the active principle of the extract. The latter's views are corroborated by Stringari and others. In as much as Kraft is the only investigator reporting the isolation of filmaron and as the method of isolation cannot be found in the literature, its identity as the chief anthelmintic constituent of the extract can hardly be said to be established.

1 Arch. d. Pharm. (1852), 120, 87-89.
5 Loc cit.
7 Arch. f. exp. Path. u. Pharm. (1897), 38, 35-38.
8 Jahresb. d. Pharm. (1904), 64, 456, from Therap. Monatsh. (1904), 18, 391.
10 Ibid. (1910), 55, 426.
With the object of arriving at the therapeutic value of the male fern rhizomes and their extract, various methods for the estimation of the above-mentioned constituents have been devised. Some of them, as the methods of Bocchi 18 and Daccomo and Scocci, 19 or the method employed by Caeser and Loretz, 20 give results corresponding to the total amount of acid substances (crude filicin) present; while the more specialized methods of Frommè 21 or Kraft 22 indicate only the filix acid 23 content. In view of the uncertainty regarding the chief taniafuge constituent of the extract and the contradictory results obtained by various investigators (see above), the determination of the filix acid for the purpose of indicating the therapeutic value seems to be of little or no importance. The determination of the crude filicin in fresh green rhizomes or extracts prepared immediately from them undoubtedly serves, in a measure, as an index to their activity; but it serves neither to detect certain classes of adulterations in commercial extracts, nor to show the true therapeutic value of deteriorated extracts or those prepared from old rhizomes for reasons that will be considered later.

METHODS OF ADULTERATION

The adulteration of the extract is not limited to the addition of foreign substances to the finished product, but begins with the drug from which it is prepared. The forms in which the drug is contaminated may be conveniently classed under three heads: (a) the substitution of old deteriorated rhizomes for the fresh active drug, (b) the admixture of the chaff and dead stipe bases with the rhizomes, and (c) the addition of the rhizomes of other species of ferns to those of the official species. The first form of adulteration is, perhaps, the most common and most widely spread.

The Pharmacopœia of the United States 24 directs that the dried rhizomes, from which the chaff together with the dead portions of rhizomes and stipes have been removed, and only such portions as have retained their internal green color, should be used. That it is almost impossible, from an economical standpoint, for the

21 Pharm. Centralhalle f. Deutschl. (1897), 38, 34.
23 Felix acid is used in this paper to represent the German "Filixsäure" rather than "filicic acid," the usual English translation, to avoid confusion with the "filicin acid" of Boehm.
24 U. S. P., 8 Rev. (1905), 62.
American manufacturer to follow these directions becomes evident from the examination of various commercial samples. During the winter and spring of 1910, a number of commercial samples of male-fern rhizomes were examined in the pharmacy laboratories of the University of Wisconsin. These samples were purchased from importers and drug millers in different parts of the United States, and comprised specimens of the whole and peeled rhizomes. Only one sample showed the presence of the green rhizomes in considerable quantity, 53.7 per cent; the remainder varied from 0 to 18 per cent. One sample of 10 kilograms purchased from a drug miller in the Middle West was not male fern at all, but was identified as the rhizomes of Osmunda.

The accompanying table shows the results of the examination:

Table I.—Content of green rhizomes in samples of male fern purchased from drug millers and jobbers.

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<tr>
<th>Sample No.</th>
<th>Purchased in—</th>
<th>Content of green rhizomes</th>
<th>Sample No.</th>
<th>Purchased in—</th>
<th>Content of green rhizomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>April</td>
<td>8.0</td>
<td>4</td>
<td>December</td>
<td>6.5</td>
</tr>
<tr>
<td>2</td>
<td>do</td>
<td>0.0</td>
<td>5</td>
<td>do</td>
<td>18.0</td>
</tr>
<tr>
<td>3</td>
<td>do</td>
<td>53.7</td>
<td>6</td>
<td>do</td>
<td>* 0</td>
</tr>
</tbody>
</table>

* Contained rhizomes of Osmunda only.

During the present year, samples have been imported from the United States and Europe and examined in this laboratory upon their arrival. The samples were purchased in 1 kilogram lots. The parts showing an internal green color on breaking were separated from those showing an internal brown color. Table II shows the percentage of green rhizomes obtained.

Table II.—Percentage of green rhizomes in samples of male fern.

<table>
<thead>
<tr>
<th>Sample No.—</th>
<th>Purchased in—</th>
<th>1.</th>
<th>2.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>0.0</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>4.25</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Manila, P. I.</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

The samples were not uniform, some consisting of the peeled rhizomes with stipe bases and chaff and, in one case, almost com-
pletely of stipe bases. Those purchased in Manila were at least 2 years old, and were badly deteriorated. The rhizomes purchased in Germany were obtained in January, and should have shown an internal green coloration had they consisted of the fresh stock harvested in the autumn; from this, it appears that the German supply is not renewed yearly as it should be, but is allowed to accumulate and deteriorate.

The use of old rhizomes cannot be detected in the extract by any of the previously mentioned assay methods as they show a crude filicin content equal to or greater than the fresh drug.

The greatest opportunity for adulteration is offered in the powdered rhizomes and, apparently, it has not been overlooked. The Belgian inspectors of pharmacies state that the powdered male fern, little used as such, is often superannuated and has completely lost its green color. In many cases, the drug miller grinds up the entire rhizome including dead portions, chaff, and stipes. Rusby reports a sample consisting of nothing but chaff and inert matter and another sample composed entirely of powdered Osmunda rhizomes. It is not necessary, however, that the drug be powdered to permit of the addition of unofficial species of fern. Pendorff who examined 20 samples of commercial rhizomes found that 12 contained 50 per cent or more of Aspidium spinulosum Sw.; 1 sample contained 90 per cent of the rhizomes of this species.

The latter form of adulteration may become evident from an assay by one of the analytical processes already mentioned, as the acid bodies present in other species of fern differ from those found in the official species both in their chemical constitution and in the quantities present. Hausmann obtained the following results upon examination of the etheral extracts of Aspidium filix mas and Aspidium spinulosum.

Table III.—Constitution of Aspidium filix mas Sw. and Aspidium spinulosum Sw.

<table>
<thead>
<tr>
<th>Species</th>
<th>Crude filicin.</th>
<th>Felix acid.</th>
<th>Aspidin.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspidium filix mas Sw</td>
<td>18.0</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Aspidium spinulosum Sw</td>
<td>8.4</td>
<td>0.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

27 Drug. Circ. (1910), 54, 616.
28 Apoth. Zeitg. (1903), 18, 150-152.
29 Arch. d. Pharm. (1899), 237, 544-556.
This investigator further affirms that the presence of aspidin in the extract is always evidence of the use of Aspidium spinulosum Sw. in its preparation. Taking into consideration the above observation of Hausmann, the report of Gehe & Co. of Dresden 30 is of interest. Upon the examination of 11 samples of the extract, 6 were found to contain aspidin, 2 to 3 per cent, but no filix acid; 4 samples contained filix acid, but no aspidin; and 1 sample showed a trace of aspidin and a small quantity of filix acid.

The adulteration of the finished extract consists in the addition of certain oils or foreign coloring materials. In the first instance, the only reason can be that of monetary gain. In the second case, the most plausible explanation is the desire to produce an extract from the deteriorated brown drug which will resemble that prepared from fresh rhizomes.

The oil usually employed in diluting the extract is castor oil, although others have been used. The quantity of oil added does not appear to bear any relation to the crude filicin content as 1 sample examined in this laboratory showed the presence of 54 per cent of castor oil and a filicin content of 8.79 per cent, while a second sample contained 62 per cent of oil and only 0.93 per cent of crude filicin.

This form of adulteration cannot be detected with certainty by any of the previously mentioned analytical processes as the crude filicin or filix acid content of the genuine extract prepared from the fresh rhizomes varies to such a great extent. Daccomo and Scoccianti 31 find the crude filicin present in quantities varying from 11.86 to 42.53 per cent, Bellingrodt 32 reports from 16.3 to 23.5 per cent present in commercial samples, while the Helvetician phar-macopeia 33 requires the presence of from 26 to 28 per cent. Madsen 31 reports the following concerning the filix acid content: Extracts from Bohemia and central Russia yielded from 0.71 to 0.97 per cent of filix acid by the Fromme method; Danish extracts with 2 exceptions (6.07 and 8.25 per cent) gave less than 2 per cent; 2 extracts from Germany showed the presence of from 6.58 to 9.59 per cent, respectively; and an extract from Wolmar in Livonia was found to contain 13.07 per cent. Furthermore, Kremel states that the adulteration with fatty oil cannot be detected by the saponification value, 1 gram of extract requiring

30 Pharm. Centralhalle f. Deutschl. (1898), 39, 298.
32 Ibid. (1898), 12, 869.
33 Pharm. Helvetia, IV Edit. (1907), 117.
34 Jahresb. d. Pharm. (1897), 32, 591.
from 116 to 165 milligrams of KOH for saponification. He proposes a solubility test instead.

For the purpose of coloring the extract, two entirely different classes of substances have been made use of; namely, copper salts and chlorophyll. Neither class can be detected by the assay methods. The addition of copper can best be detected by the examination of the ash, applying the usual tests for copper. The use of copper salts seems to be very frequent, although their presence was not detected in the samples examined in this laboratory. Weppen and Luders found 2 samples of a deep green color containing 0.056 and 0.044 per cent, respectively. Beck-urts reports 2 samples containing 0.135 and 0.044 per cent, and Pendorff states that 7 of 20 samples examined contained more or less copper.

Chlorophyll is not very generally used for the artificial coloration of the extract, although 1 sample examined in this laboratory was highly colored with it and its use is reported in at least one other instance.

**DETERIORATION OF THE EXTRACT**

As has been stated before in this paper, the extract owes its activity to various acid substances. Poulsson considered the amorphous filix acid as being the most important of these. He further states that this acid may exist in 2 forms—the amorphous or active form and the crystalline or inactive form. Upon standing, the extract becomes weaker in its action as a cataplasme owing to the conversion of the amorphous to the crystalline acid and its subsequent precipitation. Kraft, in a later investigation of the subject, concludes that the principal constituent of the extract, from a therapeutic standpoint, is an amorphous acid, "filmaron;" this exists only in the amorphous form, and is not identical with the filix acid of Poulsson. However, he

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35 The saponification values obtained by Kremel have been found to be much too low for extracts prepared in this laboratory. An extract having a filicin content of 19.18 per cent gave a saponification value of 236.7.

36 Kremel states that from 40 to 45 per cent of the pure ethereal extract is soluble in 95 per cent alcohol; less than 40 per cent means the addition of a fatty oil, more than 45 per cent going into solution means the addition of castor oil. Pharm. Post. (1887), 20, 349.

37 Pharm. Zeitg. (1893), 38, 922.

38 Apoth. Zeitg. (1893), 8, 594.

39 Ibid. (1903), 18, 150-152.

40 Pharm. Zeitg. (1893), 38, 922.


states that the acid decomposes upon the aging of the extract forming "filix acid" and "filixnigrine." In either case, it will be noticed that the investigators have found a deterioration of the extract upon standing, a fact which is corroborated by numerous others. The only fact which has been definitely established is that the extract when freshly prepared is homogeneous, but upon aging it deposits filix acid among other substances and becomes weaker in its action as a ëænizfuge. This behavior of the extract has been so universally observed that the various pharmacopoeias give specific directions for the mixing of the deposit with the liquid portion before dispensing. Viewed in the light of the observations of Poulsson or Kraft, these directions are founded upon error and are superfluous. However, Greenawalt, Reuter, and others contend that the deposit is active. Here again, we are confronted with the uncertainty of the exact nature of the process of deterioration and the futility of attempting to estimate the therapeutic value of the extract by a determination of the crude filicin or filix-acid content.

In order to establish more definitely the physical and chemical properties of the genuine extract prepared from the green rhizomes, in contrast to those of the deteriorated or adulterated product, the following constants of the genuine and commercial samples have been determined and tabulated.

**METHODS OF DETERMINING THE PROPERTIES**

**Color.**—The color of the extract was observed with the naked eye when a few drops of the substance were allowed to flow down the side of a white porcelain capsule. The results are expressed in shades of green or brown as the case demands.

**Specific gravity.**—The specific gravity of the extracts was taken at 25° C. using a 5 cubic centimeter pycnometer and an ordinary chemical balance. Owing to the thick consistency of the substance special precautions had to be taken to eliminate all air bubbles. Warming the extract before filling the pycnometer has an advantage in this connection.

**Refractive index.**—The refractive index was observed at 15° C., using an Abbé refractometer. The results given in the tables are the averages of 5 readings, and are only given to the third decimal place as the deep color of the extract prevents a more exact reading.

**Solubility.**—The solubility tests were carried out at 15° C.; those with ether and acetone being exceptions as they were ob-

\[\text{Am. Journ. Pharm. (1889), 61, 169.}\]
\[\text{Pharm. Zeitg. (1891), 36, 245-246.}\]
served at room temperature, about 30° C. In each case, with the exception of ether and acetone, the specified quantities of the extract and solvent were placed in a 25 cubic centimeter graduated, glass-stoppered cylinder and thoroughly shaken. The mixture was then cooled for two hours at a temperature of 15° C., and the volume of the oil thus separated was noted. The difference in this volume and that of the original quantity of the extract represents roughly the amount of castor oil when used as an adulterant. Where no castor oil has been added, practically the original volume of the extract was observed. For a more exact determination of the castor oil, when present, the following procedure was carried out.

Quantitative determination of castor oil.—A weighed quantity of the extract, about 10 grams, was introduced into a separator and shaken with an equal volume of petroleum ether. Part of the petroleum ether was used to aid in transferring all of the extract from the container in which it was weighed to the separator. After shaking, the mixture was allowed to stand in the ice box until the separation was complete when the oily layer was drawn off. This process was repeated. The oil was then treated with a 2 per cent solution of sodium carbonate, washed with distilled water, and dissolved in ether. A small quantity of animal charcoal was added to the ethereal solution; it was then filtered into a 200 cubic centimeter Erlenmeyer flask and evaporated to constant weight on a water bath. It was identified by a determination of its physical and chemical constants.

Determination of the crude filicin.—The crude filicin was determined according to the method employed by Caesar and Loretz \(^\text{15}\) which is essentially the same as that given in the Swiss pharmacopoeia. The following is the procedure as carried out by this firm:

Dissolve 5 grams of the extract in 30 grams of ether, add 100 grams of a saturated solution (3 per cent) of barium hydroxide, and shake the mixture vigorously during several minutes. Transfer to a separator, and run 86 grams (4 grams of the extract) of the lower aqueous layer into a flask of 200 cubic centimeters' capacity. Add 2 grams of hydrochloric acid, 25 per cent, and shake out with 3 portions of ether, 25, 15, and 10 cubic centimeters. Separate the ether, and filter each portion successively through the same plain double filter into an Erlenmeyer flask of 200 cubic centimeters' capacity which has been previously weighed. Wash the filter with 10 cubic centimeters more ether, and finally distill off the ether and dry the residue at 100° C. Weigh after allowing it to stand in a desiccator for half an hour. The weight multiplied by 25 will give the percentage of crude filicin in the sample.

\(^{15}\) Jahresb. d. Pharm. (1905), 65, 425.
In addition to the above, the color of the ethereal solution of the crude filicin was carefully noted in each case.

_Determination of aspidin._—The aspidin was determined as directed by Hausmann, the crude filicin obtained in the above assay was dissolved in absolute ether, and the solution allowed to evaporate spontaneously when the aspidin crystallized in the form of fine needles. When it was not present, the solution merely thickened without depositing any crystals and finally hardened.

_Determination of the iodine value._—The iodine value was obtained as directed in the United States Pharmacopœia. Approximately 0.2 gram of the extract was used instead of 0.3 gram as directed.

_Determination of the saponification value._—The United States pharmacopœial method for determining the saponification value of fats and oils had to be modified somewhat as the solution, after saponification, was too highly colored to distinguish clearly the end point when titrating with phenolphthalein as an indicator. In order to overcome this difficulty, only about 1 gram of the extract was taken for the determination. The mixture after saponification was diluted with 50 per cent alcohol to 100 cubic centimeters in a volumetric flask; 25 cubic centimeters of this solution were transferred with the aid of a pipette to a 500 cubic centimeter Erlenmeyer flask, diluted to 300 cubic centimeters with distilled water, and finally titrated with half-normal hydrochloric acid.

_Determination of the ash._—The ash was determined in the ordinary manner, heating carefully over a Bunsen flame until it assumed a grayish white color.

**PROPERTIES OF GENUINE EXTRACTS**

The physical and chemical constants of the genuine extracts are found to depend upon several factors; namely, the condition of the drug when extracted, the solvent used in exhausting the drug, and to some extent the method of preparation. Ether is the solvent generally employed for the extraction of the malefern rhizomes, but as the eighth decennial revision of the United States Pharmacopœia specifies the use of acetone, the properties of the official extract have been determined and are here stated for the purpose of comparison. With respect to the method of

"Arch. d. Pharm. (1899), 237, 544-556.
"U. S. P., 8 Rev. (1905), 527-528.
"Ibid., 535-536.
preparation, it has been found that a high temperature or prolonged heating causes the extract to darken somewhat in color.

The physical and chemical properties of extracts prepared from the drug in this laboratory are given in Tables IV, V, and VI. A commercial sample of 1,000 grams of the peeled drug was sorted, the rhizomes having an internal green color were separated from those having an internal brown color, and an extract was prepared from each lot using ether as the solvent.

Table IV.

<table>
<thead>
<tr>
<th>Extract from—</th>
<th>Color.</th>
<th>Specific gravity, 25°</th>
<th>Refractive index, 15°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green rhizomes</td>
<td>Yellowish green</td>
<td>1.0004</td>
<td>1.301</td>
</tr>
<tr>
<td>Brown rhizomes</td>
<td>Greenish brown</td>
<td>1.0043</td>
<td>1.490</td>
</tr>
</tbody>
</table>

Table V.

<table>
<thead>
<tr>
<th>Solubility in—</th>
<th>Ether.</th>
<th>Acetone.</th>
<th>Two volumes petroleum ether</th>
<th>Three volumes 90 per cent alcohol</th>
<th>One volume glacial acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble</td>
<td></td>
<td></td>
<td>Two volumes petroleum ether</td>
<td>Three volumes 90 per cent alcohol</td>
<td>One volume glacial acetic acid</td>
</tr>
<tr>
<td>Partially soluble</td>
<td></td>
<td></td>
<td>Mixes</td>
<td>Oil separates</td>
<td>Oil separates.</td>
</tr>
<tr>
<td>Do</td>
<td></td>
<td></td>
<td>do</td>
<td>do</td>
<td>do</td>
</tr>
</tbody>
</table>

* The term "mixes" is to be interpreted as meaning the formation of a turbid solution without the separation of oily particles.

Table VI.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per cent.</td>
<td>Per cent.</td>
<td>Per cent.</td>
<td></td>
</tr>
<tr>
<td>Green rhizomes</td>
<td>19.88</td>
<td>99.3</td>
<td>234.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Brown rhizomes</td>
<td>20.64</td>
<td>101.7</td>
<td>297.2</td>
<td>0.49</td>
</tr>
</tbody>
</table>

It will be noticed upon examination of Table VI that the iodine and saponification values vary directly as the filicin content. Such a variation, however, is not constant for extracts prepared from different samples of the drug, owing to the varied composition of the crude filicin and to the variable quantities of the fixed 49 and volatile 50 oils present. Another important item

49 Wollenweber states the fixed oil constitutes from 70 to 75 per cent of the extract. Arch. d. Pharm. (1906), 244, 466.
50 Ehrenberg found the content of volatile oil to vary with the season in which the rhizomes were collected; those gathered in September yielded 0.04 per cent of volatile oil, those gathered in April 0.008 per cent, and those gathered in June 0.025 per cent. Arch. d. Pharm. (1893), 231, 345–356.
brought out in the above table is the fact that the brown rhizomes yield an extract richer in filicin than the green. The higher filicin content of the brown rhizomes can probably be attributed to the breaking down of glucosidal bodies with the liberation of acids, or it may be due to a slight variation in the filicin content of the rhizomes themselves. (See previous discussion under adulteration with oils.)

The influence of the solvent used in the preparation of the extract upon its physical and chemical properties is set forth in Tables VII, VIII, and IX. In contrast with the ether preparation, it was noticed that the acetone extract separated into two layers, an upper oily layer having a brownish green color and a lower layer which was nearly brown and thicker than that above. Both of the extracts were prepared from the rhizomes having an internal green color. The two extracts can be distinguished by their color, specific gravity, refractive index, and solubility.

The low percentage of filicin in the acetone extract is due to the greater yield of extractive matter when acetone is used as a solvent and not to the incomplete extraction of the acid bodies as might be inferred from the table. Ether yields an extract amounting to 8.325 per cent of the drug while acetone produced 14.690 per cent.

<table>
<thead>
<tr>
<th>Table VII.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether</td>
</tr>
<tr>
<td>Acetone</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table VIII.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility in—</td>
</tr>
<tr>
<td>Ether.</td>
</tr>
<tr>
<td>Soluble</td>
</tr>
<tr>
<td>Partially soluble</td>
</tr>
</tbody>
</table>

Penndorff attributes the turning brown of the green rhizomes to the breaking down of the "filix tannic" acid into "filix red" and sugar. *Loc. cit.*
DuMecz: Oleoresin of Aspidium

Table IX.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether</td>
<td>Per cent.</td>
<td>20.37</td>
<td>99.8</td>
<td>229.3</td>
</tr>
<tr>
<td>Acetone</td>
<td>13.79</td>
<td>95.3</td>
<td>208.5</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Physical and chemical constants of commercial extracts as determined in the laboratory

The commercial extracts were purchased in different countries and subjected to the same tests as the genuine extracts prepared in the laboratory. Their properties are shown in Tables X, XI, and XII.

Table X.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Color</th>
<th>Specific gravity, 25°</th>
<th>Refractive index, 15°</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Deep green</td>
<td>1.0079</td>
<td>1.490</td>
<td>United States.</td>
</tr>
<tr>
<td>2</td>
<td>Brownish green</td>
<td>1.0085</td>
<td>1.494</td>
<td>Germany.</td>
</tr>
<tr>
<td>3</td>
<td>do</td>
<td>1.0083</td>
<td>1.493</td>
<td>England.</td>
</tr>
<tr>
<td>4</td>
<td>do</td>
<td>1.0028</td>
<td>1.492</td>
<td>Germany.</td>
</tr>
<tr>
<td>5</td>
<td>do</td>
<td>1.0012</td>
<td>1.490</td>
<td>United States.</td>
</tr>
<tr>
<td>6</td>
<td>do</td>
<td>0.9889</td>
<td>1.485</td>
<td>England.</td>
</tr>
<tr>
<td>7</td>
<td>do</td>
<td>0.9855</td>
<td>1.484</td>
<td>Do.</td>
</tr>
<tr>
<td>8</td>
<td>do</td>
<td>0.9773</td>
<td>1.489</td>
<td>Manila, P. I.</td>
</tr>
</tbody>
</table>

Table XI.

<table>
<thead>
<tr>
<th>Solubility in—</th>
<th>Ether.</th>
<th>Acetone.</th>
<th>Two volumes of petroleum ether.</th>
<th>Three volumes of 90 per cent alcohol.</th>
<th>One volume of glacial acetic acid.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Soluble</td>
<td>Partially soluble</td>
<td>Mixes</td>
<td>Oil separates</td>
<td>Oil separates.</td>
</tr>
<tr>
<td>2</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td>Do.</td>
</tr>
<tr>
<td>3</td>
<td>do</td>
<td>do</td>
<td>Partial separation.</td>
<td>Oil separates</td>
<td>Mixes.</td>
</tr>
<tr>
<td>4</td>
<td>do</td>
<td>do</td>
<td>Mixes</td>
<td>Oil separates</td>
<td>Do.</td>
</tr>
<tr>
<td>5</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td>Partial separation.</td>
<td>Mixes.</td>
</tr>
<tr>
<td>6</td>
<td>do</td>
<td>do</td>
<td>Partial separation.</td>
<td>Oil separates</td>
<td>Do.</td>
</tr>
<tr>
<td>7</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td>do</td>
<td>Do.</td>
</tr>
<tr>
<td>8</td>
<td>do</td>
<td>do</td>
<td>Mixes</td>
<td>Oil separates</td>
<td>Oil separates.</td>
</tr>
</tbody>
</table>
Table XII.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.77</td>
<td>101.5</td>
<td>240.5</td>
<td>0.49</td>
<td>Chlorophyll.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>20.32</td>
<td>100.2</td>
<td>225.5</td>
<td>0.47</td>
<td>Castor oil.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17.61</td>
<td>98.3</td>
<td>206.7</td>
<td>0.42</td>
<td>A. spinulosum.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16.55</td>
<td>97.1</td>
<td>214.6</td>
<td>0.48</td>
<td>Castor oil.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14.36</td>
<td>94.4</td>
<td>206.7</td>
<td>0.47</td>
<td>Do.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.79</td>
<td>89.4</td>
<td>202.4</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.93</td>
<td>85.8</td>
<td>195.7</td>
<td>0.18</td>
<td></td>
<td></td>
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<td>8</td>
<td>8.59</td>
<td>87.2</td>
<td>200.3</td>
<td>0.18</td>
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An inspection of the above tables indicates the use of old brown rhizomes in the preparation of the extracts. This is especially prominent in the color and refractive indices. Further evidence of this is found in the color of the ethereal solution of the crude filicin obtained in the filicin assay. All of the above extracts yielded a product which dissolved in ether with a color varying from light brown to dark reddish brown. The color of the ethereal solution of filicin obtained from fresh green rhizomes, although varying somewhat, was not of a darker shade than orange.

Extract 1 contained a considerable quantity of chlorophyll. Because of the fact that chlorophyll is a natural constituent of the extract and that there is no satisfactory method for its quantitative estimation, a determination of the amount present was not attempted. Its presence in undue quantity was based on the deep green of the preparation and the green color imparted to alcohol when shaken with a portion of the sample.

Extracts 3, 6, and 7 contained 30, 54, and 62 per cent of castor oil, respectively. This fact is indicated by the refractive index, the specific gravity, the iodine and saponification values, and the ash content.

Another fact of interest to be noted in the above tables is that the iodine and saponification values vary in the same direction as the filicin content. The one exception to this is found in sample 8. Upon an examination, however, of the physical and chemical properties of the fixed oil of male fern and those of castor oil, the apparent irregularity is readily accounted for. Tables XIII, XIV, and XV show constants of the fixed oil of fern separated from an extract prepared in the laboratory and of castor oil as specified in the United States Pharmacopoeia.\footnote{U. S. P., 8 Rev. (1905), 321.}
The index of refraction of castor oil is that given by Lewkowitsch. 53

The fixed oil of fern was obtained as follows: The extract was treated with 5 volumes of a 2 per cent solution of sodium carbonate, and the fatty oil shaken out of the mixture with several portions of petroleum ether. The petroleum-ether solution was washed with distilled water in a separator, the aqueous layer drawn off, and a small quantity of animal charcoal added to the remaining solution. The mixture was filtered into a tarred porcelain dish and evaporated to constant weight on a water bath.

<table>
<thead>
<tr>
<th>TABLE XIII.</th>
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<tr>
<td><strong>Oil</strong></td>
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<tr>
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<tr>
<td>Oil of male fern</td>
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<td>Castor oil</td>
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<table>
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<th>TABLE XIV.</th>
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<tr>
<td><strong>Solubility in</strong></td>
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<td>-------------</td>
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<tr>
<td>2 volumes of petroleum ether.</td>
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<tr>
<td>Soluble</td>
</tr>
<tr>
<td>Insoluble</td>
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</table>

<table>
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<th>TABLE XV.</th>
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<tbody>
<tr>
<td><strong>Oil</strong></td>
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<tr>
<td>Oil of male fern</td>
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<tr>
<td>Castor oil</td>
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a Four hours. b Eight hours.

**SUMMARY AND CONCLUSIONS**

The fact that the present methods of assaying the extract of male fern do not give results which indicate its therapeutic value has already been brought to notice. Neither can the properties of the extract tabulated in this paper be used as factors for indicating the degree of activity. They can only serve the physician and pharmacist in so far as they afford a means of

distinguishing between an extract recently prepared from fresh green rhizomes and that prepared from old rhizomes or in detecting a deteriorated or adulterated product. Viewed from this standpoint the following conclusions can be drawn.

The color of the extract when prepared from fresh rhizomes is yellowish green; a brownish shade indicates the use of old rhizomes; deep green means the addition of chlorophyll or other foreign coloring material, such as a salt of copper. The use of old rhizomes receives further confirmation in a low index of refraction (the refractive index is also lowered by the addition of castor oil) and in the dark color of the ethereal solution of the crude filicin obtained in the filicin assay. The presence of a copper salt can best be confirmed by an examination of the ash, applying the usual tests for copper. The addition of chlorophyll is difficult to detect chemically owing to its presence as a natural constituent of the extract. However, the amount which must be added to obscure the brown color imparted by old rhizomes is so great that it can be easily detected with the naked eye in the original sample and in the solubility tests.

The addition of castor oil to the extract produces a considerable change in all of its properties. It is especially indicated in the low specific gravity, index of refraction, and iodine and saponification values. Its presence can be most easily confirmed by the solubility tests.

The presence of aspidin can readily be determined by Hausmann’s method which serves as a practical means of detecting the use of Aspidium spinulosum Sw. in the preparation of the extract.

The use of acetone as directed in the United States Pharmacopoeia yields a product which separates in two layers upon standing—an upper oily layer and a lower layer darker in color and thicker in consistency than that above. This in itself serves as a ready means of detecting its use, which is further confirmed by the solubility tests and the high ash content.

The iodine and saponification values vary in the same direction as the filicin content, and, therefore, might be used to displace the rather long and expensive method of the present filicin assay. Especially is this true in the case of the saponification value, which requires but 1 gram of the extract and less than an hour to complete.
THE VARIABILITY OF CERTAIN STRAINS OF DYSENTERY BACILLI AS STUDIED BY THE SINGLE-CELL METHOD

By Marshall A. Barber

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

One plate and 1 text figure

During the summer of 1912, a considerable number of stools from cases of bacillary dysentery occurring in Manila came into the laboratory. Some of the cultures from these stools showed a tendency to variability, and it was decided to study some of these variations by the single-cell method.

This method, described by me in previous publications,\(^1\) enables the worker not only to obtain easily pure cultures arising from a single cell, but also to select any aberrant cell lying among myriads of normal ones and to substitute the single-cell cultivation for plate cultures in obtaining a series of selections. Further, one may more easily avoid the contaminations which are especially liable to occur in the Philippine Islands when plate cultures are used. A description of the technique is given below. The difficulties of using the method are not great. Pure cultures may be made at the rate of 50 per hour under favorable circumstances.

Two types of variations were studied; one characterized by fermentative, the other by morphological, characteristics.

I. FERMENTATIVE CHANGES WITH RESPECT TO MALTOSE

A culture, laboratory strain 105200, furnished by Dr. Otto Schöbl of this Bureau, was isolated July 7, 1912, from a typical bloody stool of a case of bacillary dysentery. This strain was mannite fermenting, and otherwise showed the characteristics of dysentery bacilli of the Flexner type. A pure culture was made by the isolation of a single bacillus, and this culture was grown on ordinary agar for about eighteen days. Transfers were made on September 12, 1912, to ordinary broth, and allowed to grow from two to five hours, in order to obtain actively growing

bacilli. Twenty-one single cells were isolated and allowed to develop overnight in hanging drops, and were then transferred to ordinary agar. After twenty-four hours' growth, they were transferred to maltose-litmus agar and incubated. Of the 21, 16 gave cultures like the original stock; that is, with blue surface growth and, at most, only slight acid formation at the bottom; while 5 gave within twenty-four hours the practically complete cherry-red color to the agar, such as is found in any maltose-fermenting organism. After twenty-four days at room temperature, 2 of the 5 red cultures remained clear red, 2 showed the lower two-thirds of the tube red with the upper part becoming blue, and the fifth had become almost wholly blue. The 16 originally blue cultures showed a deep blue, deeper than the uninoculated controls. Transfers from the originally red cultures, that had now become blue, to fresh maltose agar gave distinct red cultures like the original; while transfers from the blue gave blue cultures. So, in spite of the later change to blue through long exposure to the air, the red cultures possessed a distinct fermentative difference from the blue in that they showed complete red in forty-eight hours; and, although becoming blue later, gave a distinct red color on transfer to a new maltose-litmus medium. The change of the red coloration to blue on long exposure was not constant. In some whole series the agar remained red for weeks. Some slight variations in the composition of the medium may account for the differences. It was found that cultures remaining acid were more likely to die out.

A further characteristic of the original blue variety is the property of forming on maltose agar secondary colonies capable of fermenting maltose. These colonies are somewhat elevated, denser than the surrounding growth, and usually well defined in the substratum. They often appear within forty-eight hours, sometimes increasing in number during subsequent days, and, in this series, varying from 1 to 300 per test tube. They often increase in size, and may become 4 millimeters or more in diameter. At first they show no color, but later change the agar at their base to a dull red, and if numerous may reddens the whole tube. If a transfer is made from one of these colonies to a new tube, a permanently acidifying growth is obtained; while a transfer from the intervening substratum gives a blue culture like the original.

Many references are found in the literature to secondary colonies of this sort ("papillae," "Knöpfe," "Knötchen") possessing new fermentative characteristics. They were described
by M. Neisser 2 as occurring in *Bacterium coli mutabile*, the secondary colonies of which had the property of fermenting lactose. The secondary colonies of *B. coli mutabile* were further studied by Massini, 3 Arnold Burk, 4 Sauerbeck, 5 Kowalenko, 6 Baerthlein, 7 and Klein. 8 Burri 9 found similar secondary colonies in *Bacterium coli imperfectum*. Burri and Düggeli 10 and Hübener 11 found them in various strains of *Bacterium coli* or coli-like organisms. R. Müller 12 reported secondary colonies in many different kinds of bacteria on 18 different varieties of carbohydrate media.

In a typhoid-like organism isolated from urine, Soberneheim and Seligmann 13 found secondary colonies fermenting lactose in Conradi-Drigalski agar. Penfold 14 obtained in *Bacillus typhosus* secondary colonies which fermented dulcite. Jacobsen 15 found secondary colonies in Conradi agar plates made of an atypical strain of typhoid (*Bacterium typhi mutabile*), which had caused a typhoid epidemic in Denmark. The original strain varied from typical typhoid bacilli in that it possessed a very low degree of agglutinability to typhoid serum and a delayed acid formation in mannite broth. The secondary colonies gave cultures which grew unrestrained on Conradi agar and possessed the agglutinability and fermentation characteristic of typhoid bacilli. Seiffert 16 worked with a strain of *Bacterium coli* which had been made resistant to malachite green and had lost the power of forming red colonies on Endo agar. Secondary colonies were obtained from this modified strain which not only had regained the power of producing red cultures on Endo agar, but also exhibited a character not possessed by the original strain—that of fermenting cane sugar.

In the dysentery and paradysentery group of bacilli, R.

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2 Centralbl. f. Bakt. etc., Ref. (1906), 38, Beiheft, 98.
3 Arch. f. Hyg. (1907), 61, 250.
4 Ibid. (1908), 65, 235.
5 Centralbl. f. Bakt. etc., Orig. (1909), 50, 572.
7 Centralbl. f. Bakt. etc., Orig. (1912), 66, 21.
10 Centralbl. f. Bakt. etc., Orig. (1909), 49, 145.
Müller \cite{1} describes a strain of the Flexner type which produced secondary colonies capable of fermenting isodulcite.

Bernhardt \cite{2} investigated a strain of dysentery bacilli, type Y, which formed secondary colonies on maltose agar capable of fermenting maltose. The new characteristic was transmissible when cultures were transferred at short intervals, but, after eight weeks' growth on weakly alkaline agar or after half a year in sealed agar tubes, the power to acidify maltose was lost. On transferring to fresh agar, new secondary maltose-fermenting colonies were formed.

Baerthlein,\cite{3} in an extensive article on the so-called mutations in various bacilli, vibrios, and cocci, describes secondary colonies in strains of dysentery bacilli of both the Shiga-Kruse and of the nontoxic varieties. Secondary colonies possessing new morphological as well as fermentative qualities were obtained, although there was no constant correlation between the morphological and chemical changes. As a rule, secondary colonies were formed on transfer of old agar cultures to new media. Some mutants of the same strain of toxin-free dysentery bacilli possessed varying agglutinability to the same serum. In some cases the same strain gave two or more sorts of mutation.

Practically all of the above-mentioned writers obtained secondary colonies only in media containing the sugar fermented by the new race, and in nearly all cases the new race transmitted its characteristics indefinitely to offspring. In most cases the purity of the cultures used was established by plate cultures only, but in some the varying strain was started from a single bacillus obtained by the Burri method.

In working out the maltose-fermenting variety of dysentery bacilli observed by me, it was proposed to examine the variations carefully, not only beginning with a single cell, but testing numerous individuals, both of the stock and of the new races, by this method. It was recognized that dysentery bacilli of the Flexner type are relatively inconstant in their characteristics, and that maltose is a comparatively unreliable substance with regard to fermentation; but the characteristics of the races obtained were so well marked and constant, that they could be safely taken as a basis for a study primarily of variation.

First, the uniformity of the individuals of the red and the blue

\cite{4} Centralbl. f. Bakt. etc., Ref. (1908), 42, Beiheft, 57.
\cite{5} Zeitshr. f. Hyg. u. Infectionskrankh. (1912), 71, 229.
\cite{6} Arb. a. d. kais. Gesundheitsamte (1912), 40, 423.
types was tested by selecting individuals at random from each type and testing the characteristics of their offspring.

From a culture of the red, or maltose-fermenting, series, 10 single cells were isolated and their offspring tested on maltose agar. All gave the characteristics of the red variety. From another one of the original 5 red cultures, 12 isolations gave red cultures on maltose agar. After an interval of four days, 13 new isolations were made from one of the 12, selecting a tube which gave a deeper red than the average. All gave red cultures. A subculture of one of these 13 showed a tendency to blue, and some days later 24 new isolations were made from it, and at the same time 26 isolations were made from one of the same lot which showed a deeper red than the average. All 50 of these gave cultures which were alike red. Apparently selection in the direction of red or blue in this red series showed no progression toward either color, and it is probable that the slight variations in color were due to some inequalities in the medium.

From one of the original 16 blue, 11 cells were selected, all giving cultures of the blue type. From another one of the original 16, 16 single cells were isolated and gave blue cultures, and from one of this second group 23 isolations were made and all gave a similar blue type. All blue cultures gave secondary colonies fermenting maltose. No red cultures showed such colonies. The selections are given graphically in the chart below:

During the period of these isolations the stock cultures were kept on maltose agar except shortly before isolations, when they were transferred to ordinary broth to obtain actively growing cells for isolation. In obtaining material for selection from the blue type, transfers were made from growths between sec-
secondary colonies. Only cells of apparently normal morphology were selected. All of the above series of selections were made within a month after the original selection.

About five months after the first selections from strain 105200, a new lot of cells was selected from it. The culture had been kept on ordinary agar at room temperature with occasional transfers to keep it viable. It had never grown on a maltose-containing medium. Of 60 isolations, 52 gave typical blue cultures, 6 typical red, while 2 gave partial red with no secondary colonies. To test the composition of one of the partially red strains, 9 single cells were isolated from it, and, as a control, 13 from one of the typically red cultures. The cultures from the 9 of mixed type gave all mixed color with no secondary colonies, while the 13 red gave all pure red cultures.

Nearly ten months after the first series of selections a new lot of single cells was isolated from the original stock of dysentery strain 105200. The culture had been kept at room temperature on ordinary agar. It had been transferred at about monthly intervals to fresh agar, and had had in all about 10 transfers since the hanging drop in which the original single cell had been isolated. In this third series 123 cells were isolated, and the cultures grown from them tested on maltose agar, before a maltose-fermenting type was found. This, however, was typical, exhibiting a clear red color throughout the whole tube within twenty-four hours after sowing. No secondary colonies were formed. Of the 122 nonfermenting strains, all formed secondary colonies; in some very few and in others so many as to redden the whole tube. However, there was no possibility of confusing such cultures with a "red" variant, since the color appeared in the former only after several days and after a very large number of secondary colonies had been formed. No partially red strains were found, such as occurred in the second series.

In this third series different lots were selected at a time, the selections extending over a period of some days. In each lot of selections a fresh broth culture was made from the original agar culture in order to obtain freshly growing cells for isolation.

So, in all series, 205 isolations were made, and from these 11 (about 5 per cent) of the red variants were obtained.

Either the two varieties exist side by side for months on ordinary agar, or the maltose-fermenting variations are being continually formed from the stock. The proportion of acid formers is the smaller, 5 in 21 in the first series, 8 in 60 in
the second, and 1 in 123 in the third—the proportion becoming smaller as the months went on. In addition, there occurred varieties with less power of fermenting maltose, but otherwise corresponding to the acid type.

As a further test of the constancy of an acid-forming strain, single cells were isolated from a red strain which had been cultivated two months on ordinary agar at room temperature. It had been passed through 5 subcultures, and neither it nor the culture from which it was isolated had been grown on maltose agar. Twenty-nine such isolations gave cultures all of which completely fermented maltose-litmus agar.

In order to test the behavior of the red and blue varieties in ordinary broth alone and in competition with each other, the following experiment was arranged:

Into 4 tubes of ordinary broth, A, B, C, and D, equal amounts were sown of the red and blue strains. In order to have exactly equal sowings of the two varieties, the following method was followed: Single cells of each of the two types were isolated and grown from one to two hours in hanging drops until 3 generations had formed, that is, until 8 new individuals had formed from one. Then to a tube of ordinary broth 8 bacilli of the red and 8 of the blue strains were added, selecting such hanging drops as showed elements of approximately equal size. This was done in 4 tubes and the fifth, E, was given 5 bacilli of the blue and 6 of the red variety. For comparison, a tube was sown with a pure culture of the red and one with a pure culture of the blue type. These tubes were incubated one day, then transfers made by platinum loop to fresh broth and at the same time to maltose agar, in order to observe any fermentative changes. This was continued through 10 daily transfers.

At the end of the series, the red control remained red as before, and the blue distinctly blue with secondary colonies; while of the 4 which received mixed sowing in equal quantities, 1, A, was blue with secondary colonies like the blue control, 2, B and C, showed mixed red and blue, while the fourth, the one which had received a slightly larger amount of red strain, was as red as the red control. In order to test the composition of the cultures, a new transfer was made at the end of the series to ordinary broth to obtain freshly growing cells, and 19 single cells were isolated from A of the mixed series, a culture which showed all blue. All 19 gave typical blue colonies. Ten similar isolations from D, which showed a mixed coloration on maltose agar, gave 8 of the red and 2 of the blue; 12 isolations from the
pure blue, which had been carried through 11 daily transfers on bouillon, gave all typical blue cultures with secondary colonies.

This experiment indicates: First, that with daily transfer in broth the pure types remain pure; and, secondly, that in competition with the blue the red tends to gain the upper hand, although in 1 tube the blue predominated and in 2 tubes the red and blue persisted side by side.

After the one-day incubation, all broth tubes of this series were kept at room temperature. In order to ascertain the varying proportions of the red and the blue strains at different stages of the experiment, samples were taken from the first, fourth, and eighth transfers of A, and put into broth to get actively growing cells. From these, single cells were isolated and their descendants tested on maltose agar. Of 13 cells of the first transfer, 7 gave blue and 6 red cultures. Of 7 cells from the fourth transfer, 6 gave blue and 1 red cultures. Of 15 from the eighth transfer, all gave blue.

For confirmation, a second mixed series in ordinary broth was undertaken. Here 4 tubes were given equal mixtures by the method described above and 1 by simply adding a loopful of each type. Two pure red and 2 pure blue cultures were added to the series, and daily transfers and daily tests made as in the previous experiment. The same batch of maltose agar was used in all tests. On account of an enforced absence from the laboratory, the experiment had to be interrupted two weeks, between the first and second transfers, but all were kept in the refrigerator during the interval, and in a comparative experiment this interruption ought not to vitiate the results.

The blue and the red controls retained their characteristics after 10 transfers; and, of the mixed ones, 3 became acid at the fifth; 1, the tube inoculated with the loop mixtures, at the seventh; and 1, B, showed mixed red and blue at the tenth transfer. Twenty-one single-cell isolations from B gave 19 blue and 2 red cultures. Nineteen isolations from A, a tube which had received an equal mixture of the blue and red strains and had become red, gave 19 all red cultures.

In a third mixed series, all were passed through daily transfers in three kinds of broth—plain, maltose, and mannite. The sugar broths were made by adding to water 1 per cent peptone, 0.5 per cent salt, and 1 per cent of mannite and maltose, respectively. The bacterial mixtures were made by adding to a broth tube equal quantities (about 0.25 cubic centimeter) of each
type. The mixed tubes were done in triplicate, 3 tubes to each kind of broth. The unmixed red and blue strains retained their characteristics through 15 daily transfers in each kind of broth. Of the 9 mixed tubes, all showed mixtures of red and blue strains at the end of the fifteenth transfer, except 2, 1 maltose and 1 mannite, which became distinctly acid at the fifth transfer.

These three series indicate that, whether in plain, mannite, or maltose broth, the characteristics of the two types remain constant if daily transfers are made. If the two strains be equally mixed in the same tube, sometimes the red and sometimes the blue gets the upper hand, but there is a tendency for the red strain to predominate.

Most authors have found that a new fermentative character is acquired only in contact with the corresponding sugar and that older cultures in the sugar have given more pronounced variation than fresh cultures after repeated transfers at short periods. The older cultures afford the time necessary for the exhaustion of the preferred sources of nutrition, after which the bacteria may acquire the power of attacking the unusual food. Baerthlein 20 found that a paratyphoid-like race of bacilli, grown on lactose-containing media, kept its characteristics when transferred every twenty hours, but, transferred at somewhat longer intervals, it acquired the power of acidifying lactose. Josef Klein, 21 working with Bacterium coli mutabile, found that many generations in a lactose-free medium did not produce the lactose ferment, while relatively few generations in a medium poorly supplied with nourishment, but containing lactose, brought about lactose fermentation. Mere contact with lactose at a temperature too low for active multiplication failed to develop the lactose ferment. Twort 22 succeeded in developing the power of fermenting dulcite and lactose in a strain of typhoid by fortnightly transfers in a medium containing these substances. Hiss 23 found that a strain of dysentery, type Y, cultivated for some time in maltose, had acquired the power of fermenting that sugar. On the other hand, Lentz 24 describes a Flexner strain which, after seven years' cultivation, lost the power to ferment maltose, while retaining its specific agglutinating power.

Penfold succeeded in "training" typhoid to increased fermentative power in isodulcite.

In the race of dysentery bacilli experimented on by me, it is evident that contact with maltose is not necessary to the formation of the maltose ferment, since an ordinary agar culture of a race which had never been in contact with maltose gave, of single isolated bacilli, about 25 per cent of maltose fermenters, approximately sixty-five days after isolation from the patient, and about 13 per cent after culture on agar for five months. It may be that the agar used contained some substance which might replace maltose as an excitant of a maltose ferment, but it is more probable that in this case variations in fermentative character occur without a special food stimulus. Again, in this race of dysentery, growth in contact with maltose did not materially increase the proportion of maltose fermenters, unless kept for a long time in the same tube. Several series of experiments were carried out to test this matter.

In series 1, an alkaline and an acid-forming race were carried through 15 successive daily transfers on 3 sorts of broth: the first containing 1 per cent peptone, 0.5 per cent salt, and 1 per cent maltose; the second having the same ingredients with mannite instead of maltose; and the third consisting of ordinary beef-peptone broth. Cultures were tested on litmus-maltose agar slopes. At the end of the series the acid remained acid as before and the alkaline remained alkaline with no increase in the proportion of secondary maltose-fermenting colonies.

In a second series, an alkaline race, which had never grown on a maltose medium, was cultivated fifteen days; in one lot with daily transfers and in the other with transfers at intervals of three days, a period of time sufficient for allowing the formation of secondary colonies on maltose agar. Six different media were used: One, a broth containing peptone, 2.5 grams; sodium phosphate, 2.5 grams; calcium chloride, 5 cc.; asparagin, 5 cc.; maltose, 10 cc.; water, 1,000 cc.; 2, the same as 1, plus enough agar to make a soft medium; 3 and 4 the same as 1 and 2, respectively, but containing no sugar; 5, ordinary beef-broth peptone; 6, ordinary agar. Cultures were tested daily on maltose-agar slopes. At the end of the series both lots, the one transferred daily and the one transferred every three days, remained the same as at the start, except that the ordinary agar one showed some, although not a constant, tendency to become more acid. Since there was some suspicion that the

maltose used contained a trace of glucose, a third series was carried out, using the same media as in the second but in addition a broth and an agar made with a maltose shown by chemical test to be pure. Ten daily transfers, and 3 at intervals of three days, failed to alter the character of the race.

In addition, tests were made of the above cultures after incubating four, seven, fifteen, and nineteen days. Here a tendency to an increase of acid producers was noted in the maltose media, while in the sugar-free media the strain remained unchanged.

In summary, it may be said that there is evidence that in this race of dysentery bacillus the presence of maltose does not materially increase the proportion of maltose fermenters except after long contact with the sugar in the same tube.

To compare the effect of substances other than maltose, a typical red and a typical blue race were sown on slants of the following litmus agars: Glucose, levulose, lactose, saccharose, raffinose, glycerine, dextrin, inulin, salacin, erythrin, and dulcite. All tubes were alkaline after three days' incubation except glucose, levulose, and glycerine, which were cherry red in both the red and the blue races. After seven days' growth, each culture was transferred to a new agar of the same kind. The colors remained the same as after the first transfer. On transfer back to maltose, the originally red strain gave red cultures in all cases and the originally blue strain gave blue. Apparently growth on other substances does not affect the fermentative character of the two races with respect to maltose; and, in a short time, at least, neither race acquires the power of attacking a new substance.

For confirmation, a red and a blue strain, neither of which had been grown on a medium containing maltose or other sugar, were sown on the media used in the first test, and, in addition, on mannite, galactose, and amygdalin-litmus agars. Both races gave the same reaction on all media.

Some further experiments were made to determine the composition of the secondary colonies. These colonies have a large proportion of irregular cells, many of which are coccoid or resemble in form small yeast cells. Many of these cells were isolated and grown to determine their nature. One protocol may serve to illustrate the nature of these experiments:

From the top of a large secondary colony eight days old, 10 normal cells were isolated, of which 3 grew; and 14 coccoid forms, of which 6 grew. Grown in hanging drops of ordinary
broth, 2 of the 3 normal gave nearly normal offspring, while 1 gave a large proportion of coccoid forms. Of the 6 coccoid cells, 1 gave a large proportion of coccoid, 2 gave a small proportion of coccoid cells, and 3 nearly regular offspring. Transferred to ordinary agar and broth, all 9 gave cells with uniform, normal morphology. Sown on maltose-litmus agar, all 3 cultures of normal-cell ancestry gave acid types, and of the coccoid strains 2 gave acid and 5 alkaline cultures.

From a secondary colony of three days' growth similar to the above, cells were spread over the surface of maltose-litmus agar in Petri dishes. Forty distinctly blue and 6 bright red colonies were obtained.

From the above and similar experiments, it was proved that the secondary colonies contain a mixture of acid- and alkaline-forming cells and that there is no constant correlation between the morphology or degree of involution of cells and their fermentative properties. Further, it was shown conclusively that the first few generations from an involution cell may show a large proportion (in some cases nearly one-half) of involution cells. Later, the morphology of the cells becomes normal or nearly so.

In an ordinary transfer from a secondary colony to fresh maltose-agar one usually obtains an acid-forming growth, although in some cases not so well marked as when a "pure" acid strain is sown. It is probable that in this mixture, taken from a secondary colony, the acid formers usually outgrow the others, so as to give a more distinct red to the maltose-litmus agar.

With regard to agglutination, the parent stock and the red and blue races isolated from it gave practically the same results. A serum obtained by inoculating a rabbit with a pure culture of the stock gave agglutination to \( \frac{1}{500} \) in all three strains.

II. VARIATION IN MORPHOLOGY

In another culture of dysentery, No. 42 of the Shiga-Kruse type, a variation of a different kind was studied. This culture was kindly furnished me by Dr. C. S. Butler of the United States Navy, August 19, 1912, and came from a stool of a case of dysentery. A pure culture was obtained by isolating a single cell, August 30, 1912. Descendants from this normal cell grown in hanging drops showed, for the most part, only normal cells. From this pure culture a single cell was again isolated and grown
in a hanging drop on September 9. After twenty-four hours' growth, among many thousands of normal cells, some were found which grew chain-like with elements much more plump than the normal. Six of these variants were isolated, and of the six 1 grew. This, transferred to agar, grew apparently normally, but the cells showed a morphology decidedly different from that of the parent type (Plate I).

For confirmation, various other similar races were isolated from the stock, and all showed the same general characteristics. Not only was the morphology of cells grown on agar different, but, when transferred to ordinary bouillon, adherent masses, instead of the usually separate elements, were formed. These masses, sinking to the bottom, left the supernatant fluid clear, while the controls remained nearly uniformly cloudy. After cultivation at room temperature for over one year and passage through 54 agar transfers, the clumping tendency was as marked as before. The irregularity in the form of the cells became less marked after some months, and finally the culture became in this respect like the control.

The new race showed no fermentative characteristics different from the normal. This variation seems to be of the same general type as that previously described by me as occurring in *Bacterium coli*, where long chains, isolated, gave rise to races morphologically different from the parent stock.

Strains of dysentery which present new morphological characteristics have been described by several authors, the new strains proceeding from aberrant colonies rather than from selected aberrant cells. Kruse, Rittershaus, Kemp, and Metz found secondary colonies in dysentery and pseudodysentery cultures, which, when transplanted to bouillon, showed a growth having a tendency to clump. Baerthlein has described morphologically aberrant colonies not only in dysentery but in cultures of cholera vibrios and of typhoid, paratyphoid, and Gaertner's bacilli. In both the Shiga-Kruse and the toxin-free types of dysentery bacilli, he obtained colonies which differ in form of colony, morphology of bacilli, and in agglutinability from the parent types.

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*Loc. cit.*


28 *Centralbl. f. Bakt. etc., Ref.* (1911), 50, Beiheft, 128.

DISCUSSION

As stated above, variations in the fermentative characteristics of cultures have been observed by many writers. By most authors this phenomenon has been regarded as a mutation such as De Vries observed in higher plants, in that the new races seem to appear suddenly and in a relatively small number of individuals and the new characteristics are constantly transmissible. Pringsheim,\textsuperscript{30} however, regards a new characteristic such as the faculty of fermenting a given sugar as an adaptation to a new environment simply. Since sex and body cells are not separated in bacteria as in higher organisms, it is theoretically possible that such a new environment may impress upon the cells a characteristic which persists in the absence of the special stimulus. Such adaptive variations are not mutations in the sense of De Vries. Benecke\textsuperscript{31} holds similar views regarding the adaptive character of variations of the \textit{Bacterium coli mutabile} type. Burri\textsuperscript{32}, after extensive experiments on \textit{Bacterium coli mutabile} and \textit{B. coli imperfectum}, holds that the new fermentative characteristic may be exhibited by the descendants of all individuals of the parent stem, while in the mutations of De Vries they are present in only from 1 to 3 per cent. Further, he concludes from his experiments that the characteristic is, in strict sense, not new, but a latent characteristic become active. It is gradually acquired, while the De Vries mutation arises suddenly and fully formed. Josef Klein\textsuperscript{33}, working with \textit{Bacterium coli mutabile}, confirms the work of Burri and regards these variations as adaptations gradually acquired rather than mutations in the ordinary use of the term. Baerthlein\textsuperscript{34} holds that the above-described sudden appearance of new characteristics in strains of bacteria are true mutations. Such mutations, often indistinguishable from atavisms, have usually occurred in his experiments as the result of a change from a less favorable medium, such as is found in old agar or bouillon cultures, or in faeces, to a more favorable medium, as fresh agar. In other cases, the addition of various substances to the medium and the growth at high temperatures or the multiplication in the body of an infected animal seem to favor the production of mutations

\textsuperscript{30} \textit{Med. Klinik} (1911), 7, 144. Also, Die Variabilität niederer Organismen. Berlin (1910).


\textsuperscript{32} \textit{Centralbl. f. Bakt. etc.,} II Abt. (1910), 28, 321.

\textsuperscript{33} \textit{Zeitschr. f. Hyg. u. Infektionskrankh.} (1912), 73, 87.

\textsuperscript{34} \textit{Arb. a. d. kais. Gesundheitsamte} (1912), 40, 433.
in lower organisms. In all cases, we may conceive that, whatever the stimulus, the mutation consists in the change of latent hereditary units (Progenen) to active ones (Genen), whether the change appears as an advance or an atavism.

The maltose-fermenting race of dysentery bacilli isolated by me was obtained from ordinary agar cultures, not from secondary colonies on maltose. The appearance of the characteristic on a maltose-free medium would point to a variation of a nonadaptive character, and would indicate that we have to do with a parent race originally non-stable with regard to maltose fermentation. The new characteristic is fully formed at the start, and persists through many generations; but the comparatively large percentage of acid-forming cells in the first series and the apparent instability of the parent culture might exclude this variation from the catagory of mutation. It is true that any cell of the non-maltose fermenting type may originate an acid-producing race; but many generations may intervene before the maltose-fermenting cells arise. The final decision in this matter must depend upon the definition of the term mutation.

With regard to the other form of variation described here, that appearing in strain 42, we certainly approach more nearly to the characteristics of a true mutation. The variation certainly appears suddenly and fully formed. It appears in a relatively small number of individuals, it is not adaptive, and the new characteristics are transmissible to offspring through many generations. This new race is to be compared with the one described by me in Bacterium coli, in which the selection of certain long threads gave rise to races permanently different from the parent stock. There is the possibility of regarding both cases as degenerations, but after they became started both types showed as much vegetative vigor as the parent race, while still retaining new characteristics. Whether within the strict scope of the definition of mutation or not, both cases seem comparable to sports appearing vegetatively on higher plants and capable of indefinite propagation. Some of these variations may at first show less vegetative vigor than the parent and be none the less regarded as true sports.

SUMMARY

1. From a culture of Bacillus dysenteriae, Flexner type, derived from a single cell, 3 series of single-cell isolations were made at intervals of about five months. The first series gave 5 maltose-fermenting variants out of 21 isolations; the second, 5 out of 60;
the third, 1 out of 123. The other single-cell cultures as well as the parent culture render maltose alkaline.

2. The nonfermenting type produces secondary colonies consisting of normal and involution cells, either of which may develop acid- or alkaline-producing cultures. An ordinary transfer from a secondary colony, including many cells of both sorts, gives an acid-forming culture.

3. Selection from the acid-producing type failed to produce any but similar types, and selection from the alkaline-producing type gave only alkaline, provided secondary colonies were not chosen.

4. Mixed cultures, consisting of an equal number of cells of each type, showed that the two types may exist side by side through from 10 to 15 daily transfers, but with a tendency for the acid to outstrip the alkaline.

5. Transfer in maltose broth gave no increase in the acid-producing power except in old cultures.

6. Growth in various substances other than maltose failed to alter materially the characteristics of the two types.

7. In a specific serum, the two types showed approximately the same agglutination.

8. A permanent new race, characterized by morphological peculiarities, was obtained by the selection of an aberrant cell from a culture of dysentery of the Shiga-Kruse type. Since the special technique used in this research has been described in other publications, only the portion having to do with the making of one-cell cultures in series will be given here. A large cover glass (about 38 by 65 millimeters) is carefully cleaned, sterilized, and placed on the isolation chamber in the usual manner. Lines are ruled on the upper side with India ink or wax pencil, as shown in the illustration. Then, with a sterile pipette, bent at the tip, droplets of sterile broth about 2 millimeters in diameter are made on the undersurface of the cover, with a long drop a toward the open end of the box and a second large drop b near the other end.

Into a large drop a a small portion of actively growing culture is transferred by a loop or pipette. The chamber is now placed on the stage of the microscope, a fine-pointed pipette of the type used in isolation adjusted, and a considerable number of bacilli in medium dilution taken into the pipette from a. The

See reference, under note 1. A complete description of the pipette technique and its various applications is in preparation, and will be published in a subsequent number of this Journal.
rows of hanging drops are now brought into the field, and a small droplet ejected from the pipette in the immediate neighborhood of each (fig. 1). Each smaller droplet must be made to contain a single bacillus, and, if a proper dilution has been used, this is easily done. The small droplets must be made very small so as to be easily examined and the presence of a single cell determined. If the first droplet contains no bacilli, others may be made near it at the margin of the larger drop until one is obtained as desired. If two or more bacilli come out, all but one may be picked up and carried to the next large drop.

When as many as desired of the larger drops are supplied with the one-bacillus droplets, the pipette is withdrawn and a new sterile one introduced.

![Diagram](image)

*Fig. 1. Diagram of a microscopical slide to illustrate the method of single-cell isolation.*

This may be filled with sterile broth from drop b or from a test tube. With this pipette, broth is applied to each small droplet until, with its contained bacillus, it merges with the larger drop, or, broth is supplied to the larger drop until it overflows the smaller one.

The larger drop affords sufficient broth for good growth, and the purpose of the smaller droplet is, obviously, to permit of thorough examination in order to be sure of the presence of a single cell.

The cover is now removed from the box and sealed over a shallow moist chamber. The next day, transfers to test tubes may be made from each drop with a bent capillary pipette that is made new for each drop or by means of a fine platinum wire bent at the tip.

By this method one can quickly obtain a series of pure cultures from one source. If plump, actively growing cells are selected, practically all will grow. It is best to use a young culture grown two or three hours in the same broth as that used in the drops. Agar may be used in place of the broth in making the rows of drops. Isolations from two or more sources may be...
made on one cover, but it is usually best to employ separate covers for each. The nature of the growth in each drop may be examined microscopically before transfer, and a record kept by lettering and numbering the rows.

ADDENDUM

A special experiment was arranged to test the constancy of several different races of dysentery bacilli. All were isolated from stools of cases of dysentery occurring in Manila during the summer of 1912. There were 8 strains, 5 of them of the Shiga-Kruse type, 2 of the Flexner, and 1 with the characteristics of the Flexner but with a tendency to ferment lactose. Soon after isolation from the stools, a one-cell pure culture was made of each. This pure culture gave the same reactions as the stock on lactose-, glucose-, maltose-, and mannite-litmus agars. The cultures were now placed at room temperature on ordinary agar and transferred at about monthly intervals for about eleven months. At the end of this period fresh agar cultures were transferred to plain broth, glucose-broth fermentation tubes, litmus-lactose broth fermentation tubes, and to the following litmus agars: lactose, glucose, mannite, saccharose, maltose, levulose, dextrin, salacin, glycerine, erythrite, inulin, raffinose, galactose, amygdalin, and dulcite. The results of these cultures were the same as those observed eleven months before, and the one-cell pure culture gave the same reactions as the stock in every case. Apparently both the one-cell culture and the stock culture isolated from a colony had kept their characteristics constant during this period.

Well-marked secondary colonies were formed by 4 different strains, all of the Shiga-Kruse type. Two of these strains formed the secondary colonies only on saccharose-litmus agar, 1 on both saccharose and maltose, and 1 on saccharose and lactose. All secondary colonies were transferred to new tubes of litmus agar containing the appropriate sugar. All transfers from secondary colonies on saccharose gave acid-forming cultures, while those from the maltose and lactose gave alkali-forming cultures.

A test was made of the composition of the two strains which had produced secondary colonies only on saccharose. From a series kept on ordinary agar and never passed through saccharose, 45 single cells were isolated from one strain and 49 from the other, and the cultures from these 94 single cells were tested on saccharose-litmus agar. Only one culture proved to
be an acid-former. This culture lost its power of fermenting saccharose after several transfers on ordinary agar, and an acid-forming culture on saccharose also failed to form acid in subcultures. In order to test the composition of this originally saccharose-fermenting strain, 30 single cells were isolated from a culture which had been carried through several transfers on ordinary agar and which had never been grown on saccharose. None of the 30 cultures obtained from these cells formed acid on saccharose agar. Apparently the saccharose-fermenting variations of this strain of dysentery bacilli are less permanent than the maltose-fermenting variations of Flexner strain 105200.
ILLUSTRATIONS

Plate I.

Fig. 1. Parent culture of *Bacillus dysenteriae*, Shiga-Kruse type, strain No. 42.

2. A new race grown from a single cell isolated from the culture shown in fig. 1. Both are from 24-hour agar cultures grown under like conditions. Both had been grown about three days since the isolation of the new race, and had been passed through two agar cultures.

Text Figure

Fig. 1. Diagram of a microscopical slide to illustrate the method of single-cell isolation.

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Fig. 1. Parent culture of Bacillus dysenteriae, Shiga-Kruse type, strain No. 42.

Fig. 2. A new race grown from a single cell isolated from the culture shown in fig. 1.

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