PARASITOLOGY
PARASITOLOGY

EDITED BY

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OBSERVATIONS ON CERTAIN CESTODES OF RATS,
WITH AN ACCOUNT OF A NEW SPECIES
OF HYMENOLEPIS.

By H. A. BAYLIS, M.A., D.Sc.

(Published by permission of the Trustees of the British Museum.)

(With 4 Text-figures.)

During some investigations in which he has been engaged on the internal parasites of rats in the British Isles, Mr G. C. Dudgeon, of the Wellcome Bureau of Scientific Research, has kindly submitted to the writer examples of all the species of adult Cestodes found.

Two species of Hymenolepis are well known to occur in the brown and black rats, viz. *H. diminuta* and a smaller form most commonly referred to in literature as *H. murina*. The latter is of exceptional interest on account of the vexed question as to its identity with the human parasite *H. nana*. In more or less direct connection with this question a very extensive literature has grown up, which it is impossible to review fully here. It may be said, however, that the general result of researches by numerous authors, while still inconclusive, tends to show that the forms occurring in man and the rat are morphologically identical, while on physiological grounds there is some justification for regarding them as distinct species, subspecies, or at least varieties. This view is held on account of the many unsuccessful attempts that have been made to infect rats with *H. nana* of human origin, and on the other hand to infect man with "*H. murina*".

A few of the more important contributions to the subject may be mentioned. The celebrated researches of Grassi and his collaborators (1887–1892) along these lines led them to regard *nana* as a variety of "*murina*,” and they concluded that infection was capable of transmission from rats to man, though the experimental evidence was very scanty. On the other hand, on morphological grounds, as well as from considerations of geographical distribution, other authorities (Moniez, Blanchard, von Linstow) regarded the two forms as distinct species. The last-named author (1896) gives comparative measurements and other data compiled from his own and numerous other authors' observations, which he appears to have regarded as conclusive, but which have not proved equally convincing to later writers. Stiles (1906) favoured the separation of the forms as "host subspecies," and renamed "*H. murina*"
Hymenolepis longior n. sp.

H. nana fraterna. A recent important contribution to the question is that of Joyeux (1919). This author returns to the position taken up by v. Linstow, in regarding the forms as distinct species. He proposes to call the rat-parasite H. fraterna.

The supposed morphological differences between H. nana and "H. murina" must be discussed more fully later. It will be well, however, first to consider Mr Dudgeon's Hymenolepis material, which appears to have rather important bearings on this question.

Among the material there occur three forms, viz.

1) Hymenolepis diminuta (Rud.).
2) A form which may be called H. nana fraterna.
3) A form very closely related to the latter, but quite distinct, which does not appear to be referable to any of the other species of Hymenolepis from rats or their relatives, of which the descriptions are accessible to the writer (see list, p. 7). This form, accordingly, it is proposed to regard as a new species.

Hymenolepis longior, sp. n.

The worm is very slender, fragile and semi-transparent. Complete specimens attain a length of 45 mm., or even 60 mm. in a stretched condition. The maximum width of the strobila (usually occurring in the region of the gravid segments, but sometimes, in contracted specimens, more anteriorly), is from 0.42 to 0.53 mm. In exceptionally contracted individuals it may even reach 0.65 mm. The scolex is flattened dorso-ventrally, more or less rectangular in transverse section, and assumes very different shapes (Fig. 1)

![Fig. 1. Hymenolepis longior. Two scolices in dorsal or ventral view: A., with evaginated rostellum; B., with invaginated rostellum.](image-url)
according to the state of contraction. It is, in any case, considerably wider than the "neck" which follows it. Its diameter, measured from side to side, is from 0.21 to 0.26 mm., with a mean measurement of about 0.24 mm. The suckers, situated somewhat laterally, have an outside diameter of 0.075–0.093 mm. There is a well-developed rostellum, having a diameter of 0.07–0.08 mm., and armed with a single row of hooks of the characteristic shape, 21 or 22 in number (most commonly 22), and 19–20 μ in length.

The "neck" is unsegmented for some little distance behind the scolex. The total number of recognisable segments in the strobila may reach 600 or more. Rudiments of genital organs begin to be recognisable at about the 50th segment from the anterior end; "mature" segments—i.e. segments containing male and female organs in a state of full functional activity—at about the 250th. The number of gravid segments at the posterior end varies between (roughly) 60 and 100. The segments are broader than long throughout, though the length tends to become nearly equal to the breadth in the more posterior segments, and the ratio of length to breadth is (except in greatly contracted specimens) much higher in mature segments than in corresponding segments of H. nana or H. nana fraterna.

In a mature segment (Fig. 3) the most conspicuous organ is the ovary, which is large and compact, rather irregularly but not very deeply lobed, and occupies roughly one-third of the width of the segment, having a greatest diameter (from side to side) of about 0.15 to 0.17 mm. The ovarian ova have a diameter of 0.015 mm. The yolk-gland and shell-gland lie in the usual
positions with regard to the ovary. The three testes are relatively much smaller than in *H. nana* and *H. nana fraterna*, and, instead of being almost equal in diameter to the antero-posterior length of the segment, they occupy only a small space close to its posterior border. They are generally more oval in shape than those of the other species, which are almost spherical. Their greatest diameter varies from 0.05 to 0.075 mm. The middle testis varies

![Image of Hymenolepis longior](image1)

**Fig. 3. Hymenolepis longior.** Three mature segments; dorsal view. (From a whole preparation.)
c.s., cirrus-sac, with contained internal seminal vesicle; e., excretory canals; n., longitudinal nerve; ov., ovary; r.s., receptaculum seminis; s., shell-gland; t., testis; v., vitelline gland; v.s.e., external seminal vesicle.

![Image of Hymenolepis nana fraterna](image2)

**Fig. 4. Hymenolepis nana fraterna.** Two mature segments; dorsal view. (From a whole preparation.) Lettering as in Fig. 3.

in position with regard to the yolk-gland, and may lie dorsally to it or on either side of it. In the other species it seems to be always in the centre of the segment and dorsal to the yolk-gland. The genital pore is situated about the middle of the lateral border of the segment, or very slightly in front of it. The cirrus-sac, which measures about 0.15 mm. in length, passes, as usual in *Hymenolepis*, dorsally to both the longitudinal excretory vessels and to the
longitudinal nerve. It is club-shaped, as in the other species, and expands towards its inner end to a maximum width of 0-025–0-03 mm. It is almost completely filled by the internal seminal vesicle. There is also a pear-shaped external seminal vesicle (Fig. 3, v.s.e.), which occupies the space between the inner end of the cirrus-sac and the ovary. Ventrally to this lies a large receptaculum seminis (Fig. 3, r.s.), being the expanded inner end of the vagina, which runs along the ventral side of the cirrus-sac.

The passage from mature to gravid segments is very gradual. The latter are almost completely filled by the sac-like uterus, which contains a large number of ova. These have a thin outer membrane and a thicker, chitinised inner shell, between which there is a rather deeply-staining granular material which sometimes appears to form a third “membrane.” The inner shell is distinctly lemon-shaped, as opposed to the almost spherical inner shell of *H. nana* and *H. nana fraterna*, and it possesses at or near each pole a well-developed thickening. The terminal filaments present in the other species appear to be absent. Measurements taken by Mr Dudgeon and Dr A. C. Stevenson from ova in a fresh state in the faeces of infected rats were as follows: outer membrane, 49–60 μ × 42–48 μ; inner shell, 28-5–35-25 μ × 22-5–28-5 μ; the knobs at the poles measure 2–4 μ; onchosphere, 24–35 μ × 21–27 μ; length of embryonic hooks, 10-5–15 μ.

The following table of measurements and other data taken from Mr Dudgeon’s material will help to bring out some of the differences between *H. nana fraterna* and the new species.

<table>
<thead>
<tr>
<th></th>
<th><em>H. nana fraterna</em></th>
<th><em>H. longior</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total length</strong></td>
<td>Up to 20 mm.</td>
<td>Up to 45 mm. (60 mm. if stretched)</td>
</tr>
<tr>
<td><strong>Width of strobila (max.)</strong></td>
<td>0-27–0-51 mm.</td>
<td>0-42–0-53 mm.</td>
</tr>
<tr>
<td><strong>Width of scolex (great variation according to degree of contraction)</strong></td>
<td>0-16–0-23 mm.</td>
<td>Mean about 0-19 mm.</td>
</tr>
<tr>
<td><strong>Suckers, outside diameter</strong></td>
<td>Mean about 0-07 mm.</td>
<td>0-075–0-093 mm.</td>
</tr>
<tr>
<td><strong>Rostellum, diameter</strong></td>
<td>0-05–0-07 mm.</td>
<td>0-07–0-08 mm.</td>
</tr>
<tr>
<td><strong>Number of hooks</strong></td>
<td>22–26 (usually 22 or 23)</td>
<td>21–22 (usually 22)</td>
</tr>
<tr>
<td><strong>Length of hooks</strong></td>
<td>16–18 μ</td>
<td>19–20 μ</td>
</tr>
<tr>
<td><strong>Number of segments</strong></td>
<td>Up to 250</td>
<td>Up to, or over, 600</td>
</tr>
<tr>
<td><strong>Segment at which rudiments of genital organs begin to appear (roughly)</strong></td>
<td>35th</td>
<td>50th</td>
</tr>
<tr>
<td><strong>Segment at which mature genital organs appear (roughly)</strong></td>
<td>75th</td>
<td>250th</td>
</tr>
<tr>
<td><strong>Number of gravid segments (roughly)</strong></td>
<td>20–70</td>
<td>60–100</td>
</tr>
<tr>
<td><strong>Ova, diameter of outer membrane</strong></td>
<td>67-5–90 μ × 66–87 μ</td>
<td>49–60 μ × 42–48 μ</td>
</tr>
<tr>
<td><strong>Ova, diameter of onchosphere</strong></td>
<td>36–45 μ × 31-5–40-5 μ</td>
<td>24–35 μ × 21–27 μ</td>
</tr>
<tr>
<td><strong>Embryonic hooks, length</strong></td>
<td>15 μ</td>
<td>10-5–15 μ</td>
</tr>
</tbody>
</table>

1 These measurements were taken by Mr Dudgeon and Dr Stevenson from fresh ova in the faeces of rats.
Many authors have given measurements of *Hymenolepis nana* and “*H. murina*,” and it appears to the writer that, among the individuals ascribed to the latter form, some in reality belonging to the species now described as new have frequently been included. The new species appears, in fact, to have been confused with “*H. murina*” ever since the original description of the latter by Dujardin (1845), and to have been responsible for some of the alleged differences between it and the *H. nana* of man. The discrepancies in the measurements of total length given by different writers for “*H. murina*” are greater than could readily be accounted for by different states of contraction in complete individuals of the same species. 25 mm. is given by several authors as the maximum for *H. nana*, whereas “*H. murina*” is frequently said to reach 40 or 45 mm.—the latter figure agreeing with that given above for *H. longior*. According to Mr Dudgeon’s material, *H. nana fraterna* (the true “*H. murina*”) rarely exceeds 20 mm. in length, unless abnormally stretched. Other measurements, such as those of the maximum width of the strobila, the width of the scolex, and the diameter of the suckers, are so variable that little reliance can be placed upon them. They are certainly, as Joyeux (1919) has pointed out, useless as criteria for distinguishing *H. nana* from *H. nana fraterna*; and they seem to afford as little help in separating *H. nana fraterna* from *H. longior*. It may be mentioned that some of the extreme measurements given by previous writers, especially for the width of the strobila and the diameter of the suckers, find no parallel among the present material. Thus the width of *H. nana* has been recorded as reaching 0·7 mm., and that of “*H. murina*” 0·9 mm. Similarly the diameter of the suckers in *H. nana* is said to be sometimes as much as 0·105 mm. Reference to the table given above will show that nothing approaching these figures has been observed among Mr Dudgeon’s material, and it may be suspected that some of them, at least, are erroneous, or due to excessive artificial pressure on the specimens. Much, of course, depends upon technique in handling soft-bodied worms, and for this reason the value of such measurements is often questionable.

In the present case there is one character, depending only in part on measurements, which seems to be of great importance. The inner shell of the egg, being composed of a relatively hard, chitinoid substance, is not subject to alteration by pressure or the action of reagents to the same extent as the soft parts. It has been repeatedly insisted upon that the inner shell of the egg of “*H. murina*” is lemon-shaped, and provided at each pole with a well-developed knob-like thickening; whereas in the egg of *H. nana* the inner shell is more rounded, with the polar knobs scarcely distinguishable, but with a filamentous process at each pole. Unfortunately the measurements of the inner shell have seldom been given. Von Linstow (1896) gives the following:

*nana*: 0·028 mm. (exceptionally 0·029 × 0·024 mm.), usually spherical.

*murina*: 0·031 × 0·023 mm., lemon-shaped, with knobs at the poles.

The lemon-shaped inner shell, with polar knobs, is highly characteristic
of the ova of H. longior, and it seems almost certain that those authors who have laid stress upon this feature in their descriptions of “H. murina”—among them Dujardin himself—were dealing, as regards the ova, with H. longior.

There remains one point of considerable interest to be mentioned. As is well known, the researches of Grassi and his collaborators have led to a general acceptance of the theory that H. nana (and this covers also “H. murina”) normally passes through the whole of its life-history in the intestine of the same host, its cysticercoid living in the villi of the small intestine, and not requiring an intermediate host. Nicoll and Minchin (1911) found in the body-cavity of one of the fleas that infest rats in this country (Ceratophyllus fasciatus) a cysticercoid, of which they observe that, unless it is that of “H. murina,” it must be that of some undescribed form, the scolex of which is indistinguishable from that of “H. murina.” Johnston (1913) also found a cysticercoid in Australia in Ceratophyllus fasciatus and another rat-flea, Xenopsylla cheopis, which he regarded as that of “H. murina.” Now it seems highly probable, bearing in mind the great similarity between the scolices of H. nana fraterna and H. longior, that the latter is the adult form into which this cysticercoid develops, and that these cases cannot be taken as evidence that H. nana fraterna ever makes use of fleas as intermediate hosts.

The following species of Hymenolepis are recorded in Muridae1:

**With armed scolex:**

<table>
<thead>
<tr>
<th>Species</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>nana fraterna Stiles, 1906 (= Taenia murina Duj., 1845, c.p.)</td>
<td>Epimys rattus</td>
</tr>
<tr>
<td></td>
<td>E. norvegicus</td>
</tr>
<tr>
<td></td>
<td>E. alexandrinus</td>
</tr>
<tr>
<td></td>
<td>Mus musculus</td>
</tr>
<tr>
<td></td>
<td>M. pumilis</td>
</tr>
<tr>
<td>microstoma (Duj., 1845)</td>
<td>Micromys minutus</td>
</tr>
<tr>
<td></td>
<td>Echomys quercinus</td>
</tr>
<tr>
<td></td>
<td>Epimys rattus</td>
</tr>
<tr>
<td></td>
<td>E. norvegicus</td>
</tr>
<tr>
<td></td>
<td>Mus musculus</td>
</tr>
<tr>
<td></td>
<td>Epimys norvegicus</td>
</tr>
<tr>
<td></td>
<td>Mus musculus</td>
</tr>
<tr>
<td></td>
<td>Cricetus cricetus (C. vulgaris)</td>
</tr>
<tr>
<td></td>
<td>Mus variegatus</td>
</tr>
<tr>
<td></td>
<td>Epimys rattus</td>
</tr>
<tr>
<td></td>
<td>E. norvegicus</td>
</tr>
</tbody>
</table>

**With unarmed scolex:**

<table>
<thead>
<tr>
<th>Species</th>
<th>Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>diminuta (Rud., 1819)</td>
<td>Epimys rattus</td>
</tr>
<tr>
<td></td>
<td>E. norvegicus</td>
</tr>
<tr>
<td></td>
<td>E. alexandrinus</td>
</tr>
<tr>
<td></td>
<td>Mus musculus</td>
</tr>
<tr>
<td></td>
<td>Hesperomys pyrrhorhinus</td>
</tr>
<tr>
<td></td>
<td>Epimys norvegicus</td>
</tr>
<tr>
<td></td>
<td>? Mus musculus</td>
</tr>
<tr>
<td></td>
<td>Epimys norvegicus</td>
</tr>
<tr>
<td></td>
<td>Arvicola amphibius</td>
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**Scolex undescribed:**

<table>
<thead>
<tr>
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<th>Hosts</th>
</tr>
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<tbody>
<tr>
<td>asymmetrica Janicki, 1904</td>
<td>Micromys arvalis</td>
</tr>
<tr>
<td></td>
<td>Epimys norvegicus</td>
</tr>
<tr>
<td></td>
<td>Mus musculus</td>
</tr>
</tbody>
</table>

1 The descriptions of H. arvicolina, H. diminutoides and H. inexpectata Cholodkovsky, 1912, have unfortunately proved inaccessible. The first appears to occur in Microtus arvalis (Arvicola campestris), the two last in Epimys norvegicus.
Hymenolepis longior n. sp.

REFERENCES.


ADDENDUM.

Since the preparation of the paper on Hymenolepis from rats, the author has seen descriptions of H. diminutoides, H. inexspectata and H. arvicolina given by Cholodkovsky in Ann. Mus. Zool. Ac. Sci., Petrograd, xviii. (1913), pp. 227–229. Although the names are here marked “spec. nova,” they appear to have been first published in a catalogue of parasitic worms of the Army Medical Academy of Petrograd, in the previous year. It is this publication which was referred to as inaccessible, the redescriptions of 1913 having escaped notice.

H. diminutoides and H. arvicolina belong to the unarmed group of species. The description of H. inexspectata is very brief, and scarcely suffices to determine whether this species is distinct from that described by the writer as H. longior. The number and size of the hooks, the size of the suckers and the arrangement of the testes, as described, indicate differences which may be of specific importance, but the other differences are such as to be accounted for by a greater degree of muscular contraction, and it is impossible to lay any stress upon them.

H. diminutoides is recorded from Microtus arvalis (Arvicola campestris) as well as from the brown rat. H. straminea (Goeze, 1782), from Cricetus cricetus, should be added to the list of forms with armed scolex.
NOTE ON THE HABITAT AND STRUCTURE
OF CRASSICAUDA [NEMATODA].

By H. A. BAYLIS, M.A., D.Sc.

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(With 3 Text-figures.)

Complete specimens of this nematode are difficult to obtain, owing to the manner in which the worms bury themselves in the tissues of the urinogenital system of the Cetacea in which they are found. Mr A. G. Bennett, to whom the writer has previously been indebted on more than one occasion for Crassicauda material, has recently sent home further interesting specimens from South Georgia, accompanied by notes and a sketch which throw some light upon the burrowing habits of the worm. A special effort was made to obtain complete specimens by dissection.

In a previous note, in which an attempt was made to collect the records of the occurrence of this remarkable genus, the writer (1916) quoted an interesting passage from a report by Mr J. E. Hamilton, on the formation of "connective tissue masses" by the worms in the kidneys of Balaenoptera physalus. From Mr Bennett's observations it appears that "masses" or nodules of a similar nature may also be formed in other tissues. The accompanying diagram, which is adapted from Mr Bennett's drawing, shows the course taken by one of the worms in the tissues of the penis of a Balaenoptera (probably B. physalus). The caudal end of the worm hangs freely in the lumen of the urethra, through a perforation in the wall of which its body passes into the dense surrounding tissue. Through the kindness of Mr R. H. Burne, the writer has been enabled to compare Mr Bennett's sketch with a preparation of the penis of a Balaenoptera in the Museum of the Royal College of Surgeons. This makes it clear that the actual tissue invaded by the parasite is the dense fibrous sheath of the corpus cavernosum.

According to Mr Bennett's notes, the free portion of the worms varied from two to four inches in length. After traversing the fibrous tissue for some distance, the body passes into a dense nodule (A), where it becomes flattened and much coiled. The substance surrounding the worm in this nodule is in some cases putty-like, in others hard and apparently calcareous. The worm may be traced on again beyond this nodule for a longer or shorter distance, but eventually it passes into a second nodule (B) of pus and fibrous
tissue, in which the head is found. It is easy, therefore, to understand the difficulty hitherto experienced in obtaining an unbroken specimen of *Crassicauda*. The nodules, formed in the dense tissue of the penis or in other solid tissues, effectually prevent it from being pulled out; while its tortuous course renders the task of dissecting out the worm, without cutting it, a very difficult one, especially if the surrounding tissue has been hardened by reagents.

Mr Bennett succeeded, by cutting out one of the hard nodules (A) with the worm, in extracting the latter entire, and on examination it proved to be a male *C. crassicauda* (Crepl.). Anterior portions of females, almost certainly of the same species, were also obtained. This material enables some further details to be added to the description of *C. crassicauda*. The writer (1916, 1920) has published some notes on the anatomy, but the characters of the anterior end were still very imperfectly known. The cephalic papillae of a specimen thought to be *C. crassicauda* were described in the former paper, but in the second it was shown that at least two species exist, and this rendered the conclusions of the first less certain. In the light of the material now received, which is proved by the characters of the male tail to be *C. crassicauda*, it is possible to say that the characters of the head previously described agree with those of *C. crassicauda*. It is not, of course, possible to say whether they differ from those of *C. boops*, which is only certainly known from headless specimens. The accompanying figures (Figs. 2 and 3) show that the same papillae are present, and that they are arranged in the same way, as in the head of which an "en face" view was previously (1916) figured.

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**Fig. 1.** Diagram, adapted from a drawing by Mr Bennett, of an excised portion of the penis of *Balaenoptera*, with a specimen of *Crassicauda crassicauda* in situ. The upper part of the drawing is in transverse section. In the lower part, the skin has been cut longitudinally over the urethra and reflected on either side. The urethra is represented as slit open longitudinally.

- *c.*, cavernous core of corpus cavernosum; *f.*, fibrous sheath of corpus cavernosum; *s.*, skin of penis; *u.*, urethra; *X*, caudal end of worm hanging freely in urethra; *A, B*, successive nodules formed by the worm.
The structure of the oesophagus and other organs of the anterior region has not hitherto been described. The mouth, which is laterally compressed, leads into a similarly compressed buccal cavity, with very thick cuticular walls. This is followed by an oesophagus consisting of a relatively short anterior portion, which is scarcely, if at all, muscular, and a very long posterior portion, which is partly muscular and partly glandular. The latter portion may double upon itself several times in its course. An excretory pore has not been detected.
Measurements made from the complete male and an incomplete female were as follows (in millimetres):

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<th>Measurement</th>
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<td>0.23</td>
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<tr>
<td>Length of anterior portion of oesophagus</td>
<td>1.85</td>
<td>1.95</td>
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<tr>
<td>Thickness of anterior portion of oesophagus</td>
<td>0.11</td>
<td>0.16</td>
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<tr>
<td>Length of posterior portion of oesophagus</td>
<td>about 25.0</td>
<td>about 31.0</td>
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<tr>
<td>Thickness of posterior portion of oesophagus</td>
<td>0.3—0.4</td>
<td>0.47—0.8</td>
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<tr>
<td>Distance from anterior end to nerve-ring</td>
<td>0.5</td>
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The genus *Crassicauda* has hitherto been assigned tentatively to the family Filariidae, but the posterior position of the vulva is a character difficult to reconcile with this arrangement. It may be remarked that in this feature, as well as in the general structure of the oesophagus and buccal cavity, now described for the first time, *Crassicauda* somewhat resembles *Tetrameres* (= *Tropidocerca, Tropisurus*), a genus usually included in the Spiruridae, but placed by itself by some authors in a family Tetrameridae. The globular form characteristic of the mature females of *Tetrameres* is not, however, seen in *Crassicauda*.

REFERENCES.


OCCURRENCE OF Heligmosomum brasiliense Trav. IN ENGLAND.

By G. C. Dudgeon, C.B.E., F.E.S., etc.

Wellcome Bureau of Scientific Research.

In making the examination of a series of 400 rats, Heligmosomum brasiliense was found in 6 per cent. of those examined. The rats were all of the one species, Epimys norvegicus, and were chiefly from London and suburbs; the exceptions being one from Berkshire, one from Hampshire and two from Hertfordshire.

The worms are found, generally, in the upper third of the small intestine, and the measurements, where they differ from those previously described for this species, are given.

**Male.** In all cases, measured, the average length of the male is 2.8 mm. and the width 0.087 mm., giving a mean ratio of 32:1. The spicule averages 0.561 mm. The lobes of the bursa are asymmetrical in form; the latero-ventral and externo-lateral rays of the left lobe run parallel, and generally contiguous, throughout their length. The right lobe is much thickened and normally enrolled into a bar. The dorsal lobe is short and bears three rays, the central one terminating in two branches, which are again divided, the inner digitation bearing a small node on its outer edge, the outer digitations being slightly curved outwardly.

**Female.** The female averages 3.7 mm., with a thickness of 0.106 mm., giving a mean ratio of 34:1.

The worm has 14 longitudinal ridges visible in the median section; those of the lateral area are deeper and more prominent than either the dorsal or ventral ones. These ridges diminish in number towards the anterior end, and appear to be all present posteriorly, but of more uniform and much reduced dimensions.

A comparison of the measurements of British with those of Australian specimens may be of interest: Heligmosomum brasiliense Trav. was described and figured by T. Harvey Johnston, M.A., etc., in the Proceedings of the Royal Society of Queensland, vol. xxx. p. 56, for 1918, but with some rather remarkable differences in measurement. The general form of the worms from both continents is the same.
AN INTERESTING CASE OF CYSTICERCUS FASCIO-LARIS INFESTING THE BROWN RAT.

By A. T. HOPWOOD,

Assistant in Zoology, West Virginia University, Morgantown, W. Va.

(With Plate I.)

Cysticercus fasciolaris, the larval stage of the common tapeworm Taenia taeniaeformis (T. crassicolis), was recently found in great numbers in the liver of a brown rat. The rat measured 18 cm. in length and weighed about 275 g.; it was vigorous and apparently healthy when caught, but when killed and examined, its liver was found to be heavily infested with the cysticerci.

This cestode in itself is common enough, but the great number of cysticerci attracted attention. On the ventral surfaces of the ventral lobes of the liver which are shown in the photograph (Pl. I), 108 of the cysts were visible and this region was the least infested. The dorsal surfaces of these lobes as well as the entire surfaces of the others harboured cysts almost to their full capacity.

It is difficult to conceive how many more of the larvae could possibly have found room to encyst themselves. There was visible a total of 256 larvae, about 50 of which were visible from both surfaces. Many more may have been embedded in the tissue and invisible from either surface.

Possibly not more than nine-tenths of the liver was incapacitated and the bile ducts were not greatly obstructed, which probably explains why the animal was still apparently healthy. The cysticerci were identified in the laboratory and their determination was confirmed by reference to the Zoological Division of the Bureau of Animal Industry of the U. S. Department of Agriculture.
Brown Rat infested by *Cysticercus fasciolaris*.
THE LIBRARY
OF THE
UNIVERSITY OF ILLINOIS
ON A NEW CILIATE, BALANTIDIUM BLATTARUM, SP. NOV., INTESTINAL PARASITE IN THE COMMON COCKROACH (BLATTA AMERICANA).

By EKENDRANATH GHOSH, M.Sc., M.D.,
Professor of Biology, Medical College, Calcutta.

(With 1 Text-figure.)

The parasite herein described was found in the intestinal contents of Blatta americana at Calcutta. The species seems to be comparatively rare and was observed twice only. It may be named B. blattarum sp. nov.

Diagnosis: Body irregularly pyriform, slightly less than twice as long as its greatest transverse diameter, and circular in transverse section. Anterior end tapering, rounded and slightly bent to the side opposite to the peristome. Posterior end obliquely truncate and depressed in the middle. Side opposite to the peristome longer than the other, being slightly concave in front and convex behind. Side with the peristome short and convex. Peristome small, about one-third the body in length, somewhat cylindrical and directed backwards and medianwise. A large undulating membrane along the anterior margin of the peristome, and a row of stout cilia along the posterior margin. Body cilia small and arranged closely. Endoplasm coarsely granular and surrounded by a distinct hyaline ectoplasm. Macronucleus spherical, and placed behind the peristome in the middle of the body and somewhat towards the shorter side. A large contractile vacuole posteriorly. Length 0.09 mm. (See Text-fig.)

Balantidium Clap. and Lach., was emended by Bütschli (in Bronns, Tierreich, Protozoa, 3 Abt., pp. 1723–1725), by the removal of two species (B. duodenii and B. rotundum) which were placed together in a separate genus: Balantidiopsis. He characterised Balantidium by its round transverse section and its habitat in the large intestine, and Balantidiopsis by its flattened body and its habitat in the small intestine.
Balantidium blattarum *n. sp.*

Scheier (*Arb. d. St Petersburger naturf. Gesellsch. xxviii. No. 4*) defines the two genera in the following manner: *Balantidium*: body egg-shaped or cylindrical; contractile vacuoles numerous; macronucleus oval or horseshoe-shaped. *Balantidiopsis*: body broadly oval; contractile vacuole single, posterior; macronucleus spherical.

This year I described two new species of *Balantidium* and *Balantidiopsis* (*Bull. Carmichael Med. College, No. 2*) to which I appended synopses of the known species of both the genera. But the recognition of a large number of species of both the genera has made all the distinguishing characters fail one after another, viz. the number of contractile vacuoles, the form of the macronucleus and the nature of the habitat. Lastly, the discovery of species with oval transverse section of the body breaks the last distinction between the two genera. Under these circumstances it seems necessary to reunite the two genera into *Balantidium* as already suggested by Bezzenberger (*Arch. f. Protistenk. III. 154–156*).

As regards habitat, the already known species of *Balantidium* have been found in the gastric cavity of *Hydra* (*B. hydrae, Entz, Arch. f. Protistenk. 1912, xxvii.*) and medusa, and in the intestinal canal of the mollusc and of many vertebrates, specially the amphibians. The present species seems to be the first described from the intestinal tract of an arthropod.
INTRODUCTION.
Some observations on the biology and structure of Ornithodorus moubata have been recorded in a recent communication to this journal (Cunliffe, 1921) and in the following pages all references to this species refer to these observations, unless otherwise stated. Prof. Nuttall received living material of O. savignyi from Dr J. H. Ashworth in 1911 (from Aden, Quick Lab. Cat. No. 1575) and again in 1913 (no data, Cat. No. 2011). These stocks being available for breeding experiments, a few observations on the biology and structure of this species were made in the years 1913–14.

Even at the present time, details of the life-cycle of O. savignyi appear to be unknown. Howlett (1916) and Fletcher (1919) have both stated that this...
tick was being studied at Pusa, but their results are apparently as yet un-published. Nuttall and Warburton (1908) only note that "there are at least two nymphal stages, if not more." These facts and also that *O. savignyi* is a potential disease carrier justify the publication of these incomplete notes, made under laboratory conditions. This investigation did not yield the same amount of information as was obtained in the case of *O. moubata*, as the two were run together, and it was not possible to rear successfully large numbers of individuals of both species. As would be expected, the results of this second investigation are, for the most part, in agreement with those of the first, and where confirmation is complete, discussion of the records is omitted for economy of space, the reader being referred to the paper on *O. moubata*.

**SECTION I. BIOLOGY OF *O. SAVIGNYI*.**

It is unnecessary to recapitulate the experimental procedure, which was the same throughout as that adopted for *O. moubata*. Similar also was the aim of the investigation, namely, the determination of the number of nymphal stages passed through by this tick before reaching maturity, of the effect of temperature and moisture on the duration of the stages and of the changes taking place in the external anatomy of the ticks at each stage of development. As before, some notes were made on oviposition, copulation, longevity and engorgement. Females, with their progeny, from each stock were reared separately, but their records, being similar, are not shown separately in the following synopses of results.

*Experimental records relating to females kept at different temperatures.*

Three series of female ticks were maintained at 22°, 30° and 37° C. respectively, but only the second series bred at all successfully, as shown in Synopsis I.

*Females and progeny maintained at 22° C.*

A series of six females, which emerged from the last nymphal stage between May and July, 1913, although they fed well and were fecundated on one or more occasions, failed to oviposit until about the 400th day. The larval yield was too small to promise success in rearing and the experiment was discon-tinued. Females which emerged about the same time from the same stock, reproduced very successfully at 30° C. However, it is considered that a repetition of this experiment would show that 22° C. is not too low a tempera-ture for reproduction by this species.
**Synopsis I. Females and progeny maintained at 30° C.**

Time reckoned in days from date of emergence of female.

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<td></td>
<td>50</td>
<td></td>
<td>108</td>
<td></td>
<td>49-50</td>
<td>62 57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>78</td>
<td></td>
<td></td>
<td></td>
<td>60-61</td>
<td>76 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>155</td>
<td></td>
<td></td>
<td></td>
<td>65</td>
<td>34 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>196</td>
<td></td>
<td></td>
<td></td>
<td>67</td>
<td>20 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>203</td>
<td></td>
<td></td>
<td></td>
<td>96</td>
<td>26 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>290</td>
<td></td>
<td></td>
<td></td>
<td>118</td>
<td>8 nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>173</td>
<td>19 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>207</td>
<td>11 nil</td>
</tr>
</tbody>
</table>

* The subsequent history of these larvae is summarised in Synopsis II.*
Females and progeny maintained at 37° C.

Two separate series of females were maintained at this temperature, the first set being started in November 1912, and the second in July 1913. Out of a total of 11 females only four oviposited, the records being as follows: ♀1, 92 eggs after 19–28 days (from date of emergence) followed by 14 eggs after 69 days; ♀2, 100 eggs after 16–17 days; ♀3, 8 eggs after 46 days followed by 56 eggs after 78–86 days; ♀4, 71 eggs after 69–74 days. These females were fed for another six months without further oviposition taking place and they were then discarded. Larvae emerged from 65 per cent. of the eggs, but reference to Synopsis II, in which their subsequent history is summarised, will show that none of them succeeded in passing the third nymphal stage at this temperature.

Oviposition.

When first deposited the egg is yellow in colour; it rapidly turns brown, more slowly becomes pitchy-brown and finally, in the course of three or four days, becomes jet-black. The agglutinative coating soon loses its efficacy in this species. The data, relating to oviposition, are summarised in the following table, minimum, maximum and mean results being shown:

<table>
<thead>
<tr>
<th>Ticks maintained at 30° C.</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of days before oviposition occurred after ♀ was allowed access to ♀</td>
<td>15 (aberrant case 47)</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>No. of eggs deposited after each feed (when oviposition occurred)</td>
<td>4</td>
<td>174</td>
<td>62</td>
</tr>
<tr>
<td>No. of days between dates of feeding and oviposition commencing or recommencing</td>
<td>5</td>
<td>35</td>
<td>18</td>
</tr>
<tr>
<td>No. of days over which oviposition extended after each feed</td>
<td>1</td>
<td>50</td>
<td>9</td>
</tr>
<tr>
<td>No. of eggs deposited by a ♀</td>
<td>100</td>
<td>417</td>
<td>219</td>
</tr>
<tr>
<td>Percentage of fertile eggs</td>
<td>62 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean results are very closely comparable with those obtained in the case of *O. moubata* (at 30° C.), except that the number of eggs oviposited by the female is less by 40 per cent.

No evidence of parthenogenesis.

Parthenogenetic reproduction did not occur during the course of these experiments. Virgin females, which emerged in October 1913, were fed repeatedly, and maintained at 30° C. between meals, but they failed to oviposit during the following eight months. On the other hand, it has been shown that gorged females oviposit regularly about 17 days after the males are allowed access to them (at 30° C.).

Longevity of the female tick.

When performing their normal functions and maintained at 30° C., seven females lived for minimum, maximum and mean periods of 292, 420 and 358 days respectively. Under similar conditions at 22° C., the female had an average life of 775 days (three individuals).
Duration and number of the nymphal stages at 30° and 37° C.

(a) Experimental Data.

Some of the progeny of each female were reared in separate batches to the adult stage, to establish the duration and number of the nymphal stages. The records are summarised in Synopsis II, minimum, maximum and mean periods, together with the numbers of individuals observed, being given. As in *O. moubata*, the larva\(^1\) passes into the first nymphal stage without previous engorgement; from the first nymphal stage onwards, the periods required for ecdysis are reckoned from the date of the previous meal and not that of the previous moult.

Synopsis II. Duration of stages in days.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Ticks kept at 30° C.</th>
<th>Ticks kept at 37° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Egg to larva</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>Egg to 1st (♀)</td>
<td>13</td>
<td>32</td>
</tr>
<tr>
<td>1st to 2nd (♀)</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>2nd to 3rd (♀)</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>3rd to 4th (♀) or adult</td>
<td>(♀) 7</td>
<td>49</td>
</tr>
<tr>
<td>4th to 5th (♀) or adult</td>
<td>(♀) 11</td>
<td>25</td>
</tr>
<tr>
<td>5th to 6th (♀) or adult</td>
<td>(♀) 15</td>
<td>26</td>
</tr>
<tr>
<td>6th to 7th (♀) or adult</td>
<td>(♀) 14</td>
<td>23</td>
</tr>
<tr>
<td>(♂) 15</td>
<td>No record</td>
<td>(♂) 15</td>
</tr>
</tbody>
</table>

* These eight individuals were the only survivors out of 121 second stage nymphs and as third stage nymphs they refused to feed.

† Probably abnormal.

(b) Discussion of Data.

As with *O. moubata*, males appeared after four to six and females after five to seven ecdyses, 63 per cent. of the males appearing after the fifth ecdysis and 60 per cent. of the females after the sixth ecdysis. As, however, only 66 individuals were reared to maturity, these percentages may require correction. The mean minimum periods required for the metamorphosis of an individual from egg to adult, at 30° C., under laboratory conditions, are

1. for males, 60 (corrected), 73 or 89 days according to whether the individual undergoes four, five or six moults;
2. for females, 73, 88 or 103 days, the number of moults varying from five to seven.

These periods are longer by an average of 27 days than in the case of

1 The larva normally frees itself from the egg shell, but is otherwise inactive. In some cases, however, the species approaches *O. moubata*, inasmuch as the larva may undergo ecdysis without throwing off the shell. The feeble development of the hypostome, legs and claws and also the absence of eyes is doubtless correlated with the passiveness of this stage.
O. moubata, reared under similar conditions. An increase in temperature of 7° C. (from 30° C.) only reduced the period required for the production of third stage nymphs by 26 per cent.

**Duration and extent of engorgement.**

An experiment, similar to that carried out with O. moubata, was conducted with this species, ten nymphs of different stages being kept under observation until they reached maturity. Their weights before and after engorgement and the times of attachment to the host were accurately recorded. Again there was no regularity in the time of attachment, observation on 29 individuals of all stages giving minimum, maximum and mean times of 10, 74 and 26 minutes respectively, at room temperature, about 16° C. The figures are in agreement with those of Drake-Brockman (1915). The mean time recorded for O. moubata was 48 minutes, thus O. savignyi is apparently a more rapid feeder. Of the ten nymphs under observation, only four reached maturity, one being male and three female. In the female series, the mean increase in weight in grams was (a) before the final moult, 0.0647, and (b) after the moult 0.2202; the corresponding figures for the solitary male were (a) 0.0528, and (b) 0.0246. None of these figures were reached by O. moubata.

**The influence of moisture on vitality and ecdysis.**

A similar experiment to that carried out with O. moubata proved that, for this species also, excess of moisture is injurious to the individual. A batch of 33 first stage nymphs from the same female was divided into three lots, which between meals were maintained at 30° C. in saturated, moist and dry atmospheres respectively. A summary of the experimental records is given in the following table, in which are shown the minimum and maximum number of days required for ecdysis, with the mean in brackets and the number of individuals observed in square brackets:

<table>
<thead>
<tr>
<th>Atmosphere</th>
<th>2nd ©</th>
<th>3rd ©</th>
<th>4th ©</th>
<th>5th ©</th>
<th>6th ©</th>
<th>% of ticks which matured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>9—13 (11)</td>
<td>11—14 (13)</td>
<td>23—48 (40)</td>
<td>21</td>
<td>—</td>
<td>9%</td>
</tr>
<tr>
<td>Moist</td>
<td>7—8 (8)</td>
<td>9—32 (12)</td>
<td>10—18 (12)</td>
<td>11—17 (14)</td>
<td>—</td>
<td>27%</td>
</tr>
<tr>
<td>Dry</td>
<td>7—9 (7)</td>
<td>9—13 (10)</td>
<td>11—16 (12)</td>
<td>10—14 (12)</td>
<td>13—17 (15)</td>
<td>45%</td>
</tr>
</tbody>
</table>

Excess of moisture was therefore a decidedly unfavourable factor, as under this condition only 9 per cent. of the ticks matured, whereas in the dry atmosphere 45 per cent. matured. The ecdysis period was not markedly affected by the presence of moisture (vide Synopsis II), until after the third nymphal stage was attained, when the lack of vitality was indicated by a lengthening of this period.

1 The 1st nymphs were fed on 17. i. 13 and the 5th nymphs were fed or offered a meal on 13. vi. 13.
N. CUNLiffe

SECTION II. STRUCTURE OF O. SAVIGNYI.

Observations on the dimensions of the egg and unfed specimens of the different stages and the changes in form undergone during development by the hypostome, the fourth tarsus and the spiracle are included in this section.

Dimensions of the egg.

A total of 179 eggs were measured immediately after deposition by four different females, the minimum, maximum and mean measurements of length \( \times \) breadth being 1-0 \( \times \) 0-9, 1-8 \( \times \) 1-4 and 1-4 \( \times \) 1-1 mm. respectively. The minimum and mean measurements of the eggs of this species equal the mean and maximum measurements of those of O. moubata.

Dimensions of unfed stages.

As no records of the dimensions of the different stages appear in the literature on this species, measurements of material bred under control from one stock are summarised in Synopsis III. The number of individuals preserved was, in several cases, too small to give very accurate results, although as far as possible extreme examples were chosen for preservation.

Synopsis III. Dimensions of stages.

Measurements in mm. to nearest tenth, from unfed specimens preserved shortly after emergence in 70 % spirit.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Min. Length ( \times ) Breadth</th>
<th>Max. Length ( \times ) Breadth</th>
<th>Mean Length ( \times ) Breadth</th>
<th>No. of individuals observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larva</td>
<td>1-2 ( \times ) 1-0</td>
<td>1-5 ( \times ) 1-2</td>
<td>1-4 ( \times ) 1-1</td>
<td>52</td>
</tr>
<tr>
<td>1st</td>
<td>1-3 ( \times ) 1-0</td>
<td>1-8 ( \times ) 1-4</td>
<td>1-5 ( \times ) 1-2</td>
<td>107</td>
</tr>
<tr>
<td>2nd</td>
<td>1-5 ( \times ) 1-1</td>
<td>2-6 ( \times ) 1-9</td>
<td>2-1 ( \times ) 1-5</td>
<td>119</td>
</tr>
<tr>
<td>3rd</td>
<td>2-2 ( \times ) 1-5</td>
<td>3-6 ( \times ) 2-6</td>
<td>3-0 ( \times ) 2-1</td>
<td>16</td>
</tr>
<tr>
<td>4th</td>
<td>3-1 ( \times ) 2-3</td>
<td>5-6 ( \times ) 3-9</td>
<td>4-4 ( \times ) 3-1</td>
<td>14</td>
</tr>
<tr>
<td>( \hat{\delta} ) from 3rd</td>
<td>5-2 ( \times ) 3-8</td>
<td>6-0 ( \times ) 4-3</td>
<td>5-7 ( \times ) 4-0</td>
<td>3</td>
</tr>
<tr>
<td>5th</td>
<td>3-9 ( \times ) 2-8</td>
<td>6-5 ( \times ) 4-6</td>
<td>5-6 ( \times ) 3-9</td>
<td>7</td>
</tr>
<tr>
<td>( \hat{\delta} ) from 4th</td>
<td>5-2 ( \times ) 3-5</td>
<td>6-7 ( \times ) 4-6</td>
<td>6-0 ( \times ) 3-9</td>
<td>25</td>
</tr>
<tr>
<td>( \hat{\alpha} ) from 4th</td>
<td>7-0 ( \times ) 4-9</td>
<td>7-8 ( \times ) 5-6</td>
<td>7-4 ( \times ) 5-2</td>
<td>4</td>
</tr>
<tr>
<td>6th</td>
<td>5-0 ( \times ) 3-4</td>
<td>8-5 ( \times ) 5-7</td>
<td>6-7 ( \times ) 4-8</td>
<td>6</td>
</tr>
<tr>
<td>( \hat{\delta} ) from 5th</td>
<td>5-8 ( \times ) 3-8</td>
<td>7-4 ( \times ) 5-6</td>
<td>6-5 ( \times ) 4-7</td>
<td>15</td>
</tr>
<tr>
<td>( \hat{\alpha} ) from 5th</td>
<td>6-7 ( \times ) 4-7</td>
<td>9-2 ( \times ) 6-7</td>
<td>8-1 ( \times ) 5-7</td>
<td>14</td>
</tr>
<tr>
<td>7th</td>
<td>6-4 ( \times ) 4-4</td>
<td>9-3 ( \times ) 6-7</td>
<td>7-8 ( \times ) 5-5</td>
<td>2</td>
</tr>
</tbody>
</table>

* Probably not true minima.

As in O. moubata, there is considerable variation in the size of individuals of any one stage, maximum measurements in one stage exceed minimum measurements in the succeeding stage, and males of any one stage are only slightly larger, whereas females are distinctly larger, than nymphs of the equivalent stage. In O. moubata, the greatest growth took place in the second nymphaal stage but in this species it would appear to be spread equally over the second and third nymphaal stages, the percentage increases in length being 7-3 (larva to 1st \( \hat{\alpha} \)), 40-0, 42-8, 46-7, 27-3, 19-7 (5th to 6th \( \hat{\alpha} \)), while the
Ornithodorus savignyi
corresponding increases in breadth are 9.1, 25.0, 40.0, 47.7, 25.8 and 23.1 per cent. respectively. The percentage increases in length and breadth from nymphal to adult stages correspond closely in both species, e.g. the percentage increases in length x breadth in *O. moubata*, from fourth nymph to male, are 32 x 28—while in *O. savignyi*, the increases are 36 per cent. x 26 per cent.

Fig. 1. *Ornithodorus savignyi*, hypostomes in ventral aspect: (b) to (g) of 1st to 6th stage nymphs; (i) and (j) of females from 4th and 6th stage nymphs; (k) and (l) of males from 3rd and 5th stage nymphs. (N. C. del.)

Changes in external anatomy undergone during development.

(a) The Hypostome. Fig. 1.

The changes in the dentition of the hypostome during the development from first nymph to adult are indicated in Fig. 1, (b) to (g) representing the nymphal organs arranged in order of development, (i) and (j) the hypostomes of females emerging from fourth and sixth nymphs, and (k) and (l) of males from third and fifth nymphs respectively.

On comparison with the hypostomes of *O. moubata*, it will be observed that in this species also several proximal rows and the distal row of teeth are equally poorly developed and their arrangement difficult to determine, that in the first and second nymphal stages the number and arrangement of the teeth are very similar, but that in the later stages the number of teeth is considerably less, owing to the presence of smaller numbers of rows and files. The changes taking place during development consist of an increasing com-
plexity of dentition, correlated with an increasing size of hypostome and they are of much the same order in both species.

In many cases the teeth are not arranged symmetrically on the two sides of the hypostome, the rows on one side appearing to alternate with the rows on the other side.

(b) The Legs and Spiracle. Figs. 2 and 3.

Outlines of the terminal portions of the fourth legs and of the spiracular plates are shown in Figs. 2 and 3 respectively, for the following stages, namely, first to sixth nymph (a) to (f); males from third and fifth nymphs (h) and (i); and females from fourth and sixth nymphs (j) and (k).

The degree of development of these structures at each stage is similar in the two species, *O. savignyi* and *O. moubata* and needs no further discussion.

SUMMARY OF RESULTS.

1. The biology, as studied under laboratory conditions, of *O. savignyi* is very similar to that of *O. moubata*.
2. Females may oviposit over 400 eggs, of which at least 60 per cent. may be fertile; parthenogenesis does not occur.
3. An increase in temperature of 8° C. (from 22° C.) decreases the longevity
Ornithodoros savignyi

of the female from 775 to 358 days, i.e. by 45 per cent.; an increase of 7° C. (from 30° C.) reduces the period required for the production of third stage nymphs by 26 per cent. At 30° C., the mean minimum periods required for metamorphosis are 60 days for males and 73 days for females (cf. O. moubata, 36 days for males and 45 days for females). Reproduction was inhibited at 37° C.

4. Moisture is an unfavourable factor, decreasing the vitality of the individual at each stage of growth.

5. Changes in external anatomy undergone during development are similar to those already described for O. moubata.

REFERENCES.


A PRELIMINARY NOTE ON PARASITES INFESTING DOMESTICATED SILVER BLACK FOXES IN CANADA¹.

By J. A. ALLEN, B.V.Sc.,
*Animal Pathologist, in charge of Fox Research Station, Charlottetown, P.E.I.*

AND A. B. WICKWARE, V.S.,
*Animal Pathologist, Biological Laboratory, Ottawa, Ont.*

(From the Health of Animals Branch, Dept. of Agriculture, Canada.)

The growing importance of the silver black fox industry in Canada, and latterly in other countries, has been so marked, that scientific workers have turned their attention to a study of the maladies affecting this species of fox.

A survey has been made by one of the writers (J. A. A.) of the benign and contagious forms of disease to which domesticated foxes are subject, and papers of a popular and scientific nature have already been published in various journals.

In the course of recent investigations dealing with a distemper-like form of disease causing a high mortality amongst foxes, a determination of the species of internal and external parasites was undertaken.

As pointed out by Riley in his recent paper², "An Annotated List of the Animal Parasites of Foxes," very little literature is available on this subject, and it is for the purpose of adding a small contribution that the parasites found by us to date are herewith recorded.

We take especial pleasure in acknowledging the courtesy shown by Drs B. H. Ransom, Chief Zoologist, Bureau of Animal Industry, Washington, D.C.; Maurice C. Hall, Senior Zoologist, Washington, D.C.; and F. C. Bishopp, Entomologist, Bureau of Animal Industry, Dallas, Texas, in identifying the listed parasites, and to the various fox ranchers who have provided us with animals for autopsy.

PROTOZOA.

*Coccidium bigeminum—Isospora bigemina* (Stiles, 1891).

Small and large intestine.

ACARINA.

*Sarcoptes scabiei vulpis* (Fürstenberg, 1861) Railliet.

Body of host.

*Otodectes cynotis* (Hering, 1838).

Ears of host—external meatus.

¹ Published with the approval of Dr F. Torrance, Veterinary Director General.

Parasites infesting Domesticated Black Foxes in Canada

SIPHONAPTERA.
Ctenocephalus canis (Curtis, 1826). Body of host.

NEMATODA.
Eucoleus aerophilum (Creplin, 1839). Trachea and large bronchi. Eggs found constantly in trachea, oesophagus and faeces. Small intestine.
Belascaris cati (Schrank, 1788)
Belascaris marginata (Rudolphi, 1802)
Toxascaris limbata (Railliet and Henry, 1911)
Sometimes B. marginata penetrates the bile duct and develops in the liver. Immature ascarids found in fox pups less than one week old. Quite common to find intestinal canal from stomach to large colon completely packed with ascarids in young foxes only three to four weeks old. One seldom finds more than one or two ascarids in adult foxes. Small intestine.

Uncinaria polaris (Looss, 1911).

TREMATODA.
Echinochasmus sp. Small intestine.
ON THE DIPTEROUS GENERA PASSEROMYIA AND ORNITHOMUSCA, WITH NOTES AND BIBLIOGRAPHY ON THE NON-PUPIPAROUS MYIODARIA PARASITIC ON BIRDS.

By Professor M. BEZZI, Turin, Italy.

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I. PASSEROMYIA AND ORNITHOMUSCA.

In 1851 Macquart described two new species of the Dipterous genus Cyrtoneura from Tasmania, under the names of Cyrt. longicornis and Cyrt. analis, both species being very different from all others of the same genus in having hairy eyes and the first species also owing to its very long third antennal joint.

They were never recorded by subsequent writers until 1916 when Tyler Townsend describing his new Australian genus Ornithomusca expressed doubts as to the relationship of the above mentioned Macquart's species to his own genus (p. 145). In 1919 the late Prof. Stein in his Catalogue of the Anthomyidae of the World (p. 111) recorded these two species, showing however that they do not belong to the genus Muscina (which now comprises several species of the old genus Cyrtoneura) and that they cannot be placed in any known genus of Anthomyidae.

I have recently received from Mr E. W. Ferguson two specimens of a fly, one bred at Sydney, N.S.W., from a larva found in a nestling of the New Holland Honeyeater, the other caught in the open. Both these specimens I have recognised as belonging to the species Cyrtoneura longicornis Macquart. On the other hand, the type specimen of Ornithomusca victoria Tyler Townsend has been also found in nests of Pardalotus sp. and according to the description differ from longicornis only in the colour of the dust of the head, palpi, calypters, the base of the wings and the hairs of the body so that it is
The habit of the above-named Australian fly *Cyrt. longicornis* living upon the bird nestlings recalls that of the African species belonging to the recently established genus *Passeromyia*, and the comparative study of the specimens of the Australian and African forms shows that they are very closely allied. The wide distribution of the African species indicates moreover the importance of a careful study of specimens of this species from the Oriental region. In fact, to this group belongs the species *Muscina longicornis* from Java, previously described by Stein (1909, p. 221). He noticed in this species the elongated third antennal joint, but has overlooked the hairiness of the eyes. In 1915 Dr Villeneuve described under the name *Muscina heterochaeta* a new African fly showing aberrant characters. The larvae of this fly have been previously described by Rodhain (1914, p. 214). The same year (1915) Rodhain and Villeneuve established for this African species the new genus *Passeromyia* and in 1916 appeared a very important paper by Rodhain and Bequaert on the structure and life-history of this fly (*Passeromyia heterochaeta*).

Professor Stein in a recent letter (1920) informed me that the specimens of *Passeromyia heterochaeta* sent to him by Dr Villeneuve, are identical with his paratypes of *Muscina longicornis*. I was able myself to compare the paratype of *longicornis* of my collection with Australian specimens and found that they were strictly congeneric; it is even a matter of some difficulty to distinguish the Australian species from the African. It can be concluded now that the genus *Ornithomusca* Tyler Townsend 1916 is synonymous with the genus *Passeromyia* Rodhain and Villeneuve 1915, and that the type species *heterochaeta* Villeneuve 1915 is synonymous with *longicornis* Stein 1909. As the last name is already preoccupied by Macquart’s species, Villeneuve’s name *heterochaeta* must be retained.

The two species of the genus *Passeromyia* can be distinguished as follows:

1 (2). The dust of the entire body, chiefly that of the abdomen of the male, is of a distinct bluish tint; third antennal joint about nine to ten times longer than the second joint, and extending almost to the mouth border; bristles of all femora less numerous, shorter and thinner; hind tibiae shortly ciliated; fourth longitudinal vein with the two portions on either side of the bend, of nearly the same length; average size 7 mm. *longicornis* Macquart.

2 (1). Dust of the body grey or slightly bluish on sides of the abdomen of the male; third antennal joint seven or eight times longer than the second

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1 Another question arises in connection with the species *Cyrtoneura analis* Macquart. This species was described from a type specimen with the second and third joint of antennae missing, and it is therefore not certain that this species belongs to the genus *Ornithomusca*. The description of the abdomen recalls the fly *Synthesiomyia nudiseta* which was originally described as *Cyrtoneura* and which is present even in Australia. The hairy eyes and the black first antennal joint of *analis* do not agree with *nudiseta*. Bigot in 1877 (p. 250) placed the species *analis* in the genus *Graphomyia* but removed it in 1887 (p. 584) to the genus *Dasyphora*. 

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and not reaching the border of the mouth; bristles of the underside of the femora longer, stronger and more numerous than in previous species; hind tibiae with longer ciliation; the portion of the fourth vein in front of the bend is longer than the hinder portion; average size 8–9 mm.

*Passeromyia* Villeneuve.


1. *Passeromyia longicornis* (Macquart).

*Cyrtoneura longicornis*, Macquart, 1851, p. 228 (255), pl. 23, fig. 8; Stein, 1919, p. 111.


*Geographical distribution*. Australia; Victoria (in nest of *Pardalotus* sp.); New South Wales, Sydney; Tasmania.


*Muscina longicornis*, Stein, 1909, p. 221 (non Macquart, 1851); 1919, a, p. 111, and b, p. 68.

*Larva* (without a name), Rodhain, 1914, p. 214, fig. 1.


*Passeromyia heterochaeta*, Rodhain and Villeneuve, 1915, p. 593; Rodhain and Bequaert, 1916, p. 250, figs. 1–6, pl. xix, fig. 2; Roubaud and Van Saceghem, 1916, p. 765; Roubaud, 1918, p. 428; Stein, 1919, p. 86; Patton, 1920, p. 30, pl.; Engel, 1920, p. 258.

*Geographical distribution*. Africa: Nyasaland, Port Herald; N.W. Rhodesia, Chilanga; Katanga, Elisabethville; British East Africa, Mombasa; Belgian Congo, Ouelle, Leopoldville, Boma, etc. I have in my collection specimens from Usambara, Nguelo.

Asia: India, Madras; Java, Batavia; China, Kamsi. I have received numerous specimens from S. China, Canton, by Prof. C. W. Howard.

*Ethology*. The species has been found in the nests of various species of birds of the genera *Passer, Hirundo, Cinnyris, Spermestes, Sitagra, Ploceus*, etc.

According to Rodhain and Bequaert the larvae of *Passeromyia* have the same habits of intermittent haematophagy as observed in the floor maggot and other African Calliphorines belonging to the genera *Auchmeromyia* and *Chaeromyia* which live upon the blood of bare-skinned mammals.

It is interesting to note that *Passeromyia*, as a bird-parasite, is widely spread over the whole tropical region of the Old World only. There are however true Calliphorine flies the larvae of which live upon birds and have the same habits of intermittent haematophagy as is the case in *Passeromyia*. These flies belong to the well-known genus *Protocalliphora* which we shall examine presently.
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II. PROTOCALLIPHORA.

There is a marked tendency both in Europe and in N. America to place the three species of haematophagous Calliphorine flies (caerulea R. D., azurea Fall., and metallica Tyler Townsend) in the genus Phormia.

The genus Phormia was established by Robineau-Desvoidy in 1849 and accepted by Coqillet in 1910 (p. 589) for the species Musca regina Meigen 1826 which differs markedly from the above mentioned calliphorine species.

The calliphorine bird parasites cannot therefore be placed with the species regina Meigen in the same genus Phormia. As to the new generic name Euphormia proposed by Tyler Townsend in 1919 (p. 542) for the genotype regina it becomes quite superfluous, and was introduced only through misinterpretation of Villeneuve's statements (see 1911, p. 84).

The right generic name for the bird parasites is that of Protocalliphora Hough 1899, as it has already been used by me in the third volume of the Katalog der palaearktischen Dipteren, pp. 544-545. The true genus Phormia thus remains a monotype, with the species regina, while in the genus Protophormia Tyler Townsend 1908 must be placed the species terraeovae Robineau-Desvoidy 1830 (= groenlandica Zetterstedt 1838)\(^1\) and Boganidae Erichson 1851.

There has been some discussion about the validity of the described species of Protocalliphora. Hendel in 1901 (p. 29) distinguished in this genus five different species, while Hough, Villeneuve and Bequaert accepted only two species which were subsequently united by Roubaud (1918) into a single species. However, Kramer (1911, p. 43) has already shown that the species can be distinguished by the structure of the male genitalia; and recently Engler (1920) clearly established the existence of the two distinct European species by the study of the structure of the larval, pupal and adult stages. In addition to the two European species Tyler Townsend (1919) has added a third North American species: metallica. Of the two European species caerulea is the commoner. It shows however in addition to sexual dichroism, some variation in the breadth of the frons in the male and in the colour of the calypters. Being often the unique species of Protocalliphora present in collections, it was erroneously subdivided into two or more species under the names of sordida, azurea, braueri, etc.; the other species azurea is more uniform but very often wanting in collections.

The three known species of Protocalliphora may be distinguished as follows:

1 (4). Parafacialia without golden spot above.

2 (3). Parafacialia (viewed obliquely from above) smooth and uniformly covered with whitish pollen or dust. The two sexes differ in colour: the male has the thorax and abdomen of metallic dark blue coloration with scarce

\(^1\) In the Catalogue of Palaearctic Diptera I have erroneously reported this species under the name caerulea R.-D., which belongs to Protocalliphora.
pollen; the female showing golden-greenish thorax and metallic green abdomen with bluish reflects and a thin whitish pollen covering all the segments except the last one. Frons of the male more or less narrow, not broader than the ocellar triangle; male genitalia in lateral view show acute paralobes and straight mesolobe. Females with the middle frontal stripe three times broader than one of the parafrontalia; average size 9-11 mm.

2 (1). Parafacialia with whitish pollen distributed in irregular rows with black interspaces. In both sexes the thorax is of a dark blue metallic colour with scarce pollen and the abdomen of a glittering bluish-green colour devoid of pollen and with a distinct dark longitudinal median stripe upon the second and third segments. Frons in the male distinctly broader, being about twice as broad as the ocellar triangle; the male genitalia with the paralobes broadly obtuse and with curved mesolobe. Parafrontalia of the female broader, the middle frontal stripe being twice as broad as one of them; average size 11-14 mm.

4 (1). Parafacialia each with a conspicuous golden spot at the upper end. Thorax metallic, greenish-black to bright green colour with grey pollen and three nearly equal longitudinal stripes of the ground colour. Abdomen metallic dark bluish-green to bright cupreous or golden-green colour with scarce silvery pollen; anal segment varies always from cupreous to golden-green colour. Calypters white to buff yellow. Size 7-8.5 mm. Only female known.

The synonymy of the three above described species of *Protocalliphora* is as follows:

*Protocalliphora.*

Hough, 1899, a, p. 66 and b, p. 289.
*Avihospita*, Hendel, 1901, p. 29 and 68; b, p. 2101; Aldrich, 1901, p. 68.


*Phormia caerulea*, Robineau-Desvoidy, 1830, p. 466; 1863, p. 846.
*Lucilia caerulea*, Macquart, 1835, p. 256; Meigen, 1838, p. 295; Schiner, 1862, p. 591; de Meijere, 1902, p. 682, fig. 54.

*Protocalliphora azurea*, Falcoz, 1921, p. 139.
*Musca azurea*, Meigen (nec Fallen!), p. 63; Rossi, 1848, p. 59, note.

*Calliphora azurea*, Schiner, 1862, p. 585; Nowicki, 1867, p. 44; Brauer, 1883, p. 74; Verrall, 1886, p. 231; Strobl, 1893, p. 104; Brauer and Bergenstein, 1894, pp. 546 and 568; Pandellé, 1896, p. 214; Grimshaw, 1901, p. 27.


*Avihospita azurea*, Hendel, 1901, p. 29.

1 The *Catalogue of Palaeartic Diptera*, p. 444, contains also the genus *Philornis* Meinert as a synonym of *Protocalliphora*. The late Dr Nielsen, 1911, p. 207, pointed out this error, which is not mine (only pp. 1-397 of vol. III being my work) but that of Brauer, 1894, p. 568.
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*Phormia azurea*, Villeneuve, 1911, p. 84 and 1913, p. 132; Surcouf and Gonzalez-Rincones, 1912, p. 135; Rodhain and Bequaert, 1916, p. 245; Rodhain and Villeneuve, 1915, p. 593; Roubaud, 1918, p. 420, pl. v; Plath, 1919, a, p. 30, b, p. 191, c, p. 373; Patton, 1920, p. 30.

*Musca sordida*, Zetterstedt, 1838, col. 657, 1845, p. 1332, and 1859, p. 6185; Bonsdorff, 1866, p. 150.

*Pollenia sordida*, Rondani, 1862, p. 198; E. Corti, 1897, p. 140.

*Avihospita sordida*, Hendel, 1901, p. 29.

*Protocalliphora sordida*, Villeneuve, 1910, p. 313; Kramer, 1911, p. 43, pl. iii.

*Phormia sordida*, Villeneuve, 1911, p. 84 and 1913, p. 132; Roubaud, 1914, p. 27, and 1915, a, p. 77, b, p. 94, fig.; Rodhain and Villeneuve, 1915, p. 593; Rodhain and Bequaert, 1916, p. 244; Roubaud, 1917, p. 434; Villeneuve, 1918, p. 158; Engel, 1920, p. 256, fig. 8.

*Lucilia dispar*, Léon Dufour, 1845, p. 205, pl. ii; Zetterstedt, 1849, p. 3269, obs.


Avihospita braueri, Hendel, 1901, p. 29.

Geographical distribution. The species has been found throughout Europe, from Lapland to Sicily. Its distribution in North America is not yet thoroughly known, but from Plath's observations it seems that the species is prevalent in the west of the United States. The species was also recorded from Hawaii; if this is not an importation by man, the fly must have a very wide distribution.

Ethology. The habits of the larvae were first described by Léon Dufour and more recently Dr Roubaud has established their habit of intermittent haematophagy and that they cannot live as subcutaneous parasites

The species has been observed in nests of *Corvus*, *Passer*, *Hirundo*, *Cotyle*, *Parus*, *Ruticilla*, etc. in Europe, and of *Pipilo*, *Hylocichla*, *Merula*, *Ampelis*, *Dendroica*, *Petrochelidon*, *Passer*, *Carpodacus*, *Zonotrichia*, *Melospiza*, *Astragalinus*, etc., in North America. All these birds belong to the Passeres.

2. *Protocalliphora azurea* (Fallén).

*Musca azurea*, Fallén, 1816, p. 245 and 1821, p. 46; Zetterstedt, 1838, col. 657 and 1845, p. 1334; Bonsdorff, 1866, p. 150.

*Pollenia azurea*, Rondani, 1862, p. 197.


*Phormia azurea*, Engel, 1920, p. 257, fig. 9.

1 According to Roubaud the puparia of this fly are sometimes parasitised by a Hymenopteron *Nasonia brevicornis* Ashm.
Musca chrysorrhoea, ? Meigen, 1826, p. 60.

Calliphora chrysorrhoea, Macquart, 1835, p. 263; Schiner, 1862, p. 585; Brauer, 1883, p. 74; Strobl, 1894, p. 70; Brauer and Bergenstamm, 1894, p. 546.

Protocalliphora chrysorrhoea, Hough, 1899, p. 289; Aldrich, 1905, p. 524.

Avihospita chrysorrhoea, Hendel, 1901, p. 29.

Avihospita n. sp., Hendel, 1901, p. 30.

Phormia chrysorrhoea, Tyler Townsend, in Plath, 1919, pp. 374 and 380.

? Calliphora splendida, Macquart, 1845, p. 324.

Phormia caerulea, Kramer, 1917, p. 27.

Geographical distribution. This fly has apparently the same distribution in Europe as the other species, but is much more rare and there are only a few precise records, those of all the earlier writers being in my opinion very doubtful. It has been assumed here that the descriptions in which the sexual dichroism is not clearly indicated, apply to the true azurea, because Fallén's original description refers to a species in which the males and the females are equally coloured. But even in this last case a confusion with Protophormia terraenovae, R.-D., is always possible. The species is also recorded from North America, its distribution there being not yet determined.

Ethology. The larval stages were described by Engel in 1920, and compared with those of caerulea. The habits of these larvae seem to be the same. Engel has obtained the species from subterraneous nests of Riparia riparia, and this is the unique precise record, which indicates a very peculiar habitat.

3. Protocalliphora metallica (Tyler Townsend).

Phormia metallica, Tyler Townsend, 1919, p. 379; Plath, 1919, p. 376.


Geographical distribution. This species as far as it is known is exclusively a North American one; its range extends throughout the United States chiefly in the East, being very rare in the West. It is possible that the above mentioned splendida, Macquart, from Texas, may be synonymous with the present species.

Ethology. Plath found the larvae in nests of Merula migratoria. The larva from nests of Corvus americanus fully described by Coutant, may possibly belong to the present species; at any rate this larva cannot be that of azurea or of caerulea owing to the very important differences pointed out by Roubaud, 1918, pp. 423–424.

*       *       *       *

The larvae of the above named species of Passeromyia and Protocalliphora live in bird nests, and attracted by positive thermotropism to the naked bodies of the young nestlings, they can suck their blood. But this type of intermittent haematophagy makes it impossible for them, as shown by Dr Roubaud, to become permanent parasites. There are however several records of fly-larvae found in subcutaneous tumours on young birds and attributed
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erroneously to Protocalliphora. These records are enumerated here separately, as they do not belong to the above named flies.

In Europe they are as follows:

* Schneider, 1866, p. 89, and Kirsch, 1867, p. 245, pl. iii, figs. 3–4, on Passer domesticus, attributed to Prot. caerulea.

* Portschinsky, 1887, p. 17, pl. i, figs. 9–10, on Anthus pratensis, attributed to Prot. azurea (Pollenia).

* Meinert, 1889, p. 315, on Emberiza miliaria, attributed to Prot. caerulea.

* Brauer and Bergenstamm, 1894, p. 546, on Passer domesticus, attributed to Prot. caerulea, and on Riparia riparia, attributed to Prot. azurea.

* Pavay-Vajna, 1909, p. 288, on Motacilla alba, attributed to Melinda cognata (Onesia).

* Heinroth, 1916, p. 158, on Motacilla, attributed to Prot. caerulea.

And in North America:

* Henshaw, 1908, p. 87, on Sialis sialis, attributed to Prot. azurea.

* Miller, 1909, p. 1, the same.

* Plath, 1919, p. 377, on Astragalinus tristis.

It is probable that there are here more than one kind of parasite, belonging to the Calliphorine or other flies.

The genera Onesia and Melinda must be excluded from this list, as Dr Keilin's researches show that the former is parasitic in earthworms and the latter lives in snails (1915 and 1919). The gen. Lucilia seems to be questionable, as Hesse's observations of 1921, p. 154, are not convincing.

If we have to deal with some Calliphorine larva, the gen. Nitellia must be taken into consideration. This genus was established in 1830 by Robineau-Desvoidy, but was united with Pollenia by all subsequent writers except Hendel and Tyler Townsend. It differs from Pollenia in having the common basal stem of the Radius ciliated above, as in Protocalliphora, with which it shows moreover a notable resemblance in the shape of thorax, in the flattened body and in the whole facies. The type species (vespillo Fabricius, 1786) is common in Europe and is present even in North America; its bionomics are still unknown.

III. PHILORNIS.

Muscid larvae, typically living as subcutaneous parasites on birds, have been found only in the Neotropical Region. They belong to the Anthomyidae and not to the Calliphorinae.

It seems that in Central and South America there are several species which at present are not well distinguished. The first case was recorded by Macquart in 1853 from San Domingo under the name of Aricia pici parasitic on Picus striatus. The species was subsequently reported under the same name by Blanchard in 1896 who on Brauer’s authority established its synonymy with Mesembrina anomala Jaennicke. The birds infested were Oriolus cayennensis.

1 Macquart's assertion that Salle had seen similar tumours in Mexico on other birds (Icterus) and even on squirrels is doubtful; the latter case may have been due to some Cuterebrine larva.
and Or. mexicanus from French Guiana. Blanchard had already recorded it in 1895 under the name of Spilogaster anomalus as a subcutaneous parasite of man.

In 1901 A. de Miranda Ribeiro accepted the above synonymy on the authority of Prof. Mik of Vienna, to whom he had sent specimens for identification. These specimens were caught on the bird Peristera rufaxilla.

Meinert in 1889 had already described, from an unidentified Brazilian bird nestling, a muscid larva as Philornis molesta which evidently belongs to the same group. Other allied forms were described in 1895 by Tyler Townsend from the West Indies under the name of Mydaea spermophilae, found on birds of the genera Spermophila and Minus.

In two papers of 1911 and 1913 the late Dr J. C. Nielsen has studied very thoroughly two species of bird parasites under the names of Mydaea anomala and Mydaea torquans; the material examined was obtained from Argentina, and the larvae were found as subcutaneous parasites in tumours on various birds, both old and young ones; torquans occurring on birds of the genera Spermophila, Minus, Homorus and Pitangus, and anomala on Xiphocolaptes albicolli.

The Rev. J. Aiken (1913) has described Mydaea pici from British Guiana and has recognised four different species belonging to the present group. On the contrary Lutz and Neiva in 1912, mentioning that subcutaneous larvae are frequent on young birds in Brazil, think that they all belong to a single species, Mydaea pici Macquart. Engel in 1920 expressed a similar opinion.

In 1916 Neiva and Penna recorded Mydaea pici from numerous birds in Central Brazil, saying that the larvae are so frequent as to be commonly named "berro"; they were found on birds of the genera Cassicus, Furnarius, Molothrus, Paraoria, Amazona, Pionus, etc., mostly belonging to the Passeres.

The late Prof. Stein in 1918 revised Mik's types of 1901 under the name of Mydaea anomala; and in the World Catalogue of 1919 stated that anomala and torquans form a single species.

In my opinion there are in the Neotropical Region several species of Anthomyidae, the larvae of which live in subcutaneous tumours of birds. This opinion is supported by the fact that in a collection of Brazilian flies belonging to this group sent to me some years ago by Dr Lutz, Prof. Stein has recognised the following species of Mydaea: brevipectinata Stein, latipalpis Stein, sparsiplumata Stein and tinctinervis Stein, all described in 1918. It is probable that some of these species were bred from subcutaneous larvae.

As a conclusion, we can provisionally place the South American Anthomyidae with the larvae forming the subcutaneous tumours in birds together under the generic name of Philornis.
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Philornis.

Meinert, 1889, p. 315.

1. Philornis pici (Macquart).

Aricia pici, Macquart, 1853, p. 657, pl. xx, 2.
Hylemyia pici, Osten Sacken, 1878, p. 167; Aldrich, 1905, p. 553.
Mydaea pici, Miranda Ribeiro, 1901, p. 156, pl. i; Busck, 1906, p. 2; Lutz and Neiva, 1912, p. 133; Aiken, 1913, p. 3 (sep.), figs. 1–11; Rodhain and Bequaert, 1916, p. 247; Neiva and Penna, 1916, p. 111; Keilin, 1917, pp. 399 and 436; Stein, 1919, p. 123; Engel, 1920, p. 250.

? Hylemyia angustifrons, Loew, 1861, p. 41.
Mesembrina anomala, Jaennicke, 1867, p. 377, pl. ii, fig. 4.
Spilogaster anomala, Brauer and Bergenstamm, 1893, p. 209, note 114, 1; Blanchard, 1895, p. 118 and 1895, p. 652, pl. 17, figs. 5–9.
Mydaea anomala, Nielsen, 1913, p. 252, figs. 1–4; Aiken, 1913, p. 3 (sep.); Keilin, 1914, p. 114 and 1917, p. 399; Stein, 1918, p. 212.

Philornis molesta, Meinert, 1889, p. 315, pl. vi.

Geographical distribution. If all the above records belong really to one species, it must have a wide range of distribution over Neotropical Region, from Cuba, San Domingo and Central America to Argentina.

Ethology. Well illustrated by Meinert and others. The larva pupates in a cocoon of earth cemented by a white substance. This fact seems to be uncommon in Anthomyidae, but is observed also in the tropical Synthesiomyia nudiseta, v. d. Wulp, besides the cases recorded by Dr Keilin, 1917, p. 437.

2. Philornis torquans (Nielsen).

Mydaea torquans, Nielsen, 1913, p. 252, fig. 3; Aiken, 1913, p. 3 (sep.); Keilin, 1914, p. 114 and 1917, p. 399.

Mydaea anomala, Nielsen, 1911, p. 195, figs. 1–14.

Geographical distribution. At present only known from Argentina.

Ethology. The larva pupates freely, without producing a cocoon.

3. Philornis spermophilae (Tyler Townsend).

Mydaea spermophilae, Tyler Townsend, 1893, p. 381; 1894, p. 173 and 1895, p. 79; Aldrich, 1905, p. 543; Aiken, 1913, p. 3 (sep.).

Mydaea spermophila, Keilin, 1917, p. 399.

Geographical distribution. Only known from Jamaica; but Townsend, l.c., records a different species, which infests birds in Trinidad.

Ethology. The larva does not make a cocoon for pupation.

These three flies can be distinguished as follows:

1 (4). Third antennal joint about twice as long as the second1; abdomen

1 The figure given by Blanchard, 1896, pl. 17, figs. 5–8, if really belonging to pici (anomala), must be somewhat inaccurate, showing, in opposition with the figures of Macquart, Jaennicke, Miranda Ribeiro and Aiken, a much longer third antennal joint and a bare arista.
more or less yellowish at the base; legs as a rule entirely yellowish, at least in the male.

2 (3). Frons more narrow, the parafrontalia of the male almost touching above, and the middle stripe of the female being not broader than one of the parafrontalia; sides of the face yellowish; legs entirely yellow even in the male

\[ \text{pici Macquart.} \]

3 (2). Frons broader, the parafrontalia of the male well separated to the vertex and the middle stripe of the female broader than one of the parafrontalia; sides of the face silvery; legs of the female with blackish femora.

\[ \text{torquans Nielsen.} \]

4 (1). Third antennal joint about three times as long as the second; abdomen entirely black and the silvery pollen forms a somewhat marmorate pattern; legs brownish, with basal portion of femora darker.

\[ \text{spermophilae T. T.} \]

IV. CARNUS.

The unique non-pupiparous Dipteron, which in the adult stage is parasitic on birds, is the strange \textit{Carnus hemapterus}; the fly is haematophagous and is to be found chiefly on young birds, while the larva lives in the nests, but is saprophagous.

The fly was described and figured by Nitzsch about a century ago, was redescribed and figured by Egger in 1854 under the same name, and owing to a misinterpretation of the original description was in 1862 renamed by Schiner as \textit{Cenchridobia eggeri}. Thus for a long time it has been believed that there were two species, one belonging to the Pupipara, and one to the Acalypterata of the family Borboridae (Schiner), or Sepsidae (Brauer).

Collin in 1911, in a short but important paper, has cleared up the matter recognising but a single species and locating it in the family Milichiidae.

In the subsequent year there appeared the paper of Prof. de Meijere, with the confirmation of the singular fact that the fly emerges from the puparium fully winged, and loses subsequently the wings, short stumps of which remain only as in the Pupiparous gen. \textit{Lipoptena}. Thus in my paper of 1916 on the reduction of wings in the Diptera, I have included \textit{Carnus}, with \textit{Lipoptena, Echestypus} and \textit{Ascodipteron}, in no. 7 of my graduation, p. 108.

A supposed second species of the gen. \textit{Carnus} was described in 1913 by Stobbe, but its distinction seems to be very doubtful. Engel in his recent paper has added some new records, and therefore this interesting fly is at present known to occur on birds of the genera \textit{Aquila, Falco, Picus, Lynx, Sylvia, Sturnus} and \textit{Coloeus}. I have received from Prof. B. Grassi a couple of specimens caught at Maccarese, near Rom, by Luigioni on a nestling of \textit{Falco}; these specimens are dark coloured and bristly as in \textit{setosus}. 
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Carnus.

Nitzsch, 1818, p. 305 and p. 283.

Cenchridobia, Schiner, 1862, p. 435, and 1864, p. 334.

1. Carnus hemapterus, Nitzsch.

Carnus hemapterus, Nitzsch, 1818, p. 305; Germar, 1822 (?), pl. 24 and 25; Egger, 1854, p. 3, pl. ii, figs. 7–11; Bezzi, 1900, p. 522; Collin, 1911, p. 138; Bezzi, 1911, p. 115; de Meijere, 1912, pp. 1–18, figs. 1–12; Melander, 1913, p. 237; Stobbe, 1913, p. 193; Bezzi, 1916, pp. 108 and 177; Wasielewski and Wuelker, 1918, p. 117; Engel, 1920, pp. 249 and 258; Frey, 1921, p. 151, pl. x, fig. 125.

Cenchridobia eggeri, Schiner, 1862, p. 436 and 1864, p. 335; Brauer, 1880, p. 117, 3 and 1883, pp. 40 and 87; Bezzi, 1900, p. 521; Becker, 1905, p. 36.

Carnus setosus, Stobbe, 1913, p. 193.

Geographical distribution. Only known from Europe: Finland, Germany, Austria, Hungary, Roumania and Middle Italy.

V. Chortophila and Neottiophilum.

It may be finally recorded that in bird nests are to be found many other species of Myiodaria, which are not parasitic, but only inquiline, living probably on excrement or on organic refuse. Falcoz in 1914, p. 60, has pointed out the great number of dipterous larvae found in bird nests. It is to be noted that these flies, as far as is known, belong to the lower forms of Anthomyidae and even to the Acalypterata.

Of the former there are only the two following species, one from Europe and one from North America:


Germany; bred by Kramer from larvae in nest of Acanthis cannabina.

2. Chortophila nidicola (Aldrich).

Hylemyia nidicola, Aldrich in Plath, 1919, p. 380.

State of Washington, U.S.A.; bred by Plath from larvae found in various bird nests.

Among Acalypterate flies there is the following very interesting genus:

Neottiophilum.

Frauenfeld, 1868, p. 894.

1. Neottiophilum praeustum (Meigen).

Dryomyra praeusta, Meigen, 1826, p. 257.

Neottiophilum praeustum, Mik, 1882, pp. 98 and 194, 1887, p. 34 and 1894, p. 166; Brauer, 1883, pp. 40 and 86; v. Roeder, 1892, p. 204 and 1895, p. 270; Strobl, 1894, p. 86; Becker, 1902, p. 219 and 1905, p. 38; Bezzi, 1911, p. 115; Kramer, 1917, p. 58; Engel, 1920, p. 250.
Neottiophilum fringillarum, Frauenfeld, 1868, p. 895.
Blephariptera cortereau, Bigot, 1881, p. 370.

Geographical distribution. At present known only from Central Europe: France, Germany and Austria. The adult fly seems to be rare, and was found mostly in houses, on windows; it lives probably on trees.

Ethology. The fly was bred from puparia found in nests of Fringilla coelebs and of Passer domesticus, but almost nothing is known about the larva.

VI. CONCLUSIONS.

1. The Myiodaria living with birds show a parallelism between the grades of their parasitic adaptation and their systematic position.

2. The lower forms—Acalypterata have saprophagous larvae, living in the nests of several orders of birds: Scansores, Passeres and Raptores. In the larval stage they feed upon decaying organic matter, while in the adult stage they are, in some cases, blood-sucking (Cornus).

3. The intermediate forms—Anthomyidae show two grades of adaptation: (a) lower forms, the larvae of which are mainly saprophagous or phytophagous (Chortophila) and which, like the Acalypterata, live in the nests upon decaying substances; (b) higher forms the larvae of which are mainly carnivorous and have adapted themselves to two modes of life: (A) as subcutaneous parasites (Philornis) of Scansores, Columbae, and Passeres; (B) as intermittent haematophaga, on Passeres (Passeromyia).

4. The higher Myiodaria—the Calliphorinae show in their larval stage the two last types of parasitic adaptation, i.e. (a) intermittent haematophagy (Protocalliphora), and (b) possibly a subcutaneous mode of life on Passeres only.

5. The adult flies of all the intermediate and higher Myiodaria are non-bloodsucking. It seems to be a rule among the Diptera that the forms with haematophagous adults have non-haematophagous larvae and vice versa.

6. All these facts have to be taken into consideration in the study of other parasitic Myiodaria and especially the heterogeneous groups like Pupipara and Oestridae which, undoubtedly, are of polyphyletic origin, and are derived from lower, intermediate and higher Myiodaria.

VII. BIBLIOGRAPHY.


Parasitic Diptera


— (1883). Die Zweiflügler des Kaiserlichen Museums zu Wien. iii. Ibid. xlv, pp. 89–240.


Parasitic Diptera


— (1913). On some South American species of the genus Mydaea, parasitic on birds. Ibid. lxv, pp. 251-256, 4 figs.


PORTSCHINSKA, J. (1887). Diptera europaea et asiatica nova aut minus cognita (cum notis biologicis). Hor. Soc. Ent. Ross, xi, pp. 3-20, pl. i.


M. BEZZI


TOWNSEND, C. H. TYLER (1893). Notes from the Museum, Institute of Jamaica, Nov. 22, no. 70.


Parasitic Diptera


DESCRIPTION OF A BOX FOR COLLECTING AND TRANSPORTING LIVING INSECTS, ETC.

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(With 6 Text-figures.)

When collecting small living animals, particularly insects or other small Arthropods, for dissection, or search for parasites, etc., one encounters difficulties in choosing a suitable and convenient arrangement in which the animals may be kept without injuries until they are brought home. The usual entomological corked glass tubes and wide-mouthed jars with a gauze cover are not practicable when a large number must be carried; their bulk hinders free movement, and their fragility renders it necessary to pack them carefully in a box or other suitable containers.

In the summer of 1911, I was shown, at the Natural History Museum, Simpheropol, a very convenient apparatus for the collection and dispatching of Arthropods, which had been sent from Uriev by Prof. K. K. Saint-Hilaire. Unfortunately, I have not been able to discover the name of the inventor of the original model, a description of which I have never met with in the literature. Having convinced myself, during four years' experience of its use, of the valuable qualities of this apparatus, I have made some alteration in its construction, which I believe will render its application wider, and have decided to publish a description. If ever I should succeed in discovering the name of the author of the original model, I shall publish a supplementary note to that effect.

The model received from Prof. Saint-Hilaire consisted of a flat wooden box, 48.5 cm. in length, 34.5 cm. in breadth, and 5.5 cm. in depth. The interior of the box was divided, by eight longitudinal and five transversal partitions, into 40 rectangular compartments (Fig. I). The bottom of the box was formed of a sheet of wire gauze of 0.5–1 mm. mesh, while the lid was constructed and firmly fixed to the frame of the box. In the wood at points corresponding to the centre of each of the 40 compartments a circular aperture, 2.8 cm. in diameter, had been bored. Each of these openings was closed with a cork. The apparatus, as described, is very convenient for collecting and transporting Arthropods of cannibal habits, e.g. spiders, scorpions, myriapods.
Box for Collecting Living Insects

even, while the conditions of ventilation of the inner chambers are such as to render it a safe means for the transport of sensitive insects, as bumble-bees and other Hymenoptera, which will survive imprisonment in such a box much longer than in glass jars with gauze lids. In the summer 1914 I brought scorpions from Algeria and Tunis to Petrograd. The scorpions remained in the boxes from the 7th of July until the 28th of August, and the percentage of individuals which succumbed was very small, notwithstanding the unfavourable conditions of the voyage.

In order to render the apparatus of wider application I have made some modification in its structure which I now proceed to describe.

The external dimensions of the improved box are: length 45 cm.; breadth 31 cm.; and depth, including thickness of lid, 5.5 cm. The internal depth of the box is 4.6 cm. as in the original model.

The wooden lid is in three sections which are hinged and can be opened independently of each other (Fig. III). Each section is fastened by a pair of hooks screwed to the front wall of the box, suitable pins or eyelets being fixed into the edge of the lid for the hooks to engage (Fig. II). For greater security an additional hook is fixed at each end of the box.

The interior of the box is divided by eight continuous fixed transverse partitions (Fig. III, F) with equidistant vertical slots for the reception of quadrangular pieces of wood which divide those spaces between the continuous partitions into six compartments; space slots at the ends of the continuous partitions (Fig. III, D) serve for the reception of the quadrangular movable partitions when it is desired to increase the capacity of a compartment by the removal of one of these. If desired, pieces of tin plates may be used in place of wood for the movable partition, but in this case, the slots in the continuous partitions must be cut to fit accordingly. With all the movable partitions in their usual positions, the box is divided into 54 compartments each measuring 4 cm. x 4 cm. x 4.6 cm. In Fig. III the endmost series of compartments shows a replacement of the movable partition which results in the formation of two double compartments (C) and two single compartments. Such double compartments are suitable for the reception of solpugids or large scorpions. By further replacement it is possible to obtain compartments of triple capacity or more, and by removing all the movable partitions of one transverse compartment, space available for the reception of lizards, etc. may be obtained. The lid of the box is furnished with apertures (M) closed with corks, like that of the original model. The desired arrangement of the movable partitions should be made before the specimens are placed in the box and the latter should be introduced through one of the openings by removing the cork. It is obvious that no section of the lid should be opened when the corresponding part of the box already contains specimens.

The floor of the box is formed of wire gauze, as in the original model, and may be secured in position by a fillet of wood running round the lower edges of the box and screwed to the lateral end walls (see Fig. IV).
Fig. I. Bottom of original model, showing wire-gauze floor and partitions.
Fig. II. Lateral view of improved model showing hooks for fastening lid sections.
Fig. III. Plan of improved model showing lid sections, one of which is open: A and B, compartments subdivided into two or four parts respectively by the insertion of diagonal plates; C, double compartment formed by removal of one movable partition; D, space mortice; E, movable partitions; M, aperture of a compartment.
Fig. IV. Detail of the interior in perspective. The lid is not represented. The wire-gauze floor and wooden fillet are clearly shown.
Fig. V. Tin plates for diagonal partitions of compartments: A, incision for reception of part of cork protruding beneath the lid of the box.
Fig. VI. Pair of tin plates interlocked for fourfold subdivision of compartment.

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The rectangular compartment of the box may be subdivided by the use of tin plates, cut to the suitable shape shown in Fig. V. These plates are inserted diagonally, either singly (Fig. III, A) or cross-wise in pairs (Fig. III, B). Such small compartments serve for the reception of small spiders, myriapods, ticks, etc. The broad incision in the upper edge of each of the diagonal division plates should be cut to fit closely to the part of the cork protruding through the aperture of the lid.

Several such boxes, placed one upon the other in such a manner that the gauze bottom of one rests upon the corks of the other, are bound with travelling straps for transport. For ordinary excursions a single box is usually quite sufficient and may be carried conveniently slung by a shoulder strap.
AN ACCIDENTAL INFECTION WITH *UNCINARIA*.

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In the course of a study of a method for the recovery of *Uncinaria* larvae from the soil, one of the employees of the laboratory became infected under circumstances closely simulating experimental conditions. The history of the case is as follows:

On November 22, 1920, a quantity of faeces containing *Uncinaria* ova in large numbers was placed on the ground where it would be exposed to the sun and the rain. On December 8, many living larvae were recovered from the soil. These were placed in a tightly corked bottle out of the way on a high shelf, and all employees were warned that the specimen was not to be handled carelessly.

In spite of instructions, however, on December 11, one of the men took the specimen to demonstrate the larvae to some friends. While he was examining the preparation under the microscope, the fluid ran over the edge of the slide and came in contact with his hand. He is accustomed to hold the slide between the thumb and middle finger, allowing his hand at the base of the middle finger to rest upon the stage of the microscope.

Immediately after examining the specimen he became conscious of an intense itching at the tip of the thumb and tip and base of the middle finger.

On the following day, December 12, the three areas were inflamed and swollen and contained numbers of minute bright red points. There was no evidence of pus.

On December 13 the man noticed a marked tenderness under the arm in the region of the axillary glands. There was no evidence of lymphangitis, and the lesions on the hands remained the same as on the preceding day.

On December 14 a marked bronchitis developed. The stool was found to be negative for *Uncinaria* at this date and remained so for more than a month.

Because he had been engaged as microscopist in our hookworm campaign in Panama, he had examined his stool many times during the previous year for ova of *Uncinaria*, with negative results.

The lesions on the hand had practically disappeared by December 24.

The stool was negative on January 10, 1921, but became positive for *Uncinaria* on January 17, thirty-eight days after the infection. On January 26 the ova were increased in number. The man left the city for the interior on January 26.
Accidental Infection with Uncinaria

In answer to a request for information in regard to his condition in June, the following reply was received:

I have made other examinations and found several ova of *Uncinaria*. I am experiencing well-marked symptoms of the disease, such as general weakness, dizziness, and palpitation of the heart. My bronchitis is almost the same as when I left Panama. I have not as yet taken the treatment because of lack of time.

After repeated advice in regard to treatment, on October 31 I received the following letter:

When I received your telegram about treatment, I had already taken one treatment with chenopodium and collected the worms which were passed. I am enclosing a sample. The sample mentioned contained about twenty hookworms. On November 15 the following statement arrived:

After a second treatment I was unable to find any worms. Repeated examinations for ova have been negative. I am now feeling quite well, except for palpitation of the heart.

From the above history it seems certain that in this case the larvae after passing through the skin, travelled up the lymphatics, through the axillary glands, into the blood stream and into the lungs in three days. The parasites had reached the intestines and had matured within thirty-eight days. One treatment with chenopodium had evidently sufficed to cure this particular case.
NOSEMA APIS AND ACARAPIS (TARSONEMUS) WOODI IN RELATION TO ISLE OF WIGHT BEE DISEASE.

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I. SOME OUTBREAKS OF BEE DISEASE PREVIOUS TO 1906.

In former times when this country was dependent upon bees for its supply of sugar, a heavy mortality among these insects was of sufficient importance to be placed on record. In his Animal Plagues Fleming (1871) gives a number of references to such losses. In Ireland there was a "mortality of bees" in 950 A.D. and again in 992 A.D. there was a "great mortality upon men, cattle and bees." In 1035 A.D. the destruction of bees afflicted the whole of Bavaria. An eclipse of the sun in 1124 A.D. was followed by a great pestilence amongst oxen, sheep, pigs, and bees. During the time of the "Black Death," also, there appears to have been heavy losses of bees and at the Manor Court of Heacham in Norfolk, a statement was made on oath by the steward in the forty-fifth year of the reign of Edward III (1372 A.D.) to the effect that ten out of eleven stocks of bees had perished from the murrain. There is little doubt that this entry in the manorial court rolls refers to epidemic disease, but, as Fleming points out, "there is evidently no relationship between the morina of the bees and that of the sheep and cows."

The year 1443 A.D. was rainy and tempestuous after May which "much hurted both bees and sheep in Ireland"; while in Italy in 1690 A.D. "bees extracting no sweetness from the calyces of the flowers, but a bitter poison, either died or left the country." A great mortality among bees and carp is also recorded in 1717 A.D. in Silesia.

A well-known writer on bees, Dr Bevan (1837), states that in the winter of 1782-3, a general mortality took place among the bees in this country, which was attributed to various causes; want of honey was not one of them; for in some
hives considerable store was found after the bees were gone. Some were of opinion that it arose from the preceding being a bad breeding year and thought that the bees died of old age. Others attributed it to the moistness of the spring of 1783 which rendered the providing of pollen difficult, for without pollen no brood can be raised....The fatal influence ascribed to the wetness of the spring of 1782 seems to be improbable; though it might have affected the quantity of bees bred, it was not likely to put a stop to their breeding altogether, and the young bees ought at any rate to have escaped the desolating evil, if it were old age alone; yet wherever the mortality once made its appearance, every bee became its victim.

In the early sixties of the nineteenth century the columns of The Field newspaper supply evidence of the existence of trouble which beekeepers of the day described in language almost identical with that used to describe the Isle of Wight disease at a later period. Occasional references to a similar trouble are to be found in the bee literature of this country until the beginning of the present century.

In other countries, also, epidemic bee disease may still be heard of from time to time. Berlepsch records that in the spring of 1859, after the bees had made good use of the sallows, wholesale losses took place. The bees were to be seen in heaps, their bodies swollen with watery excrement. Many stocks were one day healthy, the next day half were dead, and the day following that all the bees were dead. The disease appeared in many places about Easter, and in others not till Whitsuntide. It was similar to human cholera, ravaged the whole of south Hanover and the lands adjoining and even extended into Denmark.

According to Dadant (1907) during the years 1901-05 entire apiaries were depopulated in the province of Ancona, Italy, by mal de Maggio, a disease of the adult bee. The losses occurred just at the opening of the honey harvest.

In Australia, whole apiaries are at times extinguished from some disease the cause of which is unknown (Garrett, 1910).

In Brazil hundreds of stocks perish in March and April, the affected bees dying outside the hives. The beekeepers attribute the trouble to the poisonous properties of the nectar of certain plants which are in bloom at the time\(^1\) (Hannemann, 1909).

In Canada and the United States epidemics occur and the following quotation from the American Gleanings in Bee Culture is an interesting description of virulent disease by Critchlow (1904):

Is this a new and strange bee disease, or is it a very malignant type of paralysis? Possibly some purely local cause is responsible for the great mortality among the bees. Who can give us some light? There seems to be great danger in Utah of a total loss of the entire bee industry. I shall give you as nearly as I can the conditions, both in the past and at the present time.

In this valley and in the one fifty miles north of here, called Cache Valley, there have been for many years a great many bees, both in the hands of skilled operators and in the

\(^1\) Very many plants have come under the suspicion of beekeepers at one time and another in various parts of the world when there was no local spraying of fruit-blossom, etc., to which the losses could be attributed.
hands of many farmers and small owners. A year ago this spring there were upwards of 2000 colonies of bees lost. It was thought a year ago that it was owing to the very cold winter weather and changeable weather in the spring; but the strange part of it is that in some localities not any warmer, but if anything, colder, the bees survived all right, with scarcely any loss at all.

I lost last year 300 colonies of bees, and in some localities in Cache Valley there were upwards of 500 colonies that went under; and in this valley (Salt Lake) last year there were fully 800 colonies that perished. It was thought by all those who were interested that it was due to the hard winter and cold spring.

This writer, who had lost nearly all his stocks, bought 225 colonies in a district that disease had not visited, and moved them into his apiary. He continues:

I was there yesterday, and a large part of the entire 225 colonies are affected with what appears to be paralysis. While there is no trembling, they drop down in the grass in front of the hives, and are unable to fly; they seem to mount the grass and twigs with great difficulty, and in taking them up in my hands they were unable to fly away, and, if thrown into the air, would drop to the ground. They seemed to have no desire even to sting. It appears to be contagious, for it seems to affect a certain part of a row, while another section of the row seems to be strong and swarming. A number of hives have all gone under. The entire yard, of course, is exposed for the reason that a few, perhaps twenty, colonies from what were left of the lot last year were put with them without any thought of anything being wrong, except that they were weak.

I am fully satisfied now that the loss last year, which would number at least 2000 colonies in Cache Valley, was due entirely to this condition.

II. The Outbreak of 1906 commonly called Isle of Wight Disease.

In 1906 we first began to hear of the bees in the Isle of Wight suffering from "paralysis," a disease of world-wide occurrence and of which the cause is unknown. On account of the deadly nature of the Isle of Wight visitation, however, it was afterwards decided that the disease was a new one and the name of Isle of Wight disease came into use. It was found to have been present on the Isle of Wight in 1904, so it was consequently assumed to have then originated and to have spread thence to the mainland. It usually manifested itself by the presence in the apiary of numbers of crawling bees with their abdomens distended with undischarged faeces. In a few months all the colonies in the apiary were dead.

III. The Symptoms of Disease in Adult Bees.

The whole subject of bee diseases teems with difficulties, and it is improbable that any method of differentiating them other than the demonstration of the causal organism is likely to prove of value. Inability to fly, which is the chief symptom in any disease of the adult bee, may originate from a variety of causes, e.g. the ingestion of fungi, mineral poisons, fermenting honey, syrup or fruit juice, or from weakness due to starvation.

Inability to fly leads to a retention of the faeces, which are voided normally by the bee in the open air while flying. We find that a crawling bee may be
Bee Disease

slow and sluggish, may run rapidly, may tremble, present a hairless appearance, drag its hind pair of legs helplessly, may be subject to dysenteric discharges, or may impart a yellow colour to the interior of the hive by passing constantly a tiny threadlet of faeces. Possibly the varying manifestation may be due to the multiplication of bacteria of different species or of other parasites in the bowel of the affected bee. Whether the condition is sometimes due to infection it is impossible to decide.

Again, the normal life of the individual bee may vary from eight weeks to as many months according to the activities of the colony, and it may be that organisms which do little harm in two months cause serious trouble where the necessities of the colony require the extension of the lives of its individual members for a longer period.

The difficulty of tracing a connection between a disease and any parasitic organism that may be found in the bee becomes very real when the investigation of disease is attempted. If, while the external conditions are favourable, the organism is fed to a colony as an experiment, the bees may fail to die or to develop symptoms; while if unfavourable conditions prevail at the time of the experiment, death may be due to unrecognised causes, such as infection with *N. apis* (see below). Apparently healthy bees, if prevented from flying, will sometimes develop symptoms (crawling with bowel distention) indistinguishable from Isle of Wight disease.

IV. Attempts to ascertain the Cause of Isle of Wight Disease.

A. *Bacillus pestiformis apis.*

The first to associate a definite causal organism with Isle of Wight disease was the late Dr Walter Malden (1909). He found no macroscopic appearances in the diseased bees that were not to be found in bees from healthy colonies. Microscopically, "no changes were discovered in the salivary glands, brain, fat-body, heart, tracheae, air-sacs, Malpighian bodies, or honey stomach." Changes were found in the chyle stomach, however, and attention was concentrated on that organ. In film preparations made from small portions of the chyle stomachs of diseased bees, teased out on glass slides and stained with methylene blue, a bacillus was found with darkly staining ends and a lightly staining central band, resembling *Bacillus pestis* in general appearance. This was suggested as having a causal relationship to the disease and the name *Bacillus pestiformis apis* was proposed. Cultures of this bacillus fed in sugar to bees did not appear to have any harmful effect and the view that it is the organism that caused the disease has been abandoned.

B. *Nosema apis.*

In 1907 Dr Enoch Zander (1911) discovered a protozoon in bees which was recognised as being closely related to *Nosema bombycis*, a parasite which did enormous damage to the silkworm industry in France about the middle
of the nineteenth century. The organism found in bees was named *Nosema apis* and Zander looked upon it as the cause of heavy losses in Bavaria. The essential feature of the trouble was a sudden and extensive mortality among the bees inside and outside the hive. Dysentery was an occasional accompaniment. Berlepsch's description of the epidemic of 1859 (see p. 54) is considered by Zander to be a typical description of the ravages of unchecked *Nosema* disease.

The discovery attracted considerable attention. Maassen (1911) found *Nosema* to be widely prevalent in German apiaries, but although he looks upon the parasite as pathogenic he considers that unfavourable conditions are necessary for the manifestation of disease.

Nussbaumer (1912) found *Nosema* in association with heavy losses in Switzerland.

Beuhne (1916) states that *Nosema* was first discovered in Australia in 1909, but that observation showed that it was doubtful whether the presence of the parasite is in itself necessarily fatal, or that it greatly interferes with the productiveness of the hives excepting under certain conditions due to climatic influence....In fact under ordinary conditions the disease is endemic, and becomes epidemic only when the vitality of the bee is impaired by the malnutrition during the bees' larval development which is caused by a dearth, or the inferior quality of the nitrogenous food which bees obtain solely from the pollen of the flowers of plants.

As the result of investigation at Cambridge (Graham-Smith, Fantham, Porter, Malden, and Bullamore 1912) a report was issued showing that *Nosema* was present in a large percentage of stocks affected with Isle of Wight disease and the disease was attributed to the presence of this parasite.

Later, Anderson and Rennie (1916) working on the Isle of Lewis took up the question of *Nosema* and Isle of Wight disease. They were unable to corroborate the findings of the Cambridge investigation and state that *Nosema* was found to be present in stocks without disease symptoms appearing. They did not find *Nosema* distributed in the bees of all the stocks but only in a few stocks at any one time, and when it occurred "Isle of Wight disease was not present."

The result is somewhat surprising as, assuming that *Nosema* is a comparatively harmless parasite, its non-occurrence in stocks suffering from Isle of Wight disease seems to require some explanation.

While the work on the Isle of Lewis was being carried on, continuous importations of bees were being made from all parts of the country. Bees, assumed to be healthy, were introduced into the apiary from Scotland, England, Wales, Ireland, America, Switzerland, Austria, and Holland. These bees developed crawling symptoms at periods varying from a week to a month or more after arrival. Consequently it is difficult to determine the disease with which Anderson and Rennie were actually working or to feel sure that importations of other diseases were not being made. The following are the particulars of what is described as a spontaneous recovery.
Three stocks of bees, one Italian, one Carniolan and one black (British or Dutch?) were imported into the apiary from Devonshire on June 4th, 1915.

During June these three stocks gave off four swarms and the seven colonies were showing crawling symptoms on June 30th. Three of the colonies died owing to faulty queens and another was destroyed by robber bees. A fifth was reinforced by the addition of three pounds of bees (source not stated) and an Italian queen was also given to this colony. Some driven bees from Wales were placed in the apiary. In December, 1915, there were four stocks alive showing no signs of disease.

Accounts of further work by Dr Rennie and Miss Harvey (1919a and b) are given in the Journal of the Scottish Board of Agriculture. With regard to Nosema the conclusion arrived at is that it “is always a weakening factor, and in the presence of other adverse conditions favourable to the development of dysentery it may become seriously pathogenic to bee stocks.” In ordinary circumstances it was not found to destroy colonies in the rapid and virulent manner generally found in Isle of Wight disease. The authors consider that in the latter disease there is no recovery of sick bees nor of affected stocks.

Mention is also made of a temporary sickness which sometimes affected all the bees of a district. “In such cases it would appear to be due to some disturbing factor in the nectar or pollen.”

C. *Acarapis (Tarsonemus) woodi*.

In November, 1920, a paper was read before the Royal Society of Edinburgh in which Dr Rennie, in association with Mr Bruce White and Miss Harvey, described a new species of mite from the tracheae of hive bees. This mite, which was named *Tarsonemus woodi*, was put forward as being the cause of Isle of Wight disease, which was henceforth to be known as “acarine disease.” Hirst (1921) considers that the new mite differs sufficiently from *Tarsonemus* to form a new genus and proposes that the species shall be renamed *Acarapis woodi*.

The mites gain entrance to the tracheae by means of the first pair of thoracic spiracles. Crawling of bees, followed by the death of the affected colony then takes place. The presence of the mite is not necessarily fatal however, for we read in the Report to the Royal Society of Edinburgh that

Of 140 stocks believed by their owners to be healthy, 50 or nearly 36 per cent. harboured the parasite. Concurrent with such discoveries we ascertained by direct examination ourselves of flying bees (1) which were members of colonies in which the disease was definitely established and (2) which were taken from colonies believed to be healthy and showing no indications otherwise, that amongst these were to be found considerable numbers harbouring the parasite. This was further complicated by the fact that in those infected flying bees certain of those pathological conditions—e.g. the blackening and hardening of the tracheal tubes—were very marked. As an example it may be quoted that this condition was found in bees entering the hive carrying pollen or nectar, both belonging to stocks in which crawling and other symptoms were well established and also in those reputed healthy stocks.
In the record of another stock we read that "the flying workers were frequently more heavily parasitised than were the bees of the same stock which were unable to fly."

In an interesting case which was under the writer's own observation in April, 1921, the crawling symptom was manifested but no mites were to be found. The crawling disappeared during the summer but reappeared in the autumn when the symptom was accompanied by the presence of mites in the tracheae.

As an example of the recovery of a stock we have the following from a letter received by Dr Rennie (1921):

On January 9th I received your report that the bees from one of my stocks had *Tarsonemus woodi*.

From this stock reported diseased on that date I had a swarm on the 23rd of May and this swarm swarmed on the 28th of June. I have taken off 150 sections and have three very strong stocks.

Dr Rennie's explanation is that early diagnosis enables us to recognise such recoveries and that formerly this was not possible. We only recognised the presence of the disease after it was irretrievably established. This "gave us an erroneous idea as to the gravity of the disease."

Such an explanation is not altogether satisfactory. When Isle of Wight disease reached an apiary the loss of colonies was usually 100 per cent. and the margin of error in forming an estimate of its gravity must have been very slight.

In the Report of the Hants and Isle of Wight Beekeepers' Association for 1906 we read:

Twenty-five years' acquaintance with bees, bee men and bee life has not revealed anything so deadly or mysterious as this so-called bee paralysis of the Island.

Silver (1907), who toured the Island in 1907, gives his impressions in these words:

The sight of whole apiaries of 10 to 20 hives standing desolate and deserted in the middle of May is a most distressing one, and standing as I did, under a horse-chestnut tree in full blossom, in the grounds of the Rev. John Vicars, of Colbourne, situated in the centre of the Island, not a bee was visible on a beautiful spring day.

Complete apiaries died out in May and June just after swarming and when the hives must have been tenanted with young bees.

In support of the thesis that *Tarsonemus woodi* has been entirely responsible for the losses known as Isle of Wight disease, Dr Rennie suggests that *T. woodi* is at present a parasite of bees in this country only. He assumes that Isle of Wight disease has never been clearly shown to exist in any other country and that no such persistent losses have ever occurred before in this or any other country, and discusses the possibility that a new disease has arisen through the migration of the mite from some other insect to the hive bee as host.

The evidence that the mite is not to be found in bees of other countries is confined to the negative results obtained by the examination of a few
hundred bees that have accompanied queen bees sent to this country by post. But if the mite is the cause of a serious disease its occurrence in such bees would be rare. It is in the dwindling and unprofitable apiaries of other countries that search must be made, and until we get definite statements from foreign workers that the mite cannot be found in any of the troubles classed together as paralysis, the chances are in favour of its occurrence.

As to the long duration of Isle of Wight disease as compared with any other epidemic, we have no knowledge as to the number of years that apiaries have been re-stocked where paralysis is causing annual losses. In this country steady losses were formerly confined to districts. That such losses now occur all over the country is due to the altered method of beekeeping. And is it safe to assume that such losses have always been due to the same cause for the last seventeen years in this country and to some other cause in the various countries from which we have imported bees? Since Isle of Wight disease was first reported nearly all the bees of this country have disappeared. Their places have been taken by foreign bees, many of which have also died and the present bees of this country are mainly the descendants of recent importations from various parts of Europe, Asia, Africa, and America.

The German epidemic of 1859 followed the importation of foreign bees. Our own losses in the early sixties of the last century came shortly after we commenced to import foreign bees and had started a crusade against the sulphuring of weak and redundant stocks. Prior to the starting of the epidemic in the Isle of Wight that island was a beekeepers' paradise, where the bees increased and gave surplus with little or no assistance from their owners. Nearly every village contained a number of skep beekeepers whose surplus colonies were sulphured in the autumn or exported as driven bees. With modern methods a steady importation of foreign bees took place. From 1898 to 1904 stocks and queens of foreign races were imported into the district where the disease is supposed to have originated. All the bees in that district were destroyed and the epidemic advanced across the island. Imms (1907) states that he was informed that the disease was so virulent that healthy swarms imported from the mainland were badly diseased within a week.

Importations of foreign bees have been so heavy and continuous that it is difficult to believe that no diseases have been introduced with these bees during the present century. In reviewing the work that has been done on Isle of Wight disease in Scotland, the possibility suggests itself that at one time

1 The danger from trading and consequent intermixing appears to have been thoroughly grasped by our forefathers who recognised that it was unlucky to trade in bees. The monks were great beekeepers and their migrations with their bees appear to have brought much bad luck. The traditional ceremonies that have been handed down among peasant beekeepers are often of pagan origin. The connection between Christianity and lack of honey is well shown in an old German adage given by Langstroth (1868):

"Bells' ding-dong and choral song
Deter the bee from industry;
But hoot of owl and wolf's long howl
Incite to toil and steady toil."
what may have been a bacterial disease was being investigated as the Isle of Wight disease.

Tinsley (1918), in a bulletin issued by the West of Scotland College of Agriculture, states that he succeeded in infecting healthy bees with Isle of Wight disease by feeding them with sugar syrup in which the liquid contents of the intestines of sick bees had been incorporated. In one of Dr Rennie’s earlier experiments (1919 a) Nosema spores in candy were fed to healthy bees in May. Crawling without Nosema was recorded as being present in June and the bees were found dead the following January. In 1915 also, pulped diseased bees were fed in honey to a stock on June 28th. The stock swarmed and both lots showed crawling in October and died out. The nearest bees were two miles away and remained healthy (Anderson and Rennie, 1916).

We assume that the Nosema spores were obtained from sick bees and that bacteria and other organisms were therefore unavoidably present in the candy. The results obtained by Dr Rennie may thus have been due to the organism that was present in the cases recorded by Tinsley. That organism is unknown, but the results suggest that it was situated in the alimentary canal.

V. Conclusions.

Acarine disease appears to be less virulent than the disease which swept across the Isle of Wight in the early years of this century. That the mite was causing damage at the same time is very probable but the investigations were centred on the acute and virulent disease.

It may be that most of the stocks affected with mites, but showing no symptoms of disease, die out sooner or later. But this does not demonstrate the existence of a new disease. It merely emphasises the soundness of the older system of beekeeping which considered it undesirable to retain any stock after the third season, the less desirable colonies being sulphured at an earlier period.

Although it may not be the cause of the Isle of Wight disease the discovery of the mite is of economic importance, revealing, as it does, one of the causes of the failure of modern beekeeping. Ever since the introduction of the “humane” system which saved the redundant bees and distributed them as “driven bees” throughout the length and breadth of the land, there has been a steady increase in disease which has helped to render the industry of honey production an unprofitable one. Before the rise of the Isle of Wight epidemic the losses were attributed usually to foul brood, although there was

1 The difficulty in classifying bee disease by symptoms is well shown by the following instance. While the work on Nosema in its relationship to Isle of Wight disease was being carried out at Cambridge there ensued a heavy mortality of humble-bees which was found to be associated with the presence in the Malpighian tubes of a protozoan closely resembling Nosema. In the year immediately past a similar mortality has been noticed in humble-bees, but the protozoan could not be found. The organism accompanying the mortality in 1921 was a nematode worm Sphaerularia bombi which undergoes development in the body cavity of the bee and eventually gives rise to huge numbers of larvae. In both years, the symptom of the trouble was inability to fly.
much difference of opinion as to the amount of harm caused by this latter malady. It is possible that *Tarsonemus* was present in the more severe cases and, consequently, a fresh series of observations are necessary.

It is the opinion of the writer that the mite will prove a comparatively harmless parasite in countries where two or more honey harvests and constant breeding activity are the rule. In some such districts we may expect to find the endemic centres of the disease.

If this opinion is confirmed, the mite will also be found in Australia, America and other countries as it is unlikely that we have been the only country to import it. In America there is a large amount of unexplained paralysis, and of winter and spring losses, while in Australia the subject of bee mortality is much discussed without anything very definite being known. Some of these troubles may be accompanied by mites.

In this country bees parasitised by mites are to be found all over the kingdom from Land’s End to John o’ Groats. The recognition of the dangers arising from modern procedure may eventually give rise to wiser and better methods of beekeeping and the discovery of the Aberdeen investigators may thus have far-reaching and unforeseen consequences.

REFERENCES.


See also Dadant (1907).


THREE NEW SPECIES OF TRICHODECTES FROM CEPHALOPHUS MONTICOLA AND PROCAVIA CAPENSIS FROM SOUTH AFRICA.

By LAURENCE HILL.

(Pietermaritzburg, Natal.)

(With Plate II.)

Trichodectes bedfordi, n.sp. Plate II, figs. 1–3.

Numerous males and females collected from a Blue Duiker (Cephalophus (Guebei) monticola) at Ngome Forest, Mt Ngwibi, Natal, by Mr J. Tustin.

Female. Total length 1.73 mm., length of head 0.37 mm., length of prothorax 0.09 mm., length of metathorax 0.11 mm., length of abdomen 1.16 mm. Width of head 0.30 mm., width of prothorax 0.25 mm., width of metathorax 0.30 mm., width of abdomen 0.42 mm.

Head. Longer than broad. Forehead elongated with a moderately deep median notch, which has a deep marginal band of chestnut-brown chitin divided into two parts by a narrow median longitudinal split, and produced into prominent trabecula-like processes in front of each antenna. General colour of head a pale yellowish-brown, with deep chestnut mandibles and bands. Antennal sinuses rather small and shallow. Ocular projections extending only very slightly beyond the temples, which are rounded. Temples with two short widely spaced hairs. Occipital margin slightly convex, rounded at the points where it is met by the faint occipital bands. Antennal bands of medium width, not reaching the base of the antennae, and tapering towards the anterior margin of the head, then broadening out into a wide clear space, interrupted by a deep narrow longitudinal line. Occipital bands joining the antennal bands just in front of the trabecula-like processes. Antenna long and narrow, extending well beyond the posterior margin of the head; third joint the longest; first and second joints of equal length. Eye pronounced. On the dorsal surface of the forehead there are three short pustulated hairs situated in a straight transverse line on each side near the antero-lateral margin of the head.

Thorax. Same colour as the head. Pronotum bare, with sides diverging, and a broad chestnut band at the lateral margins. Metathorax as wide as
the head, sides diverging, and rounded at the postero-lateral margins, with a broad band at the lateral margins and three small pustulated hairs on each side in a transverse line near the postero-lateral margins.

**Abdomen.** Elongated and narrow, white in colour, with crenulated lateral margins. Segments widely diverging towards postero-lateral borders, and with a yellowish-brown transverse band on the dorsal surface of each segment. On the dorsal surface, at the posterior margin of the band on first segment, one short pustulated hair on each side of the meson; last segment bilobed at the apex, with three to four median hairs on each lobe, and with a pair of short pustulated hairs situated on each side just within the lateral margin, and another pair mid-way between these towards the posterior indentation; remaining segments with a transverse row of minute pustulated hairs; segments two to seven with a small spiracle at each lateral angle.

**Male.** Total length 1.56 mm., length of head 0.40 mm., length of prothorax 0.07 mm., length of metathorax 0.14 mm., length of abdomen 0.95 mm. Width of head 0.28 mm., width of prothorax 0.22 mm., width of metathorax 0.26 mm., width of abdomen 0.43 mm.

**Head.** With the median notch deeper than that of the female. Antennal sinuses deep. Ocular projections small, not well defined. Temples narrow, smoothly rounded. Occipital margin markedly convex, emarginate where it is met by the occipital bands. Eye pronounced. Antennae large, and backward pointing, reaching the metathorax; the first joint large and wide (sacculatated) longer than the second and third joints combined; second and third joints of approximately equal length; on the upper surface of the first joint of the antenna, a longitudinal row of eight small pustulated hairs one beside the other, and extending from the proximal to the distal extremity; third joint slightly curved, and at the distal end two or three short stout dark-brown denticles. Mandibles situated well forward on the head.

**Abdomen.** Closely resembles the female in general outline, with the last segment oval, and having a row of medium sized hairs at the tip. On the dorsal surface, the transverse brown bands of segments four to seven are deeper (somewhat telescopic in appearance), and with well marked projecting somewhat rounded edges almost reaching the spiracles at the lateral angles. (Fig. 3 gives an excellent representation of these bands.)

**Genitalia.** Very conspicuous. The basal plate longer than the parameres, and consisting of two thickly-chitinised bars slightly thickened and rounded at their posterior end. Parameres long and narrow, of even thickness throughout their length (concavo-convex in shape), and markedly diverging posteriorly. Penis long and stout. Beneath the penis a wedge-shaped plate with the posterior end bifid. (Plate II, fig. 3.)

This species would seem to be closely allied to *Tr. lineatus* n.sp., described by Bedford in Part II, *Anop. from South African Hosts* (1920). The shape, however, of the abdominal segments of both male and female, and
终端段和生殖器的男性使该物种能够容易地与他描述的物种区分开来。

我的感谢应归于格雷夫·G.A.H.贝弗德博士，他总是慷慨地给予我帮助和协助。我非常高兴将这个物种以他命名。

*Trichodectes lindfieldi* n.sp. Plate II, figs. 4–6.

在Mtabamhlope, Estcourt District, Natal, by Mr P. Barnes.

**Female.** Total length 1.47 mm., length of head 0.43 mm., length of prothorax 0.12 mm., length of metathorax 0.15 mm., length of abdomen 0.77 mm. Width of head across temples 0.42 mm., width of prothorax 0.30 mm., width of metathorax 0.40 mm., width of abdomen, at third segment 0.71 mm.

**Head.** Light yellowish-brown. Almost as broad as long. Forehead dome-shaped, emarginated in front, with the sides slightly convex, and produced with a prominent trabecula-like process in front of each antenna. Antennal bands narrow and dark, terminating approximately midway between the anterior margin of the head and the trabecula-like processes. Frontal sinus deep, semicircular in section, with a marginal band of dark brown chitin divided into two parts by a median longitudinal narrow space; one hair situated on each side just within the emargination. Antennal sinuses small and shallow with rounded inner margin. On each side of the forehead, there are three hairs, one in front, one in the middle, and one above the antennal sinus. Ocular projections prominent, extending well beyond the temples which are rounded. Occipital margin slightly convex, very slightly emarginate at the points where it is met by the occipital bands. Temples rounded with a narrow dark marginal band and three short marginal hairs. Occipital bands conspicuous, connected at their bases by a broad band, and meeting the antennal bands just above the trabecula-like processes. Antennae long and slender, reaching beyond the posterior margin of the head; the third segment the longest with four minute hairs; first and second segments of equal length each with two short hairs. On the dorsal surface of the forehead there is a semicircular row of six small pustulated hairs; and below this semicircular row one small hair on either side just within the antero-lateral border; also two small and two medium hairs situated in a line with the trabecula-like processes. On the hind-head a semicircular row of four short hairs in the middle, and two on each temple.

**Prothorax.** Much broader than long, with a broad marginal band of deep chestnut-brown; lateral margins almost parallel, the posterior margin markedly convex, with medium postero-lateral emarginations, and one short pustulated hair on each side of the meson near the anterior margin.

**Mesothorax.** With three pustulated hairs on the dorsal surface.

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Metathorax. Wider than prothorax, with prominent projecting lateral angles reaching nearly as far as the postero-lateral angles of the first abdominal segment. On the dorsal surface two longish hairs at each lateral angle, and a row of fourteen small hairs along the posterior margin. Posterior margin markedly concave.

Abdomen. Crenate, somewhat longer than wide, widest at the third segment, with a median dark transverse band on all the segments. On the first three segments there is a large dark brown pleurite on each side, these are succeeded on the remaining segments by a smaller pleurite of the same colour. On the dorsal surface, the first segment has two pairs of small pustulated hairs on each side of the meson near the anterior margin, and twelve hairs in a row along the posterior margin; second segment with a transverse row of twenty small pustulated hairs along the posterior margin; and a lateral group of five hairs. Above, and on the inner side of this lateral group, there is a further distinct separate group of three hairs; third segment with a transverse row of twenty hairs along the posterior margin, a lateral group of five hairs, and on the inner side above each lateral group, a further group of five hairs; fourth segment with a row of twenty-four pustulated hairs, a lateral group of three hairs, and on the inner side of this group a pair of hairs; fifth segment with a transverse row of twenty-four pustulated hairs, and a pair of hairs just within the lateral margin; sixth segment with a continuous row of thirty-six pustulated hairs; seventh segment with a row of twenty-four hairs; last segment bilobed at the apex, with a transverse row of twelve hairs, and two small and two longish hairs on each lobe.

On the ventral surface segments one and two each with a row of twelve hairs; segment three with a row of fourteen hairs; segment four with a transverse row of thirty-two hairs divided into three groups, the median group consisting of twenty hairs, and each lateral group of six hairs; segment five with a row of thirty hairs, the median group consisting of twenty hairs, and each lateral group of five hairs; segment six with a row of twenty-four hairs, and each lateral group of four hairs; seventh segment with a pair of small pustulated hairs at each lateral margin; last segment with nine to ten minute pustulated hairs scattered on each side of the meson.

Male. Total length 1.52 mm., length of head 0.39 mm., length of prothorax 0.12 mm., length of metathorax 0.15 mm., length of abdomen 0.86 mm. Width of head across temples 0.39 mm., width of prothorax 0.28 mm., width of metathorax 0.35 mm., width of abdomen at third segment 0.63 mm.

Head. The general outline of the head mostly resembles that of the female. The antennal sinuses are deeper and wider. Ocular projections scarcely visible. Temples bluntly rounded, meeting the occipital margin at an angle. At the posterior margin of the temples there is a small prominent chitinised protuberance (absent in the female). Antennal bands broader than in the
female, becoming fused into the occipital bands in front of the trabecula-like processes. Antennae large, and directed backwards, reaching well beyond the occipital margin of the head; the first segment broad, and longer than the second and third combined; second segment longer than the third. On the hind-head a semicircular row of four small pustulated hairs, and three on each temple.

**Prothorax.** With lateral angles bluntly rounded.

**Abdomen.** Oval, widest across the third segment. Resembles the female in regard to colour, shape of pleurites, etc. On the dorsal surface, last segment with a semicircular row of twelve pustulated hairs, and two small hairs at the posterior margin.

**Genitalia.** Conspicuous, the basal plate consisting of two chitinous bars considerably longer than the parameres. Parameres consist of short bands, broad at the base where they are rounded, and narrowing posteriorly, becoming fused at the tip. Penis short and stout. Beneath the penis, an elongated plate with proximal end rounded. (Plate II, fig. 6.)

*Trichodectes serrations* n.sp. Plate II, figs. 7–9.

Several males and females taken in company with the previous species from a Cape Hyrax (*Procavia capensis*), shot at Mtabamhlope, Estcourt District, Natal by Mr P. Barnes.

**Female.** Total length 1.25 mm., length of head 0.33 mm., length of prothorax 0.09 mm., length of metathorax 0.12 mm., length of abdomen 0.71 mm. Width of head 0.33 mm., width of prothorax 0.22 mm., width of metathorax 0.23 mm., width of abdomen 0.49 mm.

**Head.** Light yellowish-brown, as broad as long. Forehead rounded, with four hairs on each side and a long shallow median notch produced into a medium trabecula-like process in front of each antenna. Antennal bands dark, narrow at their base, broadening out into a deep serrated band of dark brown towards the centre of the forehead. Ocular projections pronounced extending well beyond the temples which are rounded. Occipital margin slightly convex. Temples rounded, with a narrow dark marginal band, and with three short pustulated hairs on each side. Occipital bands narrow, connected at their bases by a broad, dark brown band, and meeting the antennal bands in front of the trabecula-like processes. Antennae long and narrow, reaching just beyond the posterior margin of the head; third joint the longest, with three small hairs; second joint nearly as long as the first, and each bearing two small pustulated hairs. Eye pronounced. On the dorsal surface of the forehead, there are four small hairs on each side situated just within the antero-lateral margin. On the hind-head a small pustulated hair on each side close to the ocular projection, and a semicircular row of six small hairs near the posterior margin of the head.

**Prothorax.** More than twice as broad as long, with lateral bands of dark
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brown; marginal band faint and interrupted in the middle. Posterior margin very slightly convex, becoming deeply curved towards the lateral margins, which are slightly rounded.

Metathorax. Slightly wider, and longer than the prothorax, the lateral margins curved and bordered with a narrow dark band, the posterior margin slightly convex.

Abdomen. Oval in shape, broadest at third segment, whitish in colour, with a small brown transverse band on the dorsal surface of segments 1–6; seventh segment with the transverse band involving the whole of the segment. On the dorsal surface, first segment bare, with a deep marginal band of dark brown; second segment with a median group of four hairs widely spaced, and one pair at each lateral margin; third segment with twenty pustulated hairs; fourth segment with a row of sixteen pustulated hairs; fifth segment with a row of fourteen pustulated hairs; sixth segment with a row of fourteen hairs and two short hairs at the angle of the postero-lateral margin; seventh segment with a semicircular row of twelve pustulated hairs; last segment bilobed at the apex, with a row of eight pustulated hairs, and two short hairs on each lobe.

Male. Total length 1:16 mm., length of head 0:30 mm., length of prothorax 0:11 mm., length of metathorax 0:12 mm., length of abdomen 0:63 mm. Width of head 0:30 mm., width of prothorax 0:21 mm., width of metathorax 0:22 mm., width of abdomen 0:40 mm.

Head. The head generally resembles the female in outline, except that the antennal sinuses are much wider. Ocular projections extremely small. Antennae large and backward pointing, extending well beyond the occipital margin of the head, the first segment large and broad; second segment practically the same length as the first; the third segment the longest. On the dorsal surface of the head, a row of six small pustulated hairs close to the anterior margin, two small hairs situated in the centre of the head, mid-way between the bases of the antennae and the ocular projections, and a group of three to four hairs on the temples.

Prothorax. Produced sharply posteriorly, with a deep emargination at the posterior margin; lateral margins slightly rounded.

Abdomen. Oval, widest across the fourth segment. First segment entirely lacking the deep marginal broad band of the female; a brown transverse band on the dorsal surface of segments one to five; segments six to eight with an irregularly shaped and fused transverse band; last segment oval, with five to six short hairs along the posterior margin; first segment with a row of eight hairs; second segment with a row of fourteen hairs; third segment with a row of sixteen hairs; fourth segment with an unevenly spaced row of fourteen hairs; fifth and sixth segments with a row of fifteen to seventeen hairs; last segment with a closely set row of ten hairs.

Genitalia. Conspicuous, as shown in Plate II, fig. 9.
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DESCRIPTION OF PLATE II.

*Trichodectes bedfordi* n.sp.

Fig. 1. Female.
Fig. 2. Head of male.
Fig. 3. Last abdominal segments and genitalia of male.

*Trichodectes lindfieldi* n.sp.

Fig. 4. Female.
Fig. 5. Head of male.
Fig. 6. Last abdominal segments and genitalia of male.

*Trichodectes serraticus* n.sp.

Fig. 7. Female.
Fig. 8. Male.
Fig. 9. Last abdominal segments and genitalia of male.
ON THE LARVA AND PUPA OF A PARASITIC PHORID FLY—HYPOCERA INCRASSATA MG.

By HUBERT M. MORRIS, M.Sc., F.E.S.

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(Plate III and 4 Text-figures.)

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On Jan. 27th, 1920, among a number of nearly fully grown larvae of Bibio marci which had been kept in the laboratory about two weeks, a few individuals were observed to be unhealthy or dead. These latter were found to contain parasitic Dipterous larvae, which eventually proved to be those of Hypocera incrassata Mg. Certain of these larvae were observed to be leaving the bodies of other larvae of Bibio marci at the same time.

The larvae of Hypocera incrassata pupated in the soil immediately after leaving their hosts, and only a single parasite was observed in each of the latter. The pupae were kept in soil in the laboratory, and the adults emerged between June 24th and July 1st.

The larva and pupa of Hypocera incrassata appear to be undescribed. It is noteworthy that H. vitripennis is recorded as having been reared from humble-bees' and wasps' nests(4), and H. vectabilis from dried specimens of Coleoptera (1). The present species is of additional interest as being the first definite record of an insect parasitic on Bibionid larvae.

I am indebted to Dr D. Keilin for the identification of the adult flies, and for the information regarding previous records of the rearing of species of Hypocera. I am also much indebted to Dr A. D. Imms for advice during the preparation of this paper.

1. THE LARVA.

The larvae were not observed until they were leaving, or about to leave, the body of the host, and were then fully grown. At this stage they are about 8·0 mm. to 8·5 mm. in length and about 2·0 mm. in breath, and are of a yellowish-white colour.
External Form. The larva (Plate III, fig. 1) is composed of a small cephalic region, and eleven body segments, the segmentation being fairly distinct, but somewhat obscured by the presence of supplementary folds.

On the ventral surface of the cephalic region is a small opening, the mouth, from which projects the single mandibular sclerite (Text-fig. 1). Dorsal to the mouth are a pair of hemispherical lobes, bearing sensory structures. The most conspicuous of these structures is the antennal organ, which consists of two segments, the basal segment of which is cylindrical, and the distal segment dome- or bell-shaped (Text-fig. 2a).
Close to the antennal organ, but rather ventral to it, is a minute slightly curved and somewhat club-shaped projecting organ; near the latter there is also a small group of minute papillae (Text-fig. 2 b and c).

The first segment of the body is small and rather conical, and near its posterior margin is a pair of projecting lateral spiracles which are brown in colour. Dorsally this segment bears a slight median transverse depression.

The remaining segments, except the last, are cylindrical, and increase in size to the sixth, after which they are approximately equal in size. The second and third segments are each divided dorsally into two parts by a median transverse sulcus. On each of the following segments, from the fourth to the ninth, there are three similar sulci. The second and third of the regions so formed in each segment are less clearly defined than the first and fourth, and it may be added that the third and fourth regions increase slightly in prominence on the posterior segments.

The tenth segment is divided, dorsally, into three regions, of which the third is the most conspicuous.

The terminal segment is in the form of a blunt cone, and is divided dorsally into three regions, of which the first is the largest and bears the pair of small posterior spiracles laterally. The terminal portion of this segment is rather flattened.

Ventrally, the first segment is undivided; the second segment is very faintly divided, and the third segment more distinctly divided into two regions. The fourth to tenth segments are divided into three regions, of which the first is very slightly the more prominent.

The terminal segment is similarly divided into three parts, and bears the conspicuous aperture of the anus on the foremost division. On each side of the anus is a rather prominent rounded fleshy process.

Buccopharyngeal Armature (Plate III, figs. 4 and 5). The buccopharyngeal armature is divided into three parts: (1) the pharyngeal or basal sclerite, (2) the intermediate sclerites, and (3) the mandibular sclerite.

(1) The pharyngeal sclerite is prolonged posteriorly (dorsally and ventrally) as a very delicate chitinous structure, the dorsal prolongation being much the longer. Its anterior end is strongly chitinised and bears on each side a hollow for the articulation of the intermediate sclerite.

(2) The intermediate sclerites are stout and strongly chitinised and are not fused together. They articulate with the hollows at the anterior end of the pharyngeal sclerite by means of a hook-like prolongation at their posterior end.

(3) The mandibular sclerite is a single very stout structure. It articulates with the intermediate sclerites by means of a pair of backwardly directed divergent arms at its posterior end. Ventrally, near the base, it bears several slight transverse ridges suggestive of vestigial teeth.

Articulating with a slight projection at the anterior external angle of either intermediate sclerite is a delicate process, more strongly chitinised at its base,
and having the form shown in Plate III, fig. 6. At its distal extremity each process bears several projecting spines. These processes bear some resemblance to those described by Keilin (3) as “Baguettes orales” (oral rods) in carnivorous Anthomyid larvae.

*Tracheal System.* The tracheal system is amphipneustic, a pair of spiracles being situated on both the first and last segments of the body.

Both pairs of spiracles are very alike in size and structure and are brown, chitinised outgrowths from the body-wall: each spiracle communicates with the exterior by means of four oval apertures situated at its apex (Text-figs. 3 and 4).

2. THE PUPARIUM.

The puparium is brown, with the segments fairly clearly marked. It is about 5.0 mm. in length.

Dorsally there are several conspicuous constrictions. The prothoracic spiracular horns of the pupa emerge from the puparium through a pair of holes situated in a somewhat lateral position on its dorsal side, at the posterior end of the fourth body segment of the larva (Plate III, fig. 2).

These horns are about 0.23 mm. in length, and their distal half bears two rows of minute openings, about sixteen openings in each row. Both pairs of larval spiracles remain as conspicuous dark projections from the puparium, and the mandibular sclerite also projects at the anterior extremity.

3. THE PUPA.

The pupa, seen on removal from the puparium is white, with the appendages of the adult fly closely adpressed to the body. Its general characters can be seen on referring to Plate III, fig. 3, and scarcely need detailed description.

The head is of moderate size, and the antennae are large and are produced in a long slender process. The palpi are conspicuous and conical in shape. The labrum is short and flattened. The legs lie side by side, the tarsi of the posterior pair projecting slightly beyond the apex of the abdomen.

4. EMERGENCE OF ADULT FROM PUPARIUM.

When the adult fly emerges from the puparium, the latter is split in the following way. A circular cap, consisting of the skin of the cephalic region and thoracic segments of the larva, is split off by means of a fissure passing round the body between the third thoracic and first abdominal segments. A quadrangular plate is split off in addition, and it consists of the whole skin of the dorsal surface of the first three abdominal segments.

This plate may become completely detached, or may remain slightly adhering at its posterior end to the anterior margin of the fourth abdominal segment.
The larva of *Hyypocera incrassata* differs considerably from the larvae of *Phora Bergenstammi* Mik., *P. rufipes* Mg., and *P. ruficornis* Mg., as described by Keilin (2).

The buccopharyngeal armature is very different as may be seen by comparison with Keilin’s figures. The numerous sensory structures on the cuticle of the latter larvae are not present in the case of *Hyypocera incrassata*.

The absence of sensory structures may be an adaptation to a more completely parasitic existence, as may also be the simpler buccopharyngeal armature, with the fusion of the usual two mandibular sclerites into an unpaired organ.

The study of this larva lends support to the opinions of Brues, de Meijere and Keilin that the position of the Phoridae in the classification of the Diptera should be among the Cyclorrhapha.

REFERENCES.


DESCRIPTION OF PLATE III.

Larval and Pupal Stages of *Hyypocera incrassata* Mg.

Fig. 1. Fully grown larva. Dorsal view, *as.*, anterior spiracle; *ps.*, posterior spiracle. ×20.

Fig. 2. Puparium. Lateral view. *ts.*, protruding spiracular horn of pupa. ×20.

Fig. 3. Pupa removed from puparium. Lateral view. *s.*, prothoracic spiracular horn. ×20.

Fig. 4. Buccopharyngeal armature of larva. Lateral view. *i.*, intermediate sclerite; *m.*, mandibular sclerite; *o.*, “baguette orale”; *p.*, pharyngeal sclerite. ×126.

Fig. 5. Buccopharyngeal armature of larva. Ventral view. Lettering as in previous figure. ×126.

Fig. 6. “Baguette orale” of larva ×730.
THE MALLOPHAGAN FAMILY TRIMENOPONIDAE.

By G. F. FERRIS, M.A.,
Stanford University, California.

(With 8 Text-figures.)

Three species of two-clawed Mallophaga have heretofore been recorded from South American mammals. Concerning one of these species, *Menopon extraneum* Piaget, nothing is known aside from the information included in the original description and, judging from this and the accompanying figure, it appears not to differ from the usual type of bird-infesting members of this group. The other two species have each been made the type of a genus and these two genera have been considered by Harrison as representing a well-marked family, the *Trimenoponidae*.

Through the courtesy of the authorities of the United States National Museum and the Field Columbian Museum of Chicago, the present writer has been accorded the privilege of examining for parasites the mammal skins in the collections of these two institutions. Among the wealth of material thus discovered there have appeared three new species from South American mammals that are also referable to the family *Trimenoponidae*. In connection with the description of these three species, which necessitates the description of two new genera also, it has seemed desirable to review the family as a whole.

The types of the three new species are deposited in the collections of Stanford University. Paratypes of two of them, as indicated below, are deposited in the collections of the United States National Museum.

In the accompanying figures the left half represents the dorsal aspect, the right half the ventral aspect. Owing to the fact that the male in all the species here described is practically identical with the female in general form and appearance the female only has been figured in full.

Family *Trimenoponidae* Harrison.


In proposing this family, Harrison (1915), in a key to the groups of the Amblycera based upon the respiratory system, has separated it on the basis of the presence of but five pairs of abdominal stigmata and the presence of a posterior commissure. He states further: "Of these Group A is undoubtedly of family rank, *Trimenopon* occupying a very isolated position. It shows a superficial resemblance to the Boopidae, but is without the accessory sac in
the \(\ell\) genitalia and the special sensory organs of the first three abdominal segments which characterise that family. In addition it exhibits a fusion of prothorax and mesothorax, a condition not seen elsewhere in the Mallophaga. Trimenopon must rank as the type genus of a family Trimenoponidae. Philandesia, which I have not had an opportunity to examine, probably belongs here also."

While recognising the fact that this group is rather distinct from the other Menoponoid forms I am somewhat dubious as to its deserving family rank. However, before any very definite conclusions may be arrived at a thorough review of all the members of the Menoponoid group will be necessary. For the present I am accepting the family. I present the following statement of its characters.

Menoponoid Mallophaga with abdominal stigmata present upon but five abdominal segments, the third to seventh; without a slit in the lateral margin of the head; without pharyngeal glands; with the antennae four-segmented; without combs or brushes of setae on the ventral side of the posterior femora or any of the abdominal sternites; with the mesonotum distinct or sometimes entirely fused with the pronotum, the pronotum usually strongly winged.

As has been many times pointed out one of the most interesting problems in connection with the study of these ectoparasites is that of their distribution. This is, at least in part, the problem of the genetic relationships of their hosts. Just how far the two problems are concurrent is the most fascinating aspect of it all. In the case of the South American two-clawed species infesting mammals it is evident that the two problems diverge rather early, at least if we may form any conclusions from the rather scanty amount of information that is available.

The majority of the two-clawed Mallophaga from mammals have been taken from marsupials in Australia and for these Harrison has named a distinct family the Boopidae. Two of the species herein dealt with are from marsupials, but apparently they find their nearest relatives not in the Australian marsupial-infesting species but in other species from South American rodents. The one consolation for those of us who like to see our theories work as they should is that these two species are apparently referable to the same genus. Of the other three species, one is from members of the rodent family Lagostomidae, one from the family Octodontidae and one from the family Caviidae. There is at least a suggestion that here the problem of the distribution of the parasites is in large part geographical.

A similar situation appears to exist in the case of the Mallophagan family, Gyropidae, the members of which occur upon mammals that appear to have little more in common than the circumstance that all are South American. True, one species of this group has been described from a European rodent, but I am inclined to regard this record with grave suspicion. A rather extensive collection of Gyropids is available to me, and I hope in a later paper to consider this group at length.
Genus **Trimenopon** Cummings.


The original description of this genus requires some modification. I rewrite it as follows:

Mallophaga of the family *Trimenoponidae*; with the lateral margins of the head not emarginate or notched; without spines on the ventral side of the head; with the clypeal region delimited dorsally by a distinct suture; with the antennal fossae partially covered beneath by a flap; with distinct pulvilli on the first tarsal segment; with the genitalia of the male of a very complex type.

Type of the genus, *Menopon jenningsi* Kellogg and Paine. This is the only included species.

*Trimenopon jenningsi* (Kellogg and Paine).

Figs. 1, 2 A, 3 A, 4 B.

1910. *Menopon jenningsi* Kellogg and Paine, *Ent. News*, xxi, 461; Fig.
1913. *Trimenopon echinoderma* Cummings, *Bull. Ent. Res.* iv, 40; Fig. 4.

**Previous Records.** From *Cavia cobaya*, Panama Canal Zone (Kellogg and Paine); *Cavia cutleri*, Peru (Paine); *Cavia aperea*, Paraguay (Cummings).

**Specimens Examined.** The types of Kellogg and Paine.

**Notes.** The original descriptions of this species, especially that of Cummings, are in general quite detailed and I shall content myself here principally with somewhat more detailed figures for comparison with the other species of the family.

I think there can be no question that *T. echinoderma* Cummings is a synonym of *T. jenningsi* (K. and P.) as Harrison has indicated in his Catalogue of the Mallophaga (1916). Cummings states that the antennae of his species are five-segmented, while in *jenningsi* they are certainly but four-segmented, but as the two species agree in all other respects and as all the other species of the family have the antennae but four-segmented it seems quite certain that Cummings was in error.

Genus **Philandesia** Kellogg and Nakayama.


Mallophaga of the family *Trimenoponidae*; with the lateral margin of the head deeply sinuate-emarginate; without spines on the ventral side of the head; with the clypeal region delimited dorsally by a suture; with the antennal fossae covered beneath by a flap; with the labium provided with a pair of forward-pointing processes; with distinct pulvilli on the first tarsal segment; with the genitalia of the male of a very complex type.

**Type of the Genus.** *Philandesia townsendi* Kellogg and Nakayama. This is the only included species.
Mallophagan family Trimenoponidae

Philandesia townsendi Kellogg and Nakayama.

Figs. 2 b, 3 b, 4 a, 5.


Previous Records. From Lagidium peruanum, Peru.

Specimens Examined. One female and two males taken from a skin of Viscacia inca Junin, Peru (Field Columbian Museum). The types of the species are in the Stanford Collection but appear to have been mislaid and were not examined in connection with the preparation of this paper. The hosts are members of the rodent family Lagostomidae.

Notes. There is little to add to the original description but I am presenting figures for comparison with the other members of the family. The original figure shows the mesonotum as entirely suppressed but this is in error, for the mesonotum, although small, is nevertheless present. The genitalia of the male (Fig. 4 a) are extremely complicated, so much so that with the scanty amount

Fig. 1. Trimenopon jenningsi (Kellogg and Paine).
Fig. 2. Heads of: A, Trimenopon jenningsi (K. and P.); B, Philandesia townsendi (K. and N.); C, Harrisonia uncinata n.sp.; D, Cummingsia peramydis n.sp.; E, Cummingsia maculata n.sp.

Fig. 3. Genital region of female of: A, Trimenopon jenningsi (K. and P.); B, Philandesia townsendi (K. and N.); C, Harrisonia uncinata n.sp.; D, Cummingsia maculata n.sp.; E, Cummingsia peramydis n.sp.
of material available I am unable to do more than give a general sketch of their appearance.

Genus **Harrisonia** nov.

Mallophaga of the family *Trimenoponidae*; with the posterior portion of the head strongly produced laterally, the process ending in a heavily chitinised, flattened hook and with the anterior lateral angles of the head produced into a strong, downward and backward pointing, heavily chitinised hook; with the clypeal region not delimited dorsally by a suture; with the antennal fossae not covered beneath by a flap; with the mesothorax fused with the prothorax; with small pulvilli on the first segment of the tarsi; with the genitalia of the male of a very simple type.

**Type of the Genus.** *Harrisonia uncinata* n.sp. This is the only included species.

**Notes.** The extraordinary form of the head, together with the other characters enumerated, seems adequately to justify the erection of a new genus. The single included species is one of the most remarkable forms yet described.

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Fig. 4. Genitalia of males of: A, *Philandesia townsendi* (K. and N.); B, *Trimenopen jenningsi* (K. and P.); C, *Cummingsia permydis* n.sp.; D, *Harrisonia uncinata* n.sp.; E, *Cummingsia maculata* n.sp.
among the Mallophaga but it appears to be quite definitely a member of the **Trimenopon** group.

I am naming this genus in honour of Mr Lancelot Harrison as a token of appreciation of the stimulating quality of his work on the Mallophaga.

**Harrisonia uncinata** n.sp.

Figs. 2 c, 3 c, 4 d, 6.

**Specimens Examined.** A single female, the holotype, from *Hoplomys gymnurus*; one male, the allotype, from *Nelomys mirae*; and one male paratype from *Proechimys semispinosus*; all from San Javier, North Ecuador. The specimens are from skins in the United States National Museum and a paratype of the new species will be deposited with that institution. All the hosts are members of the rodent family **Octodontidae**.

**Female** (Fig. 6). Length 1.1 mm. General form rather stout. **Head** (Fig. 2 c) as described for the genus. The clypeal region is very small, is semicircular in form and is bounded on each side both dorsally and ventrally by...
Mallophagan family Trimenoponidae

a heavily chitinised area from which rises the strong, downward curved hook at each anterior angle of the head. Slightly lateral of the clypeal region the anterior margin is deeply notched on each side. The flap covering the antennal fossa is strongly chitinised and is separated by a deep notch from the laterally produced posterior portion of the head. On the ventral side the wall of the antennal fossa is strongly chitinised, this chitinisation extending in a curve to the posterior margin of the head near the median line. The maxillary palpi are rather slender and the mouth-parts present no special characters.

Prothorax and mesothorax closely united, the mesonotum reduced merely to a chitinised, transverse bar that is concealed beneath the posterior margin of the mesonotum. The pronotum bears a pair of broad, heavily chitinised, diagonal bars and the lateral margins of the mesonotum are heavily chitinised. Dorsally both segments bear several quite stout setae and ventrally there is a longitudinal series of smaller setae at the base of the coxae.

Abdomen with the pleural plates strongly developed on the third to fifth segments, each bearing a single long and several small setae. The lateral margins of the sixth to ninth segments each bear a single long seta. Tergal
and sternal plates strongly developed on all the segments except on the first sternite. The tergal plates bear a single transverse row of four or six setae while the sternal plates for the most part bear one row of large setae and an irregular row of much smaller setae.

The genital region (Fig. 3 c) bears a cluster of setae near each lateral margin.

**Male.** Length 1 mm. In general form and structure closely resembling the female. The genitalia (Fig. 4 d) are very simple. The basal plate is composed of a pair of long, slender rods, united at the anterior extremity in the form of an inverted V. At the tip of each of these rods is a short piece terminating in a hook, these probably representing the parameres, and between the tips of the rods are a pair of flattened, pointed pieces of doubtful homology.

Genus *Cummingsia* nov.

Mallophaga of the family *Trimenoponidae*; with the lateral margin of the head at the most but slightly notched; with two pairs of strong, flattened, backward-pointing spines on the ventral side of the head, these arising from just before the bases of the antennae; with the clypeal region not delimited dorsally by a transverse suture; with the antennal fossae not covered beneath by a flap; with the mesonotum quite distinct; without pulvilli on the first segment of the tarsi; with the genitalia of the males of a simple type.

**Type of the Genus.** *Cummingsia maculata* n.sp. One other species, *C. peramydis* n.sp., is included.

**Notes.** It is with some hesitation that I refer the two species above named to the same genus, for in the structure of the head there is a rather notable difference. I would especially call attention to the apparent absence of the maxillary lobes in *C. peramydis*. However, they are not so different that their association in the same genus is entirely incongruous and in some respects they are very similar.

I am naming this genus in honour of the late Bruce Cummings, by whom *Trimenopon*, the type-genus of this family was established.

*Cummingsia maculata* n.sp.  
Figs. 2 e, 3 d, 4 e, 7.

**Specimens Examined.** One female, the holotype, and three males from *Caenolestes* sp., Cedrobamba Ruins, Peru. The specimens are from a skin in the United States National Museum and a paratype of the species will be deposited in the collections of that institution. The host is a marsupial.

**Female (Fig. 7).** Length 1-2 mm. *Head* somewhat wider than long, nearly truncate anteriorly and with a slight notch in the lateral margin. Clypeal region very small, bounded dorsally by an elongate, heavily chitinised area on each side that extends nearly to the middle of the head. Beyond this area is a smaller area at the anterior lateral angle which extends to the ventral side and from which rises the pair of flattened spines. The wall of the antennal fossa is heavily chitinised. The maxillary palpi are very short and stout.
Mallophagan family Trimenoponidae

Thorax with the mesonotum distinctly developed. The pronotum and mesonotum each bear a few small setae and one or two pairs of long setae along the posterior margin. The sternites of the thorax bear numerous scattered setae.

Abdomen with the pleural plates well developed on the second to fourth segments, very weakly so on the remainder, each plate with several small setae and with a single long seta at the dorsal posterior angle. Tergal and sternal plates strongly developed, for the most part bearing a single transverse row of ten or more slender setae. Genital region (Fig. 3 d) with two submedian clusters of small setae.

Male. Length 1.1 mm. In general closely resembling the female. Genitalia (Fig. 4 e) with the basal plate very long and moderately broad, not divided. At the apex of the basal plate are the relatively small parameres, these curved, tapering and with an expanded basal portion. Between the parameres lies the quite large Y-shaped pseudopenis. The apical third of the basal plate is overlain by the vesicula penis, the walls of which are studded with minute teeth.
Cummingsia peramydis n.sp.

Specimens Examined. Holotype, a female, four female paratypes and one male, the allotype, from Peramys domesticus, Quixada, Brazil, from skins in the Field Columbian Museum. The host is a marsupial.

Female (Fig. 8). Length 1.4 mm. Head (Fig. 2 d) somewhat broader than long, quite smoothly rounded anteriorly, with but the faintest indication of a notch in the lateral margin. The antennal fossae are quite deep and the wall is heavily chitinised, this chitinisation broadening anteriorly and extending to the anterior margin of the head. From it arises the strong ventral spines. The maxillary palpi are quite short and the maxillary lobes are apparently lacking.

Thorax with the mesonotum clearly present, the pronotum and metanotum bearing a few small setae and with two or three pairs of long setae along the posterior margin. The sternites of the thorax bear numerous short setae and near the base of each coxa is a single very long seta.
Mallophagan family Trimenoponidae

*Abdomen* with the pleural plates well developed on the second to seventh segments, each with a few small setae. Tergal and sternal plates strongly developed each bearing a transverse row of as many as twenty-four setae of variable lengths. The genital region (Fig. 3 e) bears numerous small setae.

**Male.** In general form and characters closely resembling the female. Length 1.2 mm. *Genitalia* (Fig. 4 c) with the basal plate quite long and moderately broad; parameres about a third as long as the basal plate, slender and tapering; pseudopenis large Y-shaped, attached to the vesicula penis, the walls of which are studded with small teeth.
ON ASCARIS VITULORUM GOEZE.

By C. L. BOULENGER, M.A., D.Sc.

(From the Research Laboratory in Agricultural Zoology, University of Birmingham.)

(With 3 Text-figures)

Until the appearance of Neumann’s paper “Sur l’Ascaride des Bêtes Bovines” (1883), *Ascaris vitulorum* was not generally accepted as a valid species, the form from cattle being referred by different authorities either to *Ascaris lumbricoides* or *A. megaloccephala*. Neumann’s study of this worm showed, however, that the species differs in many important anatomical characters from the common Ascarids of the pig and the horse and since the publication of his memoir the specific identity of *A. vitulorum* has not been disputed.

My attention was called to this parasite in India last year, the material used for class purposes in the Punjab University including specimens of an *Ascaris* from the Indian domesticated buffalo (*Bos bubalis*). The general macroscopic anatomy of these worms, as seen dissected by students in the laboratory, seemed to agree very well with that described by Neumann, and at the time I had no doubt that I was dealing with the same species as that found in cattle in Europe.

Since my return to Birmingham I have subjected the material to closer microscopic study which showed that the Indian specimens differ in several characters, including some of considerable systematic importance, from the *A. vitulorum* as described by Neumann.

The specific diagnosis of *A. vitulorum*, as now generally accepted, is the following given by Ransom (1911) in his monograph on the Nematodes parasitic in the alimentary tract of Ruminants, and based no doubt largely on Neumann’s account:


*Male* 15 to 25 cm. long by 3 mm. in maximum thickness. Ventral surface of tail supplied with two irregular rows of 10 to 15 papillae each, all preanal.

*Female* 22 to 30 cm. long by 5 mm. in thickness. Vulva toward the anterior sixth of the body. Eggs 75 to 80 µ in length.

Hosts.—Cattle (*Bos taurus*); zebu (*Bos indicus*).”
Ascaris vitulorum Goeze

The specimens from the Punjab fail to agree in two of the most important of these specific characters, namely the absence of cephalic papillae on the lips and postanal papillae on the male tail, the worms studied by me possessing distinct papillae on the lips in much the same position as in A. lumbricoïdes, the male individuals also bearing genital papillae behind the cloaca.

These, together with other less important points of difference, led me to believe that the worms from the Indian buffalo would prove specifically distinct from A. vitulorum from Bos taurus; I was, however, fortunately able to compare the Punjab material with some Ascarids from ordinary cattle collected by Mr. H. E. Hornby in Northern Rhodesia, these proved to agree in all essential respects with the Indian form. I have not been able to obtain any Ascarids from cattle in England, further study will therefore be necessary to settle the questions as to whether the European form is identical with that from Indian and African sources, and whether the discrepancies are due to errors of observation on the part of Neumann or not.

The following account of A. vitulorum is based solely on the material from the Punjab and from Northern Rhodesia.

Ascaris vitulorum Goeze, 1782.

Ascaris vituli Gmelin, 1790 (in part).

Length, male, 17.5 to 21 cm.; female, 21 to 27 cm. Thickness, male, 4 to 5 mm.; female, 6 mm. Body colourless in spirit material, tapering at both extremities. Body-wall thin and somewhat translucent.

Cuticle with transverse striations about 0.05 mm. apart.

Head small, 0.7 to 0.95 mm. broad, there are three simple lips, without interlabia. The shape of the lips is as described by Neumann, each consisting of a broad basal region and a much narrower distal region, the latter with a slightly emarginate anterior border.

Dorsal lip 0.45 to 0.6 mm. broad, 0.32 to 0.47 mm. long; bearing two nearly circular, flattened papillae on the basal portion (Text-fig. 1). Ventral lips slightly narrower than the dorsal lip, each bearing a single papilla. The pulp of each lip is divided anteriorly into two lobes. Dentigerous ridges well developed, with strong teeth, 0.06 to 0.075 mm. apart.

Oesophagus considerably shorter than in allied species, 3 to 4.5 mm. in length, divided into two regions, the anterior muscular and club-shaped with a maximum thickness of 0.8 to 0.9 mm., the posterior region in the nature of a "ventriculus," 0.45 to 0.6 mm. long and 0.5 to 0.8 mm. broad. The walls of the ventricular region are largely granular in structure.

Male. Posterior extremity slightly curved ventrally. Caudal region with ventral surface distinctly flattened and terminating in a small mucronate

1 e.g. the more forward position of the vulva in the female.
2 In A. lumbricoïdes from the pig the oesophagus has a length of about 9 mm.
appendix. Cuticle of tail somewhat expanded, forming narrow alae in the region of the terminal appendix (Text-fig. 2).

Cloaca 0.45 mm. from the posterior extremity of the body.

Preanal papillae in two parallel rows, each consisting of about 13. Behind the cloaca is a pair of larger flattened postanal papillae, the pulps of which have double terminations. The posterior appendix bears about five pairs of small papillae, somewhat irregularly arranged.

Spicules equal, about 0.95 mm. in length. The greatest thickness is about 0.04 mm. anteriorly, each spicule tapering gradually backwards and terminating bluntly.
Ascaris vitulorum Goze

**Female.** Tail short, the distance between the anus and the posterior extremity varies considerably in different specimens, from 0.6 to 1.1 mm. (Text-fig. 3).

Vulva situated anteriorly, 25–30 mm. from the cephalic extremity and dividing the total length of the body in the proportion 1:8. Internal female organs as described by Neumann; the uteri are fused posteriorly, forming a common tube continuous with the vagina, the latter has a length of about 10 mm., the total length of the unpaired genital duct measuring 30 mm.

Paired uteri short, about 70 mm. in length, running backwards, parallel with one another; each oviduct bears a conspicuous swelling or ampulla close to its junction with the uterus.

Eggs 0.08–0.095 mm. long × 0.07–0.075 mm. wide, less coarsely mamillated than those of *A. lumbricoides*.

The character of the oesophagus in *A. vitulorum* is of considerable interest from the systematic point of view; recent authorities, e.g. Railliet and Henry (1912) and Baylis (1920), have attached great importance to the structure.

![Fig. 3. Ascaris vitulorum. Posterior extremity of female, showing variation in length of caudal region, an. anus. ×35.](image)
of the alimentary canal in the classification of the family Ascaridae, the latter
having proposed to unite in the sub-family Anisakinae all genera in which
the oesophagus is divided into an anterior muscular portion and a posterior
ventriculus of different histological structure.

There can be no doubt that, apart from the structure of the oesophagus,
*A. vitulorum* is most closely allied to *A. lumbricoides* and *A. megaloccephala* and
must be retained in the genus *Ascaris* s. str.

The species described above is evidently a common parasite in both the
localities from which my material was obtained; when sending the specimens
from Northern Rhodesia Mr Hornby supplied the following interesting note
on the occurrence of the worms in that district:

"These parasites are extraordinarily common and, when present in large
numbers, cause scouring, wasting and death of the infested calves. Sometimes
the small intestine bulges along its whole length owing to the number of worms
present. Only sucking calves are affected. Growth after ingestion must be
very rapid, as I have found hundreds of worms, from 1 to 7 inches long, in
a calf only two weeks' old, and adult worms predominated in a six weeks' old
animal."

I can obtain no definite information as to the time required by *A. vitulorum*
to reach maturity after the ova have been ingested, in allied species, however,
development is known to be rather slow, the experiments of Epstein (1892)
on human subjects and of Ransom and Foster (1920) on pigs have shown that
for *A. lumbricoides* a period of about two and a half months is required for
full development from the egg in these hosts.

Mr Hornby's observations suggest either that the development of *A. vitu¬
lorum* in calves is much more rapid than that of *A. lumbricoides*, or that prenatal
infection must take place. The possibility of prenatal infection cannot be
excluded in view of the recent development of our knowledge of the migrations
of young Ascarid larvae within their hosts.

Neveu-Lemaire (1912) has reported two cases of prenatal infestation in
lambs with the lung-worm, *Dictyocaulus filaria*, other indisputable records of
such infections with other parasites are to be found in the literature; Cort
(1921) has recently published an interesting summary of the known cases.

REFERENCES.

**Baylis, H. A.** (1920). On the Classification of the Ascaridae. I. The Systematic Value of

**lxxvi**, 170.

**Epstein, A.** (1892). Ueber die Uebertragung des menschlichen Spulwurms (*Ascaris lum¬
v, 1–16.

Ascaris vitulorum Goeze


NOTES BEARING ON VAN BENEDEN, LEUCKART AND SONSINO WHOSE PORTRAITS APPEAR IN PARASITOLOGY, XIV, No. 1.

PORTRAIT-PLATES XII—XIV.

(Continuing the series begun in Vol. XIII.)

BY GEORGE H. F. NUTTALL, F.R.S.

(From the Molteno Institute for Research in Parasitology, University of Cambridge.)

Pierre Joseph van Beneden
1809–1894.

(Portrait-plate XII.)

Pierre Joseph van Beneden was born 19 December, 1809, at Malines, Belgium, and died 8 January, 1894, at Louvain. He graduated in medicine (1831) and became Curator of the Natural History Museum, Louvain, where he became Professor of Zoology and Comparative Anatomy in 1836. Besides doing zoological work of high value which he pursued actively to the end of his life, he established at his own expense in 1843, a marine aquarium, one of the first of its kind. In parasitology he attained great distinction through his investigations upon the biology of parasitic worms and their relation to their hosts, his work Mémoire sur les vers intestinaux (viii + 376 pp. 28 pls. 4°), published in 1858, bringing him the "grand prix des Sciences physiques" of the Institut de France. In 1875 he published his results in popular form under the title Les commensaux et les parasites dans le règne animal, as one of the International Science Series, this appearing both in English and German translations. His publications number over 200 and he was joint author with Paul Gervais of a general work on medical zoology in two volumes (1859).

He was the recipient of many distinctions and much esteemed for his high character. He was elected Foreign Corresponding Member of the Royal Society in 1875 and President of the Royal Belgian Academy in 1881. He received the Hon. LL.D. Edinburgh in 1884 and was made Grand Officer of the Order of Leopold in 1886. He left a large family of daughters and one son,
Edward (1846–1910), who became Professor of Zoology at Liège in 1870 and likewise published contributions to parasitology.


Rudolph Leuckart
1822–1898.

Karl Georg Friedrich Rudolph Leuckart was born 7 October, 1822, at Helmstedt, Brunswick, and died 6 February, 1898, at Leipzig. In 1847 he became Privat Docent at Gottingen, in 1850–69 he was Professor of Zoology at Giessen and in 1870–98 he held the like chair at Leipzig. He distinguished himself especially as a helminthologist, being attracted to the subject through the work on parasitic worms carried out by his uncle, Prof. F. S. Leuckart (1794–1843).

His first helminthological work, on Cestodes, appeared in 1848. He wrote on Linguatulidae (Pentastoma, 1857–60), Pupipara, the nematoid worms Attractonema and Sphaerularia, on Trichina spiralis (discovered by Sir James Paget when a student and named by Richard Owen), Echinorhynchus, Strongyloides, and created the class “Sporozoa” among Protozoa. One of his last discoveries was that Lymnaeus periger serves as intermediary host to Distomum hepaticum, a discovery anticipated by a few weeks by Thomas of Oxford, who later became Professor at Christchurch, New Zealand. Much of Leuckart’s original work is contained in his Menschliche Parasiten, etc., which went through two editions (1863 and 1876–1901) and was translated into English. Reference to the bibliographies below cited shows the wide range of his activities. His laboratory was a centre of keen research and many well-known parasitologists were his pupils.


For complete Bibliography see Leuckart’s Festschrift (issued on the 50th anniversary of his professorial career); Stiles and Hassall (1906), Index Catal. of Med. and Veter. Zool. pp. 1078–1088. Our portrait is reproduced from one that appeared in the München. med. Wochenschr.
PIERRE JOSEPH VAN BENEDEN

1809—1894

Separate copies may be obtained from the University Press, Cambridge.
THE LIBRARY
OF THE
UNIVERSITY OF ILLINOIS
RUDOLPH LEUCKART

1822—1898

Separate copies may be obtained from the University Press, Cambridge
ERRATUM.

*Parasitology*, xiv, p. 95, last paragraph.

For "Salmon and Stiles" read "Stiles and Hassall."
Prospero Sonsino
1835–1901.
(Portrait-plate XIV.)

Sonsino was born 6 August, 1835, at Tunis, of Italian parents, and died 9 November, 1901, at Montepiano, a village in the Apennines, Tuscany. He studied medicine at Pisa. In 1860–64 he travelled and practised his profession in Turkey and Asia Minor, then he settled in Florence, where he did public health work and edited a medical journal called the *Imparziale*, in which some of his papers appeared. In 1873–85 he resided in Egypt, practised and worked under very unfavourable conditions as micrographer to the Khedival Laboratory at Cairo, devoting himself specially to the parasitic worms of man and animals. He next travelled extensively in South America and the Far East as a ship's doctor, and returned to Pisa where he gave free courses in parasitology, revisiting Tunis (1893) and Egypt (1897) before he settled down finally in Montepiano.

As a physician he did heroic work during cholera epidemics in Italy (1865) and subsequently in Egypt. Of his 139 publications, 70 (published in 1874–1901) relate to helminthology. He laid stress on the importance of helminths as a direct cause and indirect predisposing cause of disease at a time when all too little significance was attached to them by physicians. Sonsino attached much importance to the experimental study of helminthology. He wrote on Schistosomiasis in man and animals, *Filaria bancrofti*, *Ancylostoma duodenale*, *Diotomum hepaticum*, and described a number of new and interesting species of helminths with which his name remains linked in the literature.

For a biography of Sonsino see Galli-Valerio (1906–7), *Arch. de Parasitol.* xi, 425–438, with portrait (herein reproduced) and signature (1893), a portrait on his death bed (1901), a facsimile of a letter, and bibliography; see also bibliography in Salmon and Stiles (1910), *Index Catal. of Med. and Vet. Zool.* pp. 2204–2211. Sonsino presented a collection of his papers to the writer in 1898, these papers being now in the Library of the Molteno Institute for Research in Parasitology.
NOTICE.

A Bibliography on Hookworm Disease of about 450 pages will shortly be ready for distribution as Publication No. 11 of the International Health Board. As the volume will be published in a limited edition, it is hoped that only individuals and institutions especially interested in the subject will apply for copies. Requests should be addressed to

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By GEORGE H. F. NUTTALL, F.R.S. and C. WARBURTON, M.A.

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INTRODUCTION.

The foundation of an Institute for Research in Parasitology in Cambridge is an achievement worthy of being recorded in some detail since it affords a recognition of the value of a branch of biological science that cannot but

Parasitology xiv
The Molteno Institute for Research in Parasitology

gratify all who have gained an insight into the deep significance of the many problems which parasitology presents when viewed from the standpoint of pure science.

We should, moreover, signalize the generosity of the benefactors who have rendered possible the establishment of such an Institute, for the help they have given deserves the gratitude of all who are concerned directly or indirectly in securing the welfare of man, animal or plant by combating the parasites which are the cause of so much waste and death throughout the living world.

I. EVENTS ANTECEDENT TO THE FOUNDATION OF THE INSTITUTE.

Since the writer's tenure of the Quick Professorship of Biology has had an important bearing upon the developments which led to the foundation of the Institute, it seems appropriate, in the first instance, to dwell upon the history of the Professorship and the conditions that apply to its tenure. Moreover, the work done in the Quick Laboratory during the years 1907-19 led by a process of natural growth to the inevitable necessity for the more favourable accommodation that has since been found in the Molteno Institute.

The will of Frederick James Quick⁴ bequeathed to the University of Cambridge a fund called the Frederick James Quick Fund⁵, the income thereof to be devoted to the promotion of "study and research in the sciences of vegetable and animal biology." The administration of this fund was entrusted by the testator's trustees to a Board of Managers consisting of the Vice-Chancellor and six Members of the Senate of the University appointed by Grace. The income of the fund was to serve (a) mainly for the stipend of a Chair (£1000) to be called the Quick Professorship of Biology, and (b) for the expenses of research carried on in the Professor's laboratory. The Quick Professorship is tenable for three years from the date of election, the latter taking place triennially and being open to all who may apply. The writer has been elected to five successive triennial periods of tenure. At the end of the first and second triennial periods an interval of six weeks occurred during which the chair was vacant and the stipend ceased, but the regulations governing the chair were modified in 1918³ by advancing the date of re-election so that there should be no break in the tenure of the Professorship.

The original regulations governing the Quick Professorship⁴ provided inter alia "that it shall be the duty of the Professor to devote himself to the study of the Protozoa, especially such as cause disease, and generally to promote that branch of science by research and by the superintendence of a laboratory or otherwise." The regulations provided, moreover, that the chair might "at

¹ For biographical note relating to Mr Quick, see p. 100.
³ Ibid. 23, iv. 1918, p. 619.
⁴ Cambridge University Reporter, 13, iii. 1906, p. 579.
any time after twelve years from the election of the first Quick Professor be subject to alteration by Grace of the Senate on the recommendation of the Board of Managers."

It may be mentioned here that the regulations were modified in 1920 on the recommendation of the Managers of the Quick Fund whereby the regulation cited in the previous paragraph was altered, the words "it shall be the duty of the Professor to devote himself to the study of the Protozoa, especially such as cause disease," being changed so as to read "it shall be the duty of the Professor to devote himself to the study of Parasitology." The Managers recommended that the field of study and research should be Parasitology for the following reasons:

Parasitology includes, of course, the study of Protozoa causing diseases as a special section. As a pure science it is closely linked up with vegetable and animal biology generally, while as an applied science it appeals equally to the medical man, the veterinarian and the agriculturalist.

Professor Nuttall has now been elected Quick Professor for periods of three years, five times in succession, and the work continuously carried out by him, and under his direction, has insensibly drifted into the wider field of Parasitology.

Hitherto there has been no suitable laboratory available for the Quick Professor of Biology. The authorities of the Medical School placed the unfinished part of their Museum at his disposal, temporarily, but even then the Professor and his staff have been crowded into what is practically one room. It is a happy solution of this difficulty that a new Institute has been given to the University, planned expressly for the continuation and expansion of the work of the Quick Professor.

As Professor Nuttall has been so closely associated with the gift to the University of the Molteno Institute for Research in Parasitology it seems to the Managers of the Quick Fund desirable to extend the field of activity set down for the Quick Professor so that it may coincide with that for which the Research Institute is destined.

Regulation 11 for the administration of the Quick Fund provides for alteration of the other regulations except nos. 1, 2, 7, 10 and 11 at any time twelve years after the date of the first election (16th October, 1906), "provided that in all cases the main object of the Fund, namely, the promotion of study and research in the sciences of vegetable and animal biology shall be adhered to." The alteration of regulation 8 (1), now proposed, is entirely in keeping with the main object of the Fund.

The Quick Endowment for Research. As previously mentioned (see p. 98), the Quick Fund, which provides the stipend of the Professor, also makes a certain provision for the expenses of research carried on in his laboratory. This fund, during the years 1906–18, yielded an average annual income of about £200 which has since increased to £300. Until the foundation of the Molteno Institute this represented the sole permanent source of income available for the purposes of research and the maintenance of a laboratory.

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1 Cambridge University Reporter, Date of Report 2, XII. 1920.
FREDERICK JAMES QUICK (1836-1902).

A Biographical Note.
(With portrait, Plate IV.)

Mr Quick was born in London on 22nd October, 1836, as the second son of James Carthew Quick, a wholesale coffee dealer of that city. He was educated at Harrow (1851-55) and Trinity Hall, Cambridge, where he came into residence in October, 1855, and took the degree of B.A. on 29th January, 1859. He studied for the Bar, but never practised. He learned farming at St Andrews, his father buying Woodmancole at Elmsworth, Sussex, for him. For a short time he became partner in a wholesale tea business, and in 1869 he entered (again as a partner) the firm of Quick, Reed and Smith, which his father had founded. He remained the head of this firm until his death on 21st December, 1902. Shortly before his death, having a horror of being buried alive, he requested one of the trustees of his will, Mr Williams, to see that "a surgeon probed his heart to see that death had taken place," asking, moreover, that his body might be cremated. His wishes were duly carried out, and his ashes were buried at Broadwood Cemetery. Mr Quick never married.

In business Mr Quick was a shrewd, far-seeing, and capable manager. His associates were greatly impressed with the accuracy of his judgment in affairs and in the estimation of the character of those with whom he came in contact.

He was much interested in botany and biology, and to this the founding of the Quick Professorship is mainly to be ascribed. Mr Williams has informed the writer that Mr Quick was very anxious that the Chair should be always abreast of the times, this accounting for pertinent provisions in the bequest. The full benefit of the latter will not be realized until the expiration of certain life-interests.

The writer is greatly indebted to Mr J. W. Williams for providing most of the information on which this note is based; Dr Henry Bond, Master of Trinity Hall, kindly supplied the little that is recorded in the College books.

THE QUICK LABORATORY.

After being elected to the Quick Professorship, the writer found "temporary accommodation" in the unfinished portion of the Medical School Museum, establishing the "Quick Laboratory" therein. The temporary quarters, however, became quasi-permanent for they were occupied during the years 1907-21. The laboratory consisted of one large room (Fig. 1) which was divided into cubicles by means of matchboard partitions and suitably placed cupboards or screens, the accommodation being the best that was available. A wooden floor was erected over that of the museum so as to bring the work benches near to window-level, the result being that the floor was very resonant, apart from its being creaky owing to its light sub-structure. The room grew inadequate at an early date for the increasing number of workers, collections, books and apparatus. The number of persons working in the room rendered

1 Mr Quick's second Trustee is Mr John Eagleton of London.
FREDERICK JAMES QUICK

1836—1902

Founder of the Quick Professorship of Biology, Cambridge
Fig. 1. Illustrating the cramped quarters of the Quick Laboratory during 1907–21. The entrance to the Laboratory (E) is reached by passing through the Hall (H) and small Demonstration Room (D) of the Medical School. The lettering indicates the internal arrangements: Alcohol specimen room (A), Benches (B), Desk (D), Glass blower (G), Microphotographic apparatus (M), Presses, Cupboards, Book-cases and Cabinets (P), Radiator (R), Sink (S), Thermostats (T), Ventilating shafts (V), Tables and shelves are left blank and 9 work-places are indicated by circles opposite benches.
it a very noisy place where through the shifting about and conversations going on it was difficult to collect one's thoughts and to carry on experimental work without constant interruptions, privacy being precluded. There was but one sink in the room for all the workers, there was danger of fire because of the amount of woodwork and alcohol-preserved material, and finally, especially during the war, the lack of heating and ventilation became insupportable. It was then that the writer resolved to issue an appeal for help and proceeded to write out a statement showing what a need there was in Cambridge of a suitably equipped Institute for Parasitological Research, the Quick Laboratory, in addition to all its other failings, being a place totally unfit for the prosecution of researches on organisms of an infective nature. The Quick Laboratory, as such, has ceased to exist since it is now incorporated in the Molteno Institute.

**Scientific Staff of the Quick Laboratory.**

With the aid of the grants and benefactions to be mentioned (p. 104) the following scientific staff was established, the various posts being created by Grace of the Senate: In June, 1907, a *Research Studentship in Medical Entomology* was established with an annual stipend of £100 derived from the Tropical Diseases Research Fund. In December, 1908, an *Assistantship in Protozoology to the Quick Professor* was established with an annual stipend of £100 paid during three years by the writer and subsequently by grants which have since been augmented. A second *Assistantship* was subsequently established but has lapsed. In November, 1911, a *Demonstratorship in Medical Entomology* with a stipend of £50 was established and in 1913–14 the post of *Helminthologist* was created with a stipend of £100, derived during the first year from laboratory funds.

**A List of those who worked in the Quick Laboratory 1907–21.**

Since it may be of interest in the future, a list of those who were engaged in research in the Quick Laboratory is herewith appended, the names of those who have published papers being marked with an asterisk:


1 *Cambridge University Reporter, 11, vi. 1907.*

2 *Ibid. 1, xii. 1908, p. 263; 24, xii following (Grace). Stipend now paid from the Tropical Diseases Research Fund, Medical Grant Committee and Medical Research Council.

3 *Ibid. 10 and 24, x., 14 and 21, xi. 1911. Stipend paid by the Tropical Diseases Research Fund.

4 Stipend paid in 1914 by the Tropical Diseases Research Fund. Owing to the war, the post has since been in abeyance.
*F. P. Jepson, B.A., 1908–9; *G. Merriman, 1909–12; *N. Cunliffe, B.A., 1913–14; M. E. MacGregor, B.A., 1914–15; *D. Keilin, Sc.D. (Paris), 1915–17, when he became Assistant and the Studentship was merged with the Assistantship he now holds.

Other workers in the Laboratory were: G. Beatty, M.D., Medical Officer, South Nigeria, 1911; Dr de Blieck, Director Veterinary Department, Java, 1915; Y. Bogaerts, medical student from Louvain, 1915; Major J. E. M. Boyd, M.C., R.A.M.C., sent by the War Office, 1919–20; *P. A. Buxton, M.A., now of the Health Department, Jerusalem, 1920; *Mr G. W. Bullamore, Director of the Institute for Bee-keeping, 1920–21; Major S. R. Christophers, I.M.S., Superintendent, King Institute of Preventive Medicine, Madras, 1912; *Major J. W. Cornwall, M.D., I.M.S., Pasteur Institute, Coonoor, South India, 1912; Captain J. D. Couatts, M.R.C.V.S., D.V.S.M., South Rhodesia, 1921; *Dr Lajos Gózony, Assistant, Bacteriological Institute, University of Budapest, 1914; Dr Graham, Medical Officer, Gold Coast, 1907; *Dr S. Hadwen, Pathologist, Biological Station, Ottawa, Canada, 1908–9, 1913; *L. Harrison, B.Sc., University of Sydney, Australia, 1914–16; Dr A. D. Hodges, Principal Medical Officer, Uganda, 1910; *F. M. Howlett, B.A., Assistant Imperial Entomologist, India, 1910; *Dr J. C. Johnson, Professor of Biology, New Zealand, 1913; Dr F. I. M. Jupe, Medical Officer, Gold Coast, 1910; R. C. Lewis, M.A., Cape Colony, 1911–12; Miss Jordan Lloyd, Newnham College, Cambridge, 1911; F. J. McCall, M.R.C.V.S., Veterinary Officer, British East Africa, 1914; N. MacDonald, Veterinary Service, Australia, 1909; *Miss D. Mackinnon, B.Sc., Carnegie Scholar, Aberdeen, 1908; J. E. M. Mellor, B.A., 1915, 1919; Dr C. de Meza, Veterinary Officer, Nyasaland, British East Africa, 1916; A. R. Momber, 1910–11; J. O. Muñoz, medical student from Liège, native of Mexico, 1914; *Captain J. W. Munro, B.Sc., R.A.M.C. (temp.), sent by War Office, 1917–19; *Dr Lucius Nicholls, Pathologist, Victoria Hospital, St Lucia, W. Indies, 1912–13; Dr Erik Nordenskiöld, Lecturer in Zoology, University of Helsingfors, 1910; *Captain W. S. Patton, M.B., I.M.S., 1908–9; I. M. Puri, M.Sc., Punjab, India, research student, 1921; L. P. W. Renouf, B.A., Trinity College, Cambridge, 1914; *L. E. Robinson, A.R.C.S. (London), research student, 1919–21; *Dr P. H. Ross, Government Bacteriologist, Nairobi, British East Africa, 1914; L. G. Saunders, M.Sc., McGill University, Montreal, research student, 1921; L. D. Sayers, B.A., Downing College, Cambridge, 1914; Dr J. O. Shircore, Medical Officer of Health, Nyasaland, 1914; *Dr G. S. Graham-Smith, Cambridge, 1908; *Dr G. U. Smith, Egypt, 1908; *Major F. H. Stewart, M.D., Sc.D., I.M.S. (retired), 1920–21; *Dr N. H. Swellengrebel, Assistant in Protozoology, Hygenic Institute, Amsterdam, 1910; J. F. Valladares, Senior Lecturer, Government Veterinary College, Bombay, 1910; Miss W. M. Vincent, Newnham College, research student, 1921; Major C. E. Williams, M.D., I.M.S., Rangoon, 1911; K. P. Williamson, M.A., Indian Education Service, 1911; Dr J. Y. Wood, Medical Officer, Kaballa, Sierra Leone, 1914; *Dr Wu Lien-Teh, Army Medical College, Tientsin, China, 1912;
Dr T. Yamanouchi, Tokio, Japan, 1910; Dr S. Yoshida, Department of Pathology, Osaka University, 1920.

Work carried out in the Quick Laboratory.

During the years 1906–21 the publications that emanated from the laboratory numbered 216, a large proportion appearing in this Journal. Some of the research work was done for Governments, notably that on piroplasmosis and East Coast Fever for the Governments of Cape Colony and Transvaal; that on the bionomics of houseflies, on fleas in connection with rat-plague, and on lice (in the first instance) for the Local Government Board; that on bee disease for the Board of Agriculture; that on lice and scabies for the War Office, the latter having been aided in drawing up Army Council Instructions dealing with lousiness and itch among troops and the methods of combating these widespread evils. "Combating Lousiness among Soldiers and Civilians" to the number of 250 copies (reprinted from Parasitology, x. No. 4, May 1918, pp. 411–586, 4 pls., 26 text-figs.) and issued in a special cover were presented by the writer to the British and Allied Army Medical Corps during the war.

Apart from the foregoing may be mentioned researches on bugs in relation to relapsing fever, on fleas and lice as carriers of rat trypanosomes, on the bionomics and structure of anopheline mosquitoes and their relation to malaria in England, on heartwater in sheep and goats, on piroplasmosis in the dog and horse, on the discovery of a remedy (trypanblue) for piroplasmosis and scientific observations on the effects of the drug, on the monographic treatment of the subject of ticks and of the louse, without mentioning other parasitological papers dealing with pathogenic protozoa, etc.

The activities of the Quick Laboratory were not confined to researches, for the writer, as its representative, served on various advisory bodies, notably as (a) Member of the Advisory Committee for Plague Investigations in India appointed by the Secretary of State for India, the Royal Society and Lister Institute; (b) Member of the Epizootic Abortion Committee of the Department of Agriculture and Fisheries; (c) Member of the Honorary Committee of Management of the Imperial Bureau of Entomology, Colonial Office; (d) Member of the Government Grant Committee for Scientific Investigations, Royal Society; (e) Member of the Fish Preservation Committee of the Department of Scientific and Industrial Research; (f) Member of the Army Pathology Advisory Committee, War Office. As usual, these various services have been rendered gratuitously.

REGARDING VARIOUS GRANTS AND BENEFACTIONS IN AID OF RESEARCH.

The income of the Quick Fund proving insufficient, the writer found it necessary to obtain financial assistance from outside sources, no funds being available from the University. For several years he defrayed the extra cost including the payment of the University Assistant to the chair and a Secretary.
Since 1909, annual Grants have been derived continuously from the Tropical Diseases Research Fund (Colonial Office), three grants were obtained from the Local Government Board (1913–16), three from the Board of Agriculture (1915–17) and one from the Rockefeller Institute, New York (1913–14), whilst, since, 1917, grants have been received from the War Office (once), and (repeatedly) from the Medical Research Council and Medical Grant Committee (Ministry of Education).

In addition to the foregoing, various Benefactions were received from private and other sources as follows: In 1909–10, from The Transvaal Government, £500; The Government of Cape Colony, £500; The Duke of Bedford, £100; Lord Rothschild, £100; Sir Richard Cooper, Bart., £100; Mr and Mrs P. A. Molteno, £100; Harry Mosenthal, Esq., £26. 5s.; J. Buchanan, Esq., £25; The Tropical Diseases Research Fund, £25; E. Darwin, Sc.D., F.R.S., £5. 5s.; Julius Auerbach, Esq., £5. 5s.; Fred C. Norton, Esq., £2. 2s.; making a total of £1489. In 1914, from Mr and Mrs P. A. Molteno, £400 (£100 thereof toward expenses of publication); Sir Dorabji J. Tata, £250; Mr and Mrs Henry Bubb, £25; making a total of £675.

THE FIELD LABORATORY.

In 1909–10, largely through the above-mentioned benefactions, a field laboratory was erected by the writer upon the Milton Road at a distance of two miles from the Medical School and present site of the Molteno Institute, the laboratory serving for experimental researches that cannot be carried out in the town. The buildings are of a temporary character but ample to meet the needs of the Institute in the future; they form an essential annex to the Institute. Our laboratory can accommodate two to four research workers, being adequately and economically heated by hot water radiator pipes and a coke furnace, water and gas being laid on but not electricity. The site of our laboratory forms a part (about 5 acres) of the University Field Laboratories comprising 27 acres of land. (See Fig. 2, showing the general distribution of the buildings.)

II. THE FOUNDATION OF THE MOLTENO INSTITUTE.

The highly unsatisfactory conditions prevailing in the Quick Laboratory led the writer to issue an appeal for funds with which to erect an Institute for Parasitological Research in Cambridge. The appeal was issued in printed form in May 1919 for private circulation. From Mr and Mrs P. A. Molteno it evoked the following generous response which was subsequently published in the Cambridge University Reporter by the Vice-Chancellor:

1 See Cambridge University Reporter, 22, ii. 1910 and 9, vi. 1914.
2 Nuttall, G. H. F. (1919), The Need of an Institute for Parasitological Research in Cambridge, 19 pp. 28 x 22 cm. (Illustrated with a general view and plans of the proposed Institute, Quick Laboratory and Field Laboratory. Privately printed at the University Press, Cambridge.)
Fig. 2. Showing the grouping of the buildings on a part of the land occupied by the University Field Laboratories, Milton Road, Cambridge. The dotted line (X) indicates the eastern boundary of the land belonging to our Laboratory as distinguished from that of the Agricultural and Pathological Departments; some of the buildings being held in common. Except for one building (below, on figure) to the extreme west, belonging to the Pathological Department, all buildings to the west of the dotted line belong to our Laboratory. The different structures are indicated by letters: (A) cattle sheds and stables, (C) caretaker's cottage, (D) destructor, (E) earth closet, (F) fodder store, (K) concrete platform, (L) laboratory, (O) office, (P) piggeries, (P.M.) autopsy house, (S) shed, (ST.) stable, (WT.) weighing-house.
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Mr and Mrs P. A. Molteno’s Benefaction.

Emmanuel College Lodge. 1 November, 1919.

The Vice-Chancellor has pleasure in publishing to the Senate the following letter which has been received by Professor Nuttall:

10, Palace Court,
London, W. 2.

October 23rd, 1919.

Dear Professor Nuttall,

I have for long been interested in your work on parasitology. I regard this work as of the very greatest value to the Empire and particularly to Africa, the home of so many diseases transmitted by and through parasites.

Your researches have already produced results which are of immense benefit in the control and prevention of diseases both of men and of animals. Indeed research of this nature is indispensable if Africa is to be made habitable for white men and for animals of European stock.

Your researches are however not bounded by the African field, vast as it is, but will apply all the world over in alleviating human suffering and protecting human and animal life from disease.

I am well aware how utterly unprovided you are with the necessary accommodation to carry on research work. It is really a scandal that this should have been allowed to continue for so long, and the difficulty grows continuously with the increase in the number of your specimens and the extension of your work.

Mrs Molteno has also been greatly interested in your work and desires to join me in providing funds for the erection and maintenance of a suitable building to be used in the future as an Institute for parasitological research in the University of Cambridge.

We understand from you that you consider a sum of £20,000 would provide a suitable building with its fittings for this work, and that a further sum of £10,000 would be necessary as a fund to provide an income for the upkeep and maintenance of the Institute, and that any surplus from this income could be used with the greatest advantage in furthering the purposes of research to be carried on in the Institute.

Mrs Molteno and I will be glad to present to the University these sums of £20,000 and £10,000 making £30,000 in all for these purposes, on the understanding that the University is prepared to provide a suitable site. We desire that this sum should be a benefaction to the University for the purposes outlined above.

After provision for the upkeep of the fabric, the balance of the income available from the maintenance fund should be placed at the disposal of the Director of the Institute (in the first place yourself) to be used at his discretion in furthering the work of research.

We desire further to say that we have examined the plans drawn up by Mr Harry Redfern, F.R.I.B.A.—with the general view of the proposed Institute as it would appear on the Downing site at Cambridge. These plans appear to us to afford a suitable building, with the various halls for Library, Museum, etc. and rooms for Professor and students carrying on research work.

Accommodation appears to be provided without any unnecessary superfluities in bricks and mortar, and yet to be simple and dignified, and we hope it will be found possible for this plan to be carried out.

I will be obliged to you if you will convey the contents of this letter to the Vice-Chancellor for communication to the Senate, and to the Cambridge University Association.
I may conclude by saying that I am a member of Trinity College, Cambridge, and an M.A. and LL.M. of the University, and that I have always had the highest regard for the University, and for the great work which it has done and is carrying on, and I am very glad to have the opportunity, in some small way, to assist in extending that work.

Believe me,
Yours faithfully,
P. A. Molteno.

This great benefaction was followed by a second of £6000, likewise from Mr and Mrs Molteno, to meet the greatly increased cost of building that became apparent as soon as the estimates were worked out.

It may be noted here that Mr and Mrs Molteno had already helped on the work of the Quick Laboratory financially in 1909 and 1914 (see p. 105) and that throughout the intervening years they had evinced much sympathy and interest in the researches carried out in our laboratory.

The foregoing benefaction led to another which otherwise would not have been received. It was announced in the *Cambridge University Reporter* as follows:

**Benefaction from the late Lord Strathcona and Mount Royal.**

Emmanuel College Lodge. 20 November, 1919.

The Vice-Chancellor has pleasure in informing Members of the Senate of a further benefaction received, on November 18, by Professor Nuttall:

In the *Cambridge University Reporter* of 22 February, 1910, under the heading "Benefactions for Research Work in Parasitology," the Vice-Chancellor informed Members of the Senate that various donations had been received by Professor Nuttall for the purposes of a field laboratory on the outskirts of Cambridge, adding that "£1000 has been promised, anonymously, when the fund has reached £6000."

This promise emanated from the late Lord Strathcona and Mount Royal, who visited Cambridge in July, 1909, and evinced much interest in the parasitological work that was being carried on in the University.

When Mr and Mrs P. A. Molteno generously offered the sum of £30,000 for the purpose of building and maintaining an Institute for Parasitological Research in Cambridge (*Reporter*, 4 November, 1919, page 206), an offer since accepted by Grace of the Senate, Professor Nuttall communicated with the late Lord Strathcona’s representatives, with the result that Lady Strathcona asked the Executors to pay Professor Nuttall the sum of £1000 "conditionally promised as a contribution to the Biological Research Laboratory (now the Institute for Parasitological Research) in connexion with Cambridge University."

**THE INAUGURATION CEREMONY.**

Before describing the new Institute in detail an account may be given of the ceremony of its inauguration.

The Institute was opened on the afternoon of 28th November, 1921, by Earl Buxton of Newtimber, G.C.M.G., late Governor-General of South

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1 See *Cambridge University Reporter*, 20, xii, 1920, p. 130.
2 Before the opening ceremony, visitors from the outside were entertained at lunch parties given respectively by the Vice-Chancellor and Mrs Pearce at Corpus Christi College, Sir Arthur Shipley and the writer at Christ’s College, Dr and Mrs H. K. Anderson at the Lodge of Caius College and by Mr and Mrs P. A. Molteno at the University Arms Hotel.
ELIZABETH MARTIN MOLTENO née CURRIE
1921
Africa, under the Chairmanship of the Vice-Chancellor of the University of Cambridge, Rev. E. C. Pearce, D.D., Master of Corpus Christi College. Academic dress and decorations were worn, Doctors appearing in scarlet robes in recognition of the importance of the occasion, a large and distinguished audience being present.

The Molteno family were represented by Mr and Mrs Percy A. Molteno (the founders), Sir James Molteno, K.C., Admiral Molteno, C.B. and Mrs Molteno, Mr and Mrs D. J. Molteno, Captain and Mrs Lenox Murray (née Molteno), Miss Audrey Molteno, Miss E. M. Molteno, Mr H. V. Molteno, accompanied by Mrs F. C. Selous, M. Emile Coué of Nancy, Miss E. Cowan and Mr H. B. Johnson.

Foreign institutions were represented by Professor M. Caullery of the Faculté des Sciences, la Sorbonne, Paris, and Dr M. Langeron of the Laboratoire de Parasitologie, École de Médecine, Paris, both of whom came to Cambridge to attend the ceremony; and Johan Hjort, Sc.D., Professor of Marine Biology in the University of Christiania.

Among the distinguished British visitors were Lord Pentland of Lyth, G.C.S.I., G.C.I.E., formerly Governor of Madras; Sir Richard Cooper, Bart., M.P.; Sir Herbert Read, K.C.M.G. (Colonial Office); Dr S. Monckton Copeman, F.R.S. (representing the Ministry of Health); Dr Andrew Balfour, C.B., C.M.G., and Dr C. M. Wenyon, C.M.G., both of the Wellcome Bureau of Scientific Research, London; Dr S. A. Neave of the Imperial Bureau of Entomology; Dr C. L. Boulenger and Dr F. J. Meggitt of the University of Birmingham; Lieut.-Colonel S. P. James, I.M.S. (retired) of the Ministry of Health; Dr G. A. Baylis and Rev. James Waterston of the British Museum (Natural History Department); D. Ward Cutler, M.A. of the Rothamsted Agricultural Station; Dr G. H. Macalister, Principal of Singapore Medical College; Dr P. Manson-Bahr, D.S.O., of the London School of Tropical Medicine and Mrs Manson-Bahr.

Cambridge was represented by the Mayor and Mayoress and numerous members of the University including Heads of Colleges, Professors, and others with their ladies. The total number of guests being about two hundred and fifty.

The writer was unfortunately unable to attend the opening ceremony through illness, having had five days previously a severe haemorrhage from a duodenal ulcer. His absence was especially regrettable because it prevented him from expressing publicly his gratitude to the founders of the Institute, welcoming the visitors, and participating in a ceremony that represented for him the consummation toward which he had striven for many years. His disappointment was, however, greatly mitigated by the kind expressions of sympathy received from those who attended the meeting and which emanated from all the speakers on that occasion.

The opening ceremony lasted one hour and it took place in the Research Museum of the Institute, the small platform being occupied by the Vice-
Chancellor, Mr and Mrs P. A. Molteno, Earl Buxton, Lord Pentland, Sir Arthur Shipley, Professor Caullery and the Mayor of Cambridge. The speeches were brief and may be summarized as follows:

The Vice-Chancellor welcomed the founders to the Institute they had brought into being and thanked them warmly on behalf of the University for their noble benefaction, stating that he "could conceive of no more worthy object for generous-minded men to spend their money on than an Institute for Research of that kind, which necessarily must in some sense ameliorate the lot of mankind." He read a telegram from General Smuts which Mr Molteno had received through the High Commissioner of South Africa; it ran:

The Union of South Africa welcomes the opening of the Molteno Institute, which Institute is made possible by the generosity of the Molteno family. The progress of the Institute will be sympathetically watched by South African scientists.

The Vice-Chancellor had that morning received a letter of congratulation and good wishes from Dr Edmond Sergent, Director of the Institut Pasteur of Algiers, whilst Professor Nuttall had been the recipient of a telegram which ran:

Institut Pasteur et Société Pathologie Exotique Paris adressent à Institut Molteno et à son éminent Directeur cordiales félicitations et vœux de prospérité (signed) Roux, Calmette, Mesnil.

Mr Cecil Warburton, who spoke on behalf of the writer, welcomed the representatives of the Colonial Office, Ministry of Health, and War Office, and those who had come from various universities and scientific institutions. He dwelt briefly on the main features of the new Institute and the plans that were being matured for its future development as a centre for research and advanced instruction in the whole domain of parasitology.

Mr P. A. Molteno, whose rising to speak was welcomed by loud applause, after referring in the friendliest manner to the writer, expressed his and Mrs Molteno's cordial approval of the way in which their benefaction had been applied in the construction and arrangement of the building. He referred to the very inadequate facilities which had hitherto existed for parasitological research in Cambridge and to the importance of such work for the Empire, judging by his own experiences in Africa. His eloquence, that of the practised speaker, greatly appealed to the audience.

Earl Buxton spoke as an old friend of the founders who by birth were connected with South Africa, the name of Molteno having for long been well known and respected there, "especially since the time of the first Prime Minister of Cape Province, Sir John Molteno," moreover, Mrs Molteno's father, Sir Donald Currie, had done much to assist in the development of the country. His Lordship referred to the generosity of the benefaction conferred on the

1 The following abstracts of the speeches delivered on the occasion are made from a shorthand report.
University and to his own experience of the ravages of disease in Africa, of its certainly having been high time that such an Institute, "worthy of Cambridge," should be erected and given a local habitation and a name. "Cambridge afforded ideal conditions for the establishment of a research institute of that description, because of its many-sided scientific activities and its constant stream of young and talented men who passed through the University." He emphasized the value of parasitological research and of such an institute for the Empire: "What is very greatly required is a centre where men can be trained in the scientific methods of research which bear so greatly on the health of men, animals and plants, by receiving a broad, thorough training, for at present there is very little opportunity, except for a very few men, of obtaining such training. The training is often too slight and too diffuse, the student picking up scraps here and there in professional schools, whether medical, veterinary or agricultural. The student therefore acquires no real foundation upon which to base his future research work."

"Unfortunately, in consequence of lack of training in these respects, unripe and poorly trained men went into practice who were not really fit to investigate a scientific problem. As Professor Nuttall had said: 'They travel with blinkers on their eyes along the old well-worn professional road and too often never leave it.' Thus it may come about that insufficiently trained men are frequently given posts or are sent on research expeditions, where they are expected to do scientific work, but where, not from want of zeal, but from lack of knowledge, they naturally fail.

"It would be a very great thing if the new Institute were placed in a position of being able to hold selected men to their research work until they were fit to go out as investigators and teachers. This meant, of course, considerable funds and endowments in order to keep men at work and free from financial worry for a period of years. Travelling scholarships conferred upon suitable men by their universities or Governments in different parts of the Empire would be of the utmost value to their country and mankind. If funds were available, subsidiary aid could be furnished by the Institute itself. The latter," concluded Earl Buxton, "was certain to attract men from all parts of the Empire, and scholarships or the like would be a great additional attraction. Thus a group of young and enthusiastic workers would gather round the Institute as a centre." His Lordship then declared the Institute open.

Professor Caullery, speaking in English, as the representative from France, referred to parasitology as nearly constituting a special science in the domain of biology since it bore upon an immense number of different organisms and afforded striking evidence bearing upon evolution, besides being an essential factor in the study of disease. He was glad to find one of the best of his former pupils, Dr Keilin, established at the Institute. He and his colleagues offered their congratulations and thanks to Mr and Mrs Molteno for what they had done for science and hoped that the Institute would help to bring French and British men of science into closer affiliation.
Sir Arthur Shipley, G.B.E., F.R.S., proposed a vote of thanks to those who had come from afar to help them on that memorable occasion. He wanted to add a word to what Earl Buxton had said about the necessity of further endowment. They had started a splendid building, but Cambridge had a habit of growing. The institution had grown from the Quick Laboratory to a Molteno Institute and would go on growing under the latter name. They would, and did, want further endowment. He was afraid that he was an inveterate beggar, for they had to keep the wheels going. As an American poet had said:

I hate to be a kicker.
I do not make for peace.
But the wheel that does the squeaking,
Is the wheel that gets the grease.

The assembled company then partook of tea as the guests of Professor and Mrs Nuttall and afterwards inspected the building under the guidance of members of the staff who could be readily singled out through their wearing Cambridge-blue rosettes.

THE MOLTENO INSTITUTE FOR RESEARCH IN PARASITOLOGY.

The following account of the Institute may prove useful to others who are contemplating the establishment of similar institutions elsewhere. Since the Institute was opened, and indeed before, it has been sought by numerous visitors interested in laboratory construction and arrangements who desired information on various matters, and this account will serve to answer some of the questions that have been asked by our visitors.

PREPARATIONS FOR BUILDING THE INSTITUTE.

A Syndicate was appointed on 6th December, 1919, by Grace of the Senate (Camb. Univ. Reporter, 9, xii. 1919, p. 355) to obtain plans and estimates for the erection of the Institute on the site assigned for the purpose (vide infra). This Syndicate consisted of the Vice-Chancellor (P. Giles, Litt.D., Chairman), Charles E. Grant-Ives, M.A., Rev. J. B. Lock, M.A., and G. F. C. Gordon, M.A., of the University Building Committee; G. S. Graham-Smith, M.D., F.R.S., Charles T. Heycock, M.A., F.R.S., Charles G. Lamb, M.A., and the writer (Secretary). This Syndicate held five meetings at three of which the plans were discussed with the architect, Mr Harry Redfern, F.R.I.B.A. The latter reported that the probable cost of construction, starting in March, 1920, would be £28,600 including architect’s fees and disbursements, the calculations including a sum of £1500 for fittings and contingencies, the complete building (151,200 cubic feet) being reckoned to cost three shillings and seven pence per cubic foot. The Syndicate reported this to the Senate and their report was approved (Camb. Univ. Reporter, 3, ii. 1920, p. 582; 2, iii. 1920, p. 688; 9, iii. 1920, p. 719; 16, iii. 1920, p. 746 (Grace passed)).

Subsequently various changes in the choice of materials were made whereby the cost was reduced. Tenders for the erection of the building were received.
from six contractors, that of Messrs William Saint Limited, Cambridge, being accepted (at £27,706).

The Site of the Institute.

Building operations were begun on 10th May, 1920, upon an attractive plot of land situated on the “Downing Site,” belonging to the University and so-called because it was purchased from the immediately adjoining Downing College. The south side of the building faces the grounds of the College, there being no intervening buildings, its distance from the boundary fence being 20 feet at the S.E. corner and 38 feet at the S.W. corner of the Institute, thus reserving a small strip of land to the south of the building which has in part been fenced in, and where it is hoped, with time, one or more aquarium basins, etc., may be constructed. The removal into the new Institute took place toward the end of October, 1921, whilst workmen were still engaged in parts of the building.

The Institute is very favourably situated with regard to space and remoteness from the noise, tremor and dust of street traffic. In its immediate vicinity there is being erected a Low Temperature Station, built at Government expense and intended primarily for the scientific study of refrigeration in its bearing on food preservation and allied problems. The Schools of Forestry, Agriculture, and Botany are close by, likewise the Physiological and Biochemical Laboratories, the latter being now in course of construction. Access to the Downing Site, upon which these buildings stand, is gained from Downing Street on whose opposite side lie the Medical School, Zoological and Chemical Laboratories and other University buildings which harbour the Zoological Museum, Balfour Library (Zoological) and the Library of the Cambridge Philosophical Society which the workers at the Institute frequently seek. Apart from departmental libraries in the schools and laboratories mentioned as being near at hand, the great University Library may be finally noted as being at a distance of but 10 minutes’ walk from the new Institute.

Description of the Institute.

The Exterior of the Building.

The external features of the Institute have been described at my request as follows by the architect, my friend Mr Harry Redfern, F.R.I.B.A.:

“In designing the elevations of any building it is incumbent upon the architect to express, as well as he can, the use to which that building is to be put. Such limits as may be imposed upon him by the nature of the materials employed—whilst often confining his problem within some narrow limits—not infrequently exercise a restraint which is beneficial to the result.

“If the building has been planned strictly to suit its purpose, and if the elevations truly reflect the plan (as they should do) it would seem inevitable that the external appearance is predestined, and that little remains for the designer but to model the various features with care—emphasizing here,
Fig. 3. General view of the Molteno Institute for Research in Parasitology, north side and east end, showing the large plate-glass windows.
toning down there—until the result satisfies his fancy. In any building the fenestration is a very powerful factor in the design.

"The buildings used by scientists for their highly technical operations present certain difficulties to their architect when designing the exterior: for example, it not infrequently happens that adjacent rooms on the same front demand entirely different conditions as to the size, number and arrangement of the windows, etc. It is such difficulties as these which make the planning of laboratories an extremely interesting problem in design. Furthermore, a building should not only be true to itself but should harmonize with its environment. It has a duty to its immediate neighbours, especially if these are buildings of a University.

"The above considerations had weight in the designing of the Molteno Institute. The architect was influenced by the expressed desire to build in brick of a certain colour and texture and to avoid the use of an undue amount of stone dressings. Moreover, the roof had to be flat, in order to admit of its being used in connection with certain work in the laboratories.

"The accompanying plans show that, broadly speaking, there are two types of room: a series of research laboratories, with one side of each consisting almost entirely of windows glazed with large sheets of plate glass; the other type a series of rooms where large windows were unnecessary and undesirable. In the midst of this series is a large library, and the top floor is, for all practical purposes, an enormous room—the Research Museum. How to combine these elements into a composition in which the large glass area of the research room windows should not throw the remainder out of scale, nor the numerous smaller windows fritter away the wall space—how they should be arranged in some rhythmical way—that was the problem of the elevations.

"It seemed best to group the research rooms together on the two floors and to combine the two storeys into one 'order' by treating the piers between the windows as pilasters, the intervening wall-surface being kept low in tone; by this means the dark masses of the large windows do not unduly assert themselves but are kept in a relatively subservient position in the strong vertical emphasis of the pilaster groups. The regular series of four-light windows on the top storey are intended to indicate the existence of one large room; whilst the treatment of the south front with its smaller windows with leaded panes and the brick piers, qua pilasters, between is intended to present a kindly front to the green Court of Downing College.

"The colour scheme of the exterior has received some consideration: it is quiet and subdued and somewhat low in tone. The brick walls are in broken tints varying from purple-brown to brownish-grey; the copings, cornices and pilasters are grey-white; the woodwork (all of teak) is fast approaching a similar tone.

"This simple building, standing four-square against a pleasant background of old trees, is an attempt to house a modern scientific department in a manner not unsuited to its needs and the ‘atmosphere’ of its environment."
Fig. 4. Plans of the Institute as completed.
The interior of the Institute was planned by Mr Redfern and the writer with a view to securing a maximum of convenience in the matter of general arrangement, light, ventilation and heating, whilst having regard to considerations of economy in view of the greatly increased cost of building operations consequent upon the great war.

Basements. These are restricted to both ends of the building, the basement on the east, serving for the accommodation of bicycles, being entered by a gate set in an arched doorway; this basement has a transversely ridged floor sloping downward to a level portion partly occupied by bicycle racks. The size of this basement corresponds to that of the combined area occupied by the staircase shaft, vestibule and lavatories above.

The basement to the west corresponds in size to the area covered on the ground floor by the photomicrographic room, research room, vestibule, part of the corridor, sterilizing room and staircase shaft. This basement contains the furnace and coal cellars on the north, the exit to the chute from the roof (see p. 120), and to the south a small animal room and storage space for a carpenter's bench, refrigerator, boxes, etc.

A space 4 feet in height, with concrete floor, lies beneath the rest of the building, whereby access can readily be gained to all drains, etc. beneath the ground floor.

General arrangement. To economize space, and to have as many research room windows as practicable fronting north whilst keeping the rooms adjacent to each other, the entrances are placed at both ends of the building. Two staircases are provided for safety in case of fire, and a straight corridor runs midway along the length of the building on two floors. There are eight research rooms occupying the ground floor and first floor, these rooms accommodating eight to sixteen workers.

The main entrance is to the east through double doors whose upper panels are glazed and guarded by a grill. The vestibule contains a commemorative tablet of bronze in a stone setting of appropriate design (see Fig. 5), whilst a small telephone chamber is situated at the bottom of the staircase shaft.

The corridors (ground floor and first floor) are cut off from the vestibules at each end by double swing-doors with glazed panels, being well lit by day through borrowed lights and transoms at their sides and from their ends by the light entering the vestibules through doors and windows.

Near the western staircase on all floors a space has been used to advantage by erecting light partition walls enclosing store-cupboards and closets for housemaids' sinks where brooms, pails, etc. can be kept. On the ground floor the sink and attendants' lavatory, etc., occupy but one space.

In the western vestibules on three floors there are hoist holes provided with teak trapdoors through which heavy articles can be raised from the basement to the top floor by means of a chain and pulley. This simple arrangement
functions in place of a lift whilst not cramping the floor space when out of use.

The ground floor, on the south side, contains cloak rooms and lavatories for both sexes, an office, a small waiting room for visitors, an incubator room, a laboratory assistants’ room, a washing-up and sterilizing room. A photomicrographic room and four research rooms occupy the north side. The photomicrographic room and adjoining dark room are each supplied with a radiator so that they are comfortably warm in winter and always free from damp. Through vents leading respectively from the ceiling of the dark room to the roof and from the photographic room to the hall these rooms are satisfactorily ventilated whilst trapped against light, the air entering from the outside, as elsewhere, near the radiators.

The first floor, on the south side, contains a tea and rest room, a library, a lavatory, a laboratory assistants’ room and chemical room. Four research rooms and an aquarium room occupy the north side.

The tea and rest room is regarded by the writer as a most useful feature in an institution of this kind. It serves several purposes: (a) as a gathering place for sociable intercourse among the workers at the Institute during the short interval when tea is provided, (b) as a place where those who may bring a meal with them can retire when they desire, (c) as a room where workers may rest or spend the night should this be necessary in connection with their researches. The room, in its arrangement and furniture, has nothing reminiscent of a
laboratory about it; being provided with comfortable chairs, a fire-place, a cupboard and hidden sink for storage and the washing up of eating utensils which are kept out of the laboratory entirely. A long, cushioned, bench which can be widened by a flap affords if need be a sleeping place; the writer has in the past spent many an uncomfortable night in laboratories that lacked such a resting place for workers who have to make periodic observations at night in connection with their researches.

The aquarium room, as shown in the plan, is on the first floor and it faces north. It has a slate window bench that is drained by a white glazed channel. The asphalted floor slopes to one corner where there is a large drain to prevent the danger of flooding. The sink is large and shallow to facilitate the cleansing of large glass vessels which may be used as aquaria. Three stopcocks deliver tap-water to one side of the sink, one of them being fitted with a hose coupling. The rain-water supply to the aquarium flows down vertically through a glazed pipe from the slated collecting area on the roof (see below) and is stored in a slate cistern (position indicated on the plan by a dotted line), held in a steel frame beneath the ceiling of the aquarium room. The cistern measures $8' \times 4' 6'' \times 2''$, its capacity being about 350 gallons. The top of the cistern is covered with slate and access thereto is gained through a teak trapdoor in the floor of the room above, a movable slate slab preventing the entry of dust into the cistern. To obviate undue condensation of moisture upon the steel bearers and slate surface of the cistern in the room, the whole is enclosed in sheets of uralite with an interposed air-space. The water used for the aquaria is conducted out of the cistern in a tin pipe provided with two tinned stopcocks with tapered nozzles, one over the sink, the other over the end of the window-bench nearest the sink, a glass gauge joined to the tin delivery-pipe near the cistern serving to indicate the stored water level. A vulcanite standpipe conducts any overflow from the cistern into a large pipe opening near the floor drain above-mentioned.

The second floor is almost entirely devoted to the research museum whose north side may serve for demonstrations, practical instruction, or for research work, being, if necessary, divided up by light movable partitions to form cubicles. Museum cases, cabinets and various cupboards occupy the south side. A preparation room opens into the museum at one end, whilst the northwest corner of the building contains a store-room.

The roof, to which access is gained at the west end, is flat and of reinforced concrete finished with asphalt, with a closed brick parapet, 4 feet high, forming a court in which there can be accommodated terraria, aquaria, and small animals in hutches, etc. Extending along the middle of the roof is the research museum skylight, constructed on the weaver-shed principle, glazed on the north and slated on the south. A glazed earthenware gutter collects the rain-

1 The writer is much indebted to his friend Mr Edward Bles, M.A., for advice in practical matters relating to the arrangement of the aquarium room and its rain-water supply.

2 See footnote, p. 121.
water from the whole length of this slated portion and voids it down the
erminal vertically to the slate cistern in the aquarium room. At the west end of the
roof is a hatchway to a vertical chute leading down to a closed space opening
with a door near the furnace in the basement; this chute, finished in smooth
cone internally, serves for throwing down refuse from the top to the
bottom of the building where it can be incinerated or otherwise dealt with.

The stairs outside the building are of stone; those within are granolithic
with powdered carburundum (non-slip) finish, they measure 3' 5'' in width.
The bannisters are of iron painted dull black. The internal surface of the walls
is lime-washed in the basements, plastered and white, cream, or otherwise
distempered throughout, except in the photographic room and dark room
which are painted black overall; in the lavatories the walls are coated with
white enamel paint to a height of 5 feet above the floor. The skirtings are of
concrete and usually 3 inches high, being mostly blackened, the concrete
being preferred to wood. The floors are of concrete, partly reinforced, their
surfaces being covered by red tiles in the vestibules, corridors and lavatories,
black tiles and stone covering an area about the fireplace in the tea room;
otherwise the floors are finished in Portland cement trowelled smooth, or
asphalted (as on the roof) in the chemical, aquarium, sterilizing and dark
rooms; a few of the rooms have the floors covered by dark green linoleum
similar to that on the work-benches; as linoleum is very expensive its use
necessarily been (we hope temporarily) restricted. Woodwork. The external
doors and gate to cycle basement, window frames and ledges, and trap-doors
to hoist are of teak; a few window ledges are of slate. The woodwork of the
internal doors, borrowed lights, etc., is white enamelled throughout except in
the basements; the doors, where not glazed, are smooth, unpanelled, consisting
of a frame covered with boards of three-ply wood; three pairs of research rooms
intercommunicate by sliding doors conveniently placed. The woodwork in
the tea room and library (shelves 10'' deep) are of teak. Appropriate legends
and numbers are painted in black on the doors. Windows. The frames and
casements of the seven research rooms and aquarium (ground and first floors)
are of steel; the central large fixed casement holding a single sheet of plate
glass as in shop windows, the two casements flanking the central one being
arranged to open so that their upper portion swings horizontally, the top edge
falling inward, whilst the lower portion is hung vertically swinging one-third
in and two-thirds out whereby they are readily cleaned; the lower sections of
the flanking windows open in opposite ways so as to secure suitable ventilation
according to the direction of the wind. The windows on the north side of the
research museum, consisting of four lights each, have their lateral casements
movable like those in the research rooms, the casements being of steel set in
teat frames. The remaining windows of the building are leaded, the steel case-
ments being set in teak frames. In all cases the upper line of the glazed portion
of the window runs at a distance of about 6 inches from the ceiling whereby
the rooms are brightened by the large amount of reflection from the ceiling.
Work benches extend across the width of the rooms and for a short distance to one side in the form of an L, being supported on steel T-bars set in the outer wall in cement. The tops are covered with green linoleum glued down to the thick deal boards beneath and all are edged with teak having rounded edges. The teak border on the free side nearest the worker is slightly broader than the thickness of the bench, the bottom margin thus affording a drip-edge in case fluids are spilled on the bench. The short arm of the L-shaped bench serves for the reception of an incubator, embedding bath, or the like. The benches are provided with electric light plugs and gas fittings conveniently placed so as to be as much as possible out of the way.

The sinks are white glazed earthenware throughout.Measured on the inside, those in the research rooms are 1' 9" x 1' 4" by 6" in depth; those in the sterilizing room (9" deep) and photographic room (6" deep) measure 2' 4" x 1' 5"; that in the aquarium room 3' 4" x 1' 9" by 5" in depth. Tap nozzles are usually placed 14" above the bottoms of sinks. As shown in the plans, the research room sinks and similar sinks elsewhere are each provided with a teak draining board and adjacent stone shelf, each measuring 2' x 1' 6". The sinks empty into 3" glazed drains, whose large calibre precludes stoppages if larger objects escape into the drains from the sinks. A considerable economy was effected by placing sinks on opposite sides of a wall so that they empty into a common downfall drain, such paired sinks and their accompanying draining boards and stone shelves being supported by but one set of T-steel rods which transfix the wall.

Metal fittings. There is little to note regarding these, but it may be mentioned that gun-metal has been used in place of brass wherever possible so as to save unnecessary labour in cleaning. Most of the door handles are of the "push, pull and turn" pattern and therefore specially to be commended for laboratory purposes.

Some measurements relating to the building.

The following measurements are noted since some of them may be of use as a guide to others who contemplate constructing laboratories: Externally the building measures 92' x 44' and 35' in height to the top of the stone coping. The corridors are 6' wide. The four research rooms on the ground floor and the corresponding rooms above measure 14' in depth, the length of the middle pair being 15' 6", that of the other two being 15' 10". The photographic room (designed for a Zeiss optical bench) and the adjacent dark room are 16' deep and have a width of 4' 9" and 5' 6" respectively, being separated by a light partition wall of hollow bricks. The corresponding small research room at the N.W. corner of the floor above measures 10' 6" in width. On the ground and first floors, south side, the rooms are 14' deep except for the tea room which has a depth of 16'; the widths of these rooms being as follows: On the ground floor: Sterilizing and Laboratory Assistants' room 15' 10" and 15' 6" respectively, Incubator room 6' 6", Waiting room 7' 7", Office 12' 3"; on the first floor: Chemical room 15' 10", Laboratory Assistants' room 11' 9", Library 26' 10", Tea room 15' 9". On the second floor, the Research museum measures 65' x 36', and both the preparation and store rooms measure 10' 6" x 16'. The size of the basements is indicated on p. 117. The

The signs ' and " denote feet and inches respectively.
basements are 6' 9" high, the ground and first floor rooms 10' high, the second floor rooms 9' high. Where there are no basements, the ground floor is suspended 4' above the concreted surface of the ground-level. The roof parapet is 4' high, the chimneys being but slightly higher. The skylight is 65' long and consists of 34 lights (4' x 2') framed in teak, the glazing being double with a closed air-space between the pairs of panes; the slated surface which serves for the collection of rain-water measures 66' 8" x 7' (area 467') and is tilted at an angle of 30°, whilst the glazed surface is 4' wide and stands at an angle of 60°. The internal measurement of the chute is 2' x 2', that of the hoist-hole 4' x 4'.

The work benches throughout are 27" wide, their surface being 33" above floor-level. The slate bench in the aquarium window is 30" wide and 21" above floor-level. The chemical bench in the centre of the room measures 7' x 4'. The doors measure 6' 7" x 3' as a rule. The windows in seven research rooms and aquarium occupy almost the whole north side of the room, the fixed central pane measuring 5' 11" x 5' 9". The borrowed lights (3' 4" x 22") are placed 6' 11" above floor-level, fifteen borrowed lights being on the ground floor and nine on the first floor, occurring either as transoms over doors or fixed in corridor walls into which they mostly open.

The Molteno Institute for Research in Parasitology

Heating, Ventilation and Lighting. The building is heated by hot water on the low pressure system, the boiler ("Coronation") being situated in the basement alongside the coal cellars. There are 44 radiators placed in rooms, corridors and landings, 18 being on the ground floor, 14 on the first floor, and 12 on the second floor. The radiators are mostly placed near windows beneath work benches but to one side so as not to incommode persons working at the bench; they draw fresh air through apertures from the outside and the warmed air passes up through a cast-iron grating in the bench beside the window. The radiators have been found to heat the building very efficiently. There are three open fireplaces in the building, two with modern gas hearths (in library and tea room), and one in the office where it is at times convenient to be able to burn papers. Apart from these sources of heat, arrangement was made whereby gas-stoves may be introduced into the building at some future period if required. For this purpose, small triangular gas-fume flues were built in eleven rooms by means of flat bricks, measuring 9" x 9", diagonally placed across one corner of the room, these flues leading up to the roof alongside the columns in the research museum; the small chimney-like vents to these flues are shown at five points on the plan of the roof (Fig. 4). The corner position to be occupied by gas-stoves, if required, is indicated on the plans. To install gas-stoves it is merely necessary to cut a hole through the plaster and thin brick for the purpose of inserting the stove-pipe.

Ventilation is secured by opening windows, transoms, or borrowed lights, and by allowing the air to enter from the outside through the apertures near the radiators. Reference has already been made to the character of the windows (see p. 120).

Owing to the large windows, glazed tops of swing-doors at the ends of corridors, borrowed lights suitably placed, and to the general whiteness of the interior of the building, the latter appears singularly well illuminated during the daytime. Thoroughly efficient artificial illumination is secured by means of electric light distributed in the form of drop-lights, wall-brackets and wall-
plugs serving table lamps where they are most useful whilst having regard to economy.

It will suffice to mention that each research room is provided with (a) a central drop-light that can be moved up and down above the writing table, (b) a bracket-light above the sink, (c) a bracket-light above the short arm of the L-shaped bench used for the embedding bath, etc., (d) two plugs to table lamps on the work bench, these supplying two or four lamps as required. The bracket-lights are placed at a height of about 3 feet above the bench or sink. The drop-lights are ½-watt, 60-candle power, the others 30 c.p. throughout; the corridors are lit by 30 c.p. fixed drop-lights, corresponding lights in the research museum being 60 c.p. The wiring is all carried in flattened lead pipes attached to wooden strips, the pipes being protected by iron guards where liable to injury, this method of wiring having various advantages over that in which iron pipes are used for carrying the insulated wires.

Electric power plugs have been placed in five situations: the sterilizing room, chemical and photographic rooms and research museum.

Equipment. The Institute is well provided with all requisite apparatus including modern appliances for the study of the biology of parasites. Special attention is being given to securing facilities for the study of the best and most recent methods of conducting research in parasitology.

Scientific Collections.

The collections of the Institute comprise numerous specimens acquired by the writer during many years, others presented to the Quick Laboratory and more recently to the Molteno Institute. The collections are intended primarily to aid the workers at the Institute and are housed in the research museum, the latter affording space for the considerable expansion of the collections which is expected in the future.

The collection of Ixodoidea, which is the most valuable at present, is one of the largest existing, besides being rich in types. There are many specimens of blood-sucking and parasitic insects and acari. The helminthological collection is rapidly growing in value, and, like the preceding, comprises types. Protozoological and mycological specimens are being steadily acquired, and there is a bacteriological cabinet. We hope to augment our collections by means of exchanges and through the further generosity of donors in different parts of the world.

A considerable amount of illustrative material, largely original, including diagrams prepared for the purpose of instruction in parasitology, is already contained in our museum.

Portraits of scientific men. A collection of about 300 portraits of those who have distinguished themselves in the domain of parasitology has been gathered by the writer and presented to the Institute. The portraits are mostly framed and hung chiefly in the well-lit corridors, the grouping being by nations and as far as possible by subjects. A number of these portraits are being published
in the form of a series appearing in *Parasitology* with accompanying biographical notes. The collection is of considerable interest to students of parasitology and others.

**The Library.**

The basis of a departmental library has been formed by the gift to the Institute of the whole of the writer’s scientific books, brochures, reports, journals and some thousands of reprints which are being suitably classified, bound and catalogued, new publications being continually added. Gifts and exchanges are also helping on the library.

The nucleus of a *Library Fund* has been formed through a benefaction received from Mrs Stella Churchill in memory of her late brother Dr Walter Myers, who died in Pará, Brazil in 1901 whilst investigating yellow fever\(^1\).

![Fig. 6. The book-plate of the Institute designed by the writer.](image)

The accompanying figure (Fig. 6) illustrates the book-plate designed by the writer for the library. The heavily parasitized border shows a reckless disregard for the relative size of the parasites depicted, most of the forms figured being readily recognizable to the initiated.

**Facilities for Publication.**

The publication of the scientific work carried out at the Institute is greatly facilitated by the circumstance that the writer is the founder and editor of the two journals, *Parasitology* and *The Journal of Hygiene*, the first having now attained its 14th and the second its 21st volume. Both journals are

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\(^1\) For biography and portrait, see *Journal of Hygiene*, 1901, i. pp. 285–287.
PLANS FOR THE FUTURE DEVELOPMENT OF THE INSTITUTE AND THEIR DEPENDENCE ON FURTHER ENDOWMENT.

The Institute was founded for the advancement of our knowledge of Parasitology in all its branches, being in this respect the first of its kind. Hitherto Parasitology has been taught in a limited way in professional schools, either medical or veterinary, in zoological and botanical laboratories, or in conjunction with agriculture and fisheries; consequently the student has been precluded from obtaining a general survey of the subject as a science. Similarly, in the matter of research, the work carried on in the various schools has been largely confined to problems having only a practical professional bearing. The Molteno Institute proposes to deal with Parasitology on a broad biological basis, the research and instruction offered therein being directed both to purely scientific and practical problems because pure and applied science are inseparably interwoven and many of the most important practical discoveries in the past have had their origin in purely scientific investigation.

Parasites and parasitic affections are widely distributed in Nature, the chief forms among the innumerable parasites affecting animals and plants being found among Protozoa, Vermes, Insecta, Acarina, and Fungi including Bacteria. It is evident therefore that the student desiring to obtain a broad survey of Parasitology will require to familiarize himself with a large number of widely divergent groups of organisms and that he should have corresponding facilities for their study placed at his disposal.

The well-equipped Institute herein described is an instrument of research which cannot be used to full advantage without ample funds. To render the work of the Institute efficient and to carry out the plan which governed its design and equipment it is necessary that an endowment fund or funds shall be established.

The present permanent endowments yield a total annual income of £861 derived from (a) the Quick Fund (£300 for research and laboratory), (b) the Molteno Benefaction (£546 for maintenance of Institute), and (c) the Walter Myers Fund (£5 for Library). Of this amount £500 go to meet the exorbitant rates and taxes at present demanded on the building, leaving but £351 for general purposes. Needless to say this sum represents but a fraction of the annual expenditure required to run the Institute and pay stipends, salaries, maintenance, cost of research, apparatus, chemicals, books, etc.

The main expenditure in running the Institute is met by grants for research derived from various sources as in the case of the Quick Laboratory during previous years. It will be readily understood that it is a grave drawback to any Research Institution to be dependent on grants because these are
The Molteno Institute for Research in Parasitology necessarily obtained at irregular intervals and for specified purposes. Under the most favourable conditions, grants are but made from year to year, a circumstance that introduces a distracting element of uncertainty into the policy of the Institute. Moreover, since grants can only be obtained, as a rule, for specific researches that appeal to the judgment of the grantors, a most undesirable limitation is placed upon the Institution in its scientific work. Means should therefore be found in the near future to obtain funds wherewith scientific work may be conducted in a more unfettered manner.

In connection with the Institute, the position of Parasitology requires consideration. Founded in 1911 by the writer, this Journal has been carried on by him at a heavy personal loss during most of the years of its existence (1911-21). It is hardly to be expected that a publication of this kind should be self-supporting, essential though it be for the coordination and development of research, its circulation being necessarily limited, but it is clear that the cost of maintenance is small in proportion to its usefulness. It would be well if the Journal were made the official organ for the publication of the work done by investigators at the Institute whilst still serving for the presentation of papers derived from other sources. A suitable sum should therefore be made available toward the support of this Journal from endowments which may accrue to the Institute in the future, for, in view of the continued loss in publication, its continued existence is at stake.

It is hoped, therefore, that in the near future adequate funds will be found for the permanent endowment of the Institute so that it may develop as it should and perform the functions for which it is intended.

ACKNOWLEDGMENTS.

In concluding, the writer would express his deep sense of gratitude to Mr and Mrs Percy Alport Molteno for the splendid gift they have made to Science, the Empire and the University, a gift which will be appreciated to a growing degree in the future. Our great indebtedness to the late Mr Frederick James Quick, founder of the Quick Professorship of Biology, should be acknowledged anew, for, thanks to his foresight and the provisions of his legacy, wisely carried out by his Trustees and the Managers of the Quick Fund, the means provided by both the Molteno and Quick benefactions serve one and the same excellent end. We are, moreover, under great obligations to those public bodies and private persons who have helped on our work in the past (see p. 104) and, more recently, especially to the late Lord Strathcona and Mount Royal for his benefaction (see p. 108).

Our warmest thanks are due to Mr Harry Redfern, F.R.I.B.A., the architect of the Institute, for the extremely able manner in which he designed and carried out the building plans and for his ever friendly consideration of our wishes. The good work of the builders, Messrs William Saint and Son of Cambridge, speaks for itself, and we are grateful to their excellent foreman Mr Brain for the painstaking manner in which he carried out his multifarious duties.
THE DEVELOPMENT OF HELIGMOSOMUM MURIS YOKOGAWA, A NEMATODE FROM THE INTESTINE OF THE WILD RAT.

By SADAMU YOKOGAWA, M.D.

Professor of Pathology, Medical School of Formosa.

(With Plates VII—XII and 8 Text-figures.)

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I. INTRODUCTION.

The post-embryonic development of nematodes is very insufficiently known. A surprisingly small number of forms have been studied and in many of these investigations the work is entirely inadequate. Leuckart (1887) investigated

1 This paper is a contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University.
the post-embryonal stages of *Allantnema mirabile* and Neuhaus (1903) studied these stages in a free-living nematode, *Rhabditis nigrovenosa*. The most notable study on the post-embryonal development of a nematode was made by Looss (1897, 1911) on the hookworm, *Ancylostoma duodenale*. More recently in South Africa Veglia (1915) has made extensive studies on the post-embryonal development of *Haemonchus contortus* from the sheep, and Theiler and Robertson (1915) have investigated these stages in *Trichostrongylus douglasi*, the wire-worm from the ostrich. In the studies which have been made of the post-embryonal development of the Strongylidae the free-living stages, especially of the hookworms, have been very completely investigated. While in only a comparatively few forms have the free-living stages been studied in detail, there is found to be a considerable degree of uniformity in the development and relationship of these stages, so that we have an adequate conception of what the stages of development of this stage are. It is entirely otherwise with the parasitic development in this family. The literature gives an entirely inadequate conception of the morphogenesis of the various organ systems, and on some phases of this development there is no information whatsoever. This lack of knowledge is probably partly due to the fact that in most nematodes this period is of considerable length and many of the hosts studied are large animals.

*Heligmosomum muris*, which I described from the rat (Yokogawa, 1920), proved to be remarkably good material for the study of development during the parasitic stages. This form is small and develops in ordinary culture rats as well as in wild rats. But best of all infection can be easily obtained and the development in the rat requires only a few days. This made it possible to obtain an abundance of material of the stages of development and to obtain at any time material of any particular stage on which further observations were needed.

All the observations recorded in this paper were made from living specimens. To quiet the larvae the slide could be heated or exposed to direct sunlight. To find the larvae in the lungs of the experimental rats it was necessary to tease the tissue into very small pieces in normal salt solution. These pieces were then carefully crushed in a mortar and filtered through a fine wire screen. The filtrate was then centrifuged and the larvae if present would be recovered. After the larvae had made their way to the intestine it was not difficult to pick them from the surface of the mucous membrane.

In the present paper no attempt is made to study in detail the embryonic development which many authors have studied in nematodes. While the free-living larval stages were studied as fully as the time would permit no attempt has been made to discuss critically these stages for the Strongylidae. Looss (1911) gives a very complete and critical analysis of these stages in *Ancylostoma duodenale*. The main emphasis in the paper is placed on the study of the parasitic development, and especially on the development of the reproductive organs.
II. THE FREE-LIVING LARVAL STAGES.

1. Development of the Egg.

(a) Morphology of the Egg.

The eggs of *Heligmosomum muris* are ellipsoidal in shape and have a very thin shell. The average size is 0.05824 mm. by 0.03276 mm.; a minimum size is 0.0546 mm. by 0.0309 mm. and a maximum is 0.06188 mm. by 0.03458 mm. Segmentation of the egg begins within the uterus of the female. Eggs in the one-cell stage are found in the upper part of the uterus, while in the lower part of the uterus they are in the 4 to 16 cell stages. In fresh faeces, the embryo is found most frequently in the 16 to 20 blastomere stage, while eggs which have just been given off by the female are usually in the 4 to 16 cell stages. Exceptionally, eggs may develop to the morula stage in the intestine of the host but they are usually found in earlier stages.

(b) Formation and Hatching of the Embryo.

The embryonic development of the larva and the hatching of the egg of *Heligmosomum muris* are very similar to these stages as described for other members of the family Strongylidae, therefore I will include only a brief outline of these phases of development.

For the observation of the embryonic development, I placed eggs which had just escaped from the female in a liquid culture. These experiments were carried out in the beginning of the summer of 1920 in Baltimore, at temperatures favourable for the development. The period of embryonic development varies according to the temperature and the media in which the eggs are placed. The following notes are the summary of the observations from these experiments. In 4 to 6 hours after placing the eggs in the culture a large number of them were found in the morula stage and some even in the advanced morula stage. In 8 to 10 hours the majority of the eggs had reached the "tadpole" stage, and some already showed a few structural details. At this stage the anterior end is broader than the posterior and somewhat conical and the cylindrical, buccal-cavity is visible. The beginning of the oesophagus and the intestine can be seen as a strand of dark cells.

After 12 to 15 hours many of the eggs were in the advanced "tadpole" stage, while others already contained the rhabditiform larva. In some cases the embryo had attained three times the length of the egg, and in a few of the eggs it was four times this length. The shape of the body of the larva is cylindrical, the head is conical, and the tail is long. The mouth cavity shows clearly in optical section as two longitudinal lines, each terminating posteriorly in a point. The rhabditiform oesophagus and the chyle intestine are also well-developed. At this period a few free larvae are occasionally found already hatched.

In 17 to 20 hours about 20 per cent. of the eggs are found already hatched and the majority of eggs seem to hatch between 20 and 24 hours.

(a) Method of Culture.

In my studies of the formation of the embryo and the free development of the larvae I made many cultures of the faeces of wild rats infested with Heligmosomum muris. I found that cultures made in liquid media are best for the study of the formation of the embryo and the earlier stages of larval development, while those made in solid media are most suitable for the study of the development of the later larval stages.

The best results with cultures in liquid media were obtained by using a filtered decoction of faeces prepared in the following manner. One teaspoonful of normal faeces from white rats was boiled in 100 c.c. of tap water. The mixture was then filtered, and the decoction poured into a glass dish in a very thin layer. To this were added many eggs fresh from the females. The glass dish was then kept at room temperature, since this work was carried on in the summer time and was covered to prevent evaporation. For the purpose of examining the progress of development the dish can be placed from time to time under the low power of a microscope. In this way the hatching of the egg or any of the developmental stages can easily be observed on a number of larvae at the same time. By means of a pipette the larvae can be picked out, transferred to a drop of water on a glass slide and examined under high power. This method of cultivation is very convenient for the studying of the early stages of larval development. It is not suited, however, for the study of the development of the infective larvae, since the majority of the larvae cannot complete the first moult in liquid media.

The method of making the cultures of solid media was very simple. Fresh faeces from rats infected with H. muris was smeared on moist filter paper in a covered dish. This culture must be kept moist and the faeces must be spread out well on the filter paper to give sufficient air. It is not necessary, however, to add charcoal to avoid fermentation as in the cultures of human faeces containing hookworm eggs. The mature infective larvae appear on the filter paper or on the walls of the dish four to five days after the making of the culture. They are later found in great numbers along the edge of the filter paper giving the appearance of minute threads.

(b) A General Description of the Stages of the Post-embryonal Development.

In the literature on the development of nematodes the statement is made that four moults are necessary for the complete development. Looss (1911) makes this definite statement: "the number of the moults in the case of the nematodes is apparently always four. It remains to be seen whether further experience will confirm or overthrow this apparent rule." In the case of H. muris only three moults were observed. Early in my work I was struck with the fact that I could find only one moult in the free-living stage and suspected that I had overlooked the first moult. After repeated examina-
tions of the free development of the larvae I finally became convinced that there was only one moult. Finally the finding of the double layers of the cuticula in the adult stage struck me as important in this connection. The separation of the outer cuticula at this stage goes on just as if the worm were preparing for a moult but it remains always attached. It is only a short time after the completion of the third moult that the double conditions of the cuticula develops, and the space between the two layers of cuticula gradually becomes larger. The outer cuticula of the female is much looser than that of the male and extends posteriorly, forming a peculiar sac (Yokogawa, 1920). The outer cuticula is thick and has the transverse striations limited to the prominent longitudinal ridges, while the inner one is thinner and has only the transverse striations. No other case of a double cuticula has been found recorded for nematodes. It is probable that this condition is a characteristic of the genus *Heligmosomum*, which has escaped notice in the other species that have been described. This condition undoubtedly represents an incomplete moult. Therefore it seems to me that we can safely conclude that in *H. muris* the condition is the same as in other nematodes but that the second moult is transferred into the parasitic life and the fourth is never completed.

Looss (1911) divided the post-embryonal development of the nematodes into five periods which are separated by four moult. This method of division is commonly used. This division does not hold good for *H. muris*, since if in this form the stages were separated by the moult the development would include only four stages divided by three moult, the second stage including both a free and a parasitic stage and two distinct periods of development. In this case, it seems to be necessary to consider as a separate stage the time from the entrance into the final host to the second moult. This stage is then the third stage or the first parasitic stage and is separated from the second stage by the penetration of the larvae through the skin.

The post-embryonal development of *Heligmosomum muris* is divided into five stages by three moult. The first two stages are passed outside the host, as free-living larvae, and are separated by a relatively long mouling period, in which there are many structural changes. There exists some difference of opinion in regard to exactly when the second larval stage in the free life of nematodes commences. Previous authors have described the second stage as beginning after the larva has completed its first moult. From the biological point of view larvae are already in the second stage when the structural changes which go on during the moult have taken place. In the case of *H. muris*, the duration of the first moult is relatively long and remarkable structural changes occur during this period. Since it seems necessary for the purposes of description to establish a definite line of demarcation between the first and the second stages, I will here describe as a part of the first stage those changes which occur up to the completion of the first moult.

The three last stages are passed inside the host. The third larval stage or the first parasitic stage is separated from the second larval stage not by a
moult as in the hookworm and other forms but by the penetration of the larvae through the skin of the host. Entering the fifth stage the worm is sexually mature and is usually spoken of as the adult worm.

(c) The First Larval Stage.

In this stage, which extends from the hatching of the eggs to the first moult, the growth and development of the larvae are very remarkable. The changes were so great that it seemed probable that this stage must be passed without the lethargic stage which has been described for the larvae of other Strongylidae. Repeated examinations failed to show a lethargic stage so that the conclusion was reached that the first stage in the development of *H. muris* was passed in continuous development and activity.

I will now describe the structure of the larvae in the first stage of development.

*Size of the larvae.* The larvae (Fig. 1, Plate VII) hatched under natural conditions vary from 0·28 mm. to 0·30 mm. in length and from 0·015 mm. to 0·018 mm. in thickness, measured just behind the base of the oesophagus. They gradually grow until the time of the first moult, when they measure from 0·68 mm. to 0·78 mm. in length and from 0·032 mm. to 0·036 mm. in thickness. The larvae at this stage (Figs. 2, 3) belong to the rhabditiform type. The form of the body is cylindrical, decreasing in thickness from the base of the oesophagus to the tail. The tail is extremely slender, ending in a hair-like point.

*Body-cavity.* In the larva just hatched from the egg the body-cavity is very small, consisting of a slight space around the digestive tract. Anteriorly the body-cavity is slightly expanded around the anterior part of the buccal-cavity and surrounds the digestive tract to the region of the anus (Fig. 2). Towards the end of the first larval stage this space is almost obliterated by the expansion of the chyle intestine. The lateral and the ventral and dorsal bands cannot be distinguished at this stage.

*Cuticula.* The cuticula of the newly hatched larvae is very thin and without markings. The only change which it undergoes in the first stage is an increase in thickness as the size of the larva increases.

*Buccal-cavity.* The buccal-cavity is quite long, having a length of 0·012 mm. to 0·016 mm. and is cylindrical in shape.

*Oesophagus.* The oesophagus is rhabditoid in shape. The lumen appears as a clear line, and the "Y-shaped mark" in the centre of the bulb is quite clearly visible. The walls of the oesophagus are thick and transparent, containing some nuclei of muscle cells and have fine transverse striations. The length of the oesophagus varies from 0·06 mm. to 0·146 mm. according to the length of the larvae (see Table I).

*Chyle intestine.* The chyle intestine extends through the body from the oesophagus to the rectum. When observed from the side its lumen has a slightly irregular zigzag course. The wall of the intestine consists of two rows of eight cells, one row on the dorsal side and the other on the ventral side.
These cells are irregular flattened, cuboidal in shape, with oval nuclei. They jut into the lumen of the intestine reducing it to a mere line. The cells of the intestinal wall vary in size, even in the same specimen, according to their position, viz., the cells situated at the anterior and the posterior part of the intestine are much smaller than those in the middle region. The protoplasm of these cells contains fine granules which increase in number with the growth of the body. Accordingly the nuclei become difficult to see in the later development of the first larval stage. At the boundary line between the chyle intestine and the oesophagus are situated four cells (Fig. 2, iv, Plate VII), dorsally, two on each side, which represent the primordium of the intestinal valves. These cells are very small and clear and each contains a round nucleus.

**Rectum.** The rectum is a very fine slit-like canal lined by a thin chitinous membrane. Its length varies from 0.015 mm. to 0.055 mm. according to the length of the body. At the point where it joins the chyle intestine two to three small cells are visible, which seem to be the primordium of the rectal ligament (Fig. 2, rc). The rectum runs diagonally and is surrounded by a cell-group. The distance of the anus from the tip of the tail varies in the different sizes from 0.052 mm. to 0.11 mm. (see Table I).

**Nervous system.** At the constriction of the oesophagus of the youngest larva of the first stage there are found a large number of round cells surrounding the oesophagus. The majority of these cells belong to the nervous system. The nerve ring appears as a band surrounded by a number of these cells toward the end of this stage (Fig. 4, n, Plate VII).

**Excretory apparatus.** In the larvae just hatched from the eggs a number of round cells are found on the ventral side at the base of the oesophagus and at the anterior limit of the chyle intestine. This cell group is not at this stage sharply separated from the nerve cell group. In the last period of the first stage, two pear-shaped relatively large cells can be clearly seen at the same place occupied in the earlier stages by the cell-group mentioned above. These two cells are apparently separated from the nerve cell group, and are situated on the ventral side of the anterior part of the chyle intestine, with their narrow parts pointing anteriorly (Fig. 3, eg, Plate VII). In the front of these cells, a short canal is visible which enters into a group of cells. This canal is apparently the beginning of the excretory canal. The excretory pore cannot yet be distinguished.

**Genital primordium.** The germ anlage is recognizable as a small elongated body, measuring 0.006 mm. × 0.004 mm. to 0.01 mm. × 0.06 mm., according to the growth of the larvae (Figs. 1, 2 and 4, gp). In the youngest larvae it apparently consists of two cells. It is located on the ventral side between the chyle intestine and the body wall near the middle body.

Table I shows the increases in body length and in the length of certain other parts during the first stage of development.
Table I.
Increases in Size of the Body of H. muris during the First Stage of Development.

<table>
<thead>
<tr>
<th>Specimen number</th>
<th>Length (mm.)</th>
<th>Width (mm.)</th>
<th>Buccal-cavity (depth) (mm.)</th>
<th>Oesophagus (length) (mm.)</th>
<th>Tail (length) (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.28</td>
<td>0.015</td>
<td>0.014</td>
<td>0.072</td>
<td>0.052</td>
</tr>
<tr>
<td>2</td>
<td>0.300</td>
<td>0.018</td>
<td>0.012</td>
<td>0.060</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>0.395</td>
<td>0.018</td>
<td>0.013</td>
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<td>0.016</td>
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</table>

Activity during first larval stage. The newly hatched larvae are small and move very slowly. After a few hours they move more actively and commence to feed. In the larvae at this stage a contraction of the intestine can be noted, causing a bulging of its posterior end. During the second day, the growth of the larvae is remarkable and constant movements are noted. Usually about two days after hatching when the chyle intestine becomes packed with granules the movements become somewhat slower. After this they start in on the first moult.

(d) The First Moult.

At the time of the beginning of the first moult, the chyle intestine appears to contain a great amount of refractive granular material. These granules are the globules of food material stored in the cells lining the intestine. These (Fig. 3, Plate VII) intestinal cells seem to be entering into a period of division, since many nuclei can be seen in them. This division of the cells proceeds from the anterior region backwards. Therefore the cells in the posterior region of the intestine at times will show no evidence of cell division, while in its anterior region this process is apparently going on. By this cell division, the cells lining the intestinal wall are modified into flattened cells containing fine yellowish brown pigment granules, and transparent ellipsoidal nuclei (Fig. 4). This cell division in the intestinal wall is a revolutionary process, since the cells do not increase in number. Those cells which jut into the lumen of the intestine break off after division and seem by degeneration to set free food material which is utilized during the moulting period. This revolutionary modification greatly increases the width of the intestinal lumen, which now loses its irregular zigzag character (Fig. 4, Plate VII).

During the first moult, the constriction of the oesophagus becomes less marked and it loses its rhabditoid shape. Also a newly formed cuticula gradually makes its appearance. After a while the tail becomes separated within the old cuticula and the beginnings of the lateral bands of the new cuticula appear (Fig. 5, Plate VIII). The newly formed tail is blunter and shorter than during
the former stage. Therefore, when these structural changes have been accomplished the larvae themselves have decreased considerably in length.

It is very difficult to determine exactly when the first moulting begins and when the sheathed larvae cast their old skins, since the structural changes proceed very slowly, and the ecdysis usually does not occur in liquid media. The casting of the old cuticula is a purely mechanical process and is effected by the activity of the larvae at the beginning of the second stage. The old cuticula, however, is so thick and rigid that the larvae cannot easily rid themselves of it without mechanical assistance. Accordingly the larvae after the completion of the structural changes described above prefer to penetrate into the filter paper or into pieces of faeces or to climb up the wall of the container in order to bring themselves in contact with some surface which will offer resistance. At this time the posterior end of the old cuticula usually becomes bent in the form of a hook (Fig. 5, Plate VIII). Occasionally a larva can be seen to be firmly attached by means of this hook to a piece of faeces, as it begins to free itself from the sheath.

In solid cultures, the structural changes of the larvae can be noted in 60 to 80 hours after the beginning of development, and some mature larvae (in the second stage) are found at the edge of the filter paper or on the sides of the container in 100 to 120 hours.

Measurements of the larvae toward the end of the moulting period show that while the length of the sheath is 0.77 mm. to 0.82 mm., the larvae within the sheaths are only 0.62 mm. to 0.75 mm. in length. In the moulting period, the movements of the larvae are slow and sometimes they are very quiet. They always react, however, to any stimulus. If they are removed from the culture into water about 5 mm. in depth, they will become quiet within several hours and finally die.

(e) The Second Larval Stage. (The infective stage.)

The larvae after the first moult are usually found at the edge of the filter paper or on the wall of the container. This stage is reached under favourable conditions five days after the cultures are made. Since the structural changes which occur during the first moult are very pronounced, the structure of the larvae during the second stage differs fundamentally from that of the first stage. Those larvae (Fig. 6, Plate VIII) are now ready to infect the host and differ from the larvae of other known Strongylidae in not having any sheath.

Shape and size. In shape, they do not differ greatly from the first stage except that the tail is short and blunt, having various shapes as shown in Text-fig. 4. These variations are nothing but individual differences. The larvae in this stage do not vary much in size, averaging 0.69 mm. by 0.027 mm.

Body-cavity. The body-cavity is so narrow that it can only be detected with difficulty.

The cuticula. The cuticula shows faint transverse striations. On each side of the body there is a sharp narrow projection of the cuticula extending from
the region of the oesophagus to the posterior end. In the anterior region of the body three to four small highly refractile specks, like spines, are found on the surface of the cuticula at the region about the middle part of the oesophagus. Similar structures are recognizable in the tail region.

Subcuticle and musculature. It is difficult to understand the details of the structure of subcuticle and muscle layers in the optical sections of the living larvae. However, the lateral bands can be distinguished easily as two longitudinal strands at the sides of the body, since they are more transparent than the two neighbouring muscular areas. The dorsal and the ventral bands are not clearly distinguished.

The buccal-cavity. The buccal-cavity is narrower and shorter than in the larvae in the first stage. Its structure is shown in Text-fig. 1 a.

Oesophagus. The oesophagus is more or less claviform in shape and a little more slender than in the previous stage. Its lumen appears as a straight line. At the posterior end of the oesophagus there are three pairs of clear cells, each containing a vesicular nucleus (Fig. 6, eg, Plate VIII). These cells seem to correspond to the "oesophageal glands" described by Looss (1911) for the hookworm larva. They first become visible in larvae which have completed their metamorphosis.

The intestine. The intestinal lumen is very wide and contains a certain quantity of fine granules. The intestinal wall is composed as described above of two rows of eight flat cells, each containing an ellipsoidal nucleus. The protoplasm of these cells has increased somewhat in thickness over the condition just after the division of the cells. They have a uniform thickness along the whole length of the intestine. The nuclei in the cells are sometimes difficult to find, being covered by pigment granules.

Nervous system. The oesophageal ring is more clearly defined and has the same position as in the first stage. Details of the structure of the nervous system are difficult to make out in the living specimens.

The excretory system. In the mature larvae, the cervical glands become clearly recognizable by their large transparent ellipsoidal nuclei (Fig. 6, neg,
Plate VIII). They are found in the dorso-lateral regions of the body. One of them is located at a distance of 0.06 to 0.08 mm. from the base of the oesophagus, and the other about the same distance behind the anterior one. These glands seem to have developed from the large pear-shaped cells, which were found in the first stage on the ventral side of the region where the oesophagus joins the intestine. The excretory pore opens at the ventral side at a distance of 0.13 mm. to 0.135 mm. from the anterior end of the body. Although the cervical gland-cells may be connected with the excretory pore at this stage, I could not trace any such connection clearly.

The genital anlage. The genital anlage (Fig. 6, gp, Plate VIII) is represented by an ellipsoidal or somewhat spindle-shaped transparent body which is situated ventrally in the region of the fourth or the fifth cells of the intestinal wall. Usually there can be seen beside the genital anlage a dark granular body as shown in the figures (Text-fig. 2). This body is so close to the genital anlage that it seems almost to be a part of it. I have, however, not succeeded in identifying this body in later development. The genital anlage consists of 6 to 8 cells and measures 0.0012 to 0.0017 mm. by 0.007 to 0.008 mm. It is impossible to distinguish the sexes at this stage.

Table II shows the size of the various parts of the larvae at this stage.
Table II.

Measurements of Larvae of H. muris in the Infective Stage.

<table>
<thead>
<tr>
<th>Length Position</th>
<th>Width Position</th>
<th>Buccal-cavity</th>
<th>Oesophagus</th>
<th>Excretory pore</th>
<th>Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mm.)</td>
<td>(mm.)</td>
<td>(depth)</td>
<td>(length)</td>
<td>(from ant. end)</td>
<td>(mm.)</td>
</tr>
<tr>
<td>18 days after culture</td>
<td>14 days after culture</td>
<td>18 days after culture</td>
<td>14 days after culture</td>
<td>18 days after culture</td>
<td>14 days after culture</td>
</tr>
<tr>
<td>(in faeces)</td>
<td>(in faeces)</td>
<td>(in faeces)</td>
<td>(in faeces)</td>
<td>(in faeces)</td>
<td>(in faeces)</td>
</tr>
<tr>
<td>0.680</td>
<td>0.028</td>
<td>0.0125</td>
<td>0.140</td>
<td>0.130</td>
<td>0.047</td>
</tr>
<tr>
<td>0.715</td>
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<td>0.135</td>
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<td>0.0111</td>
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<td>0.132</td>
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<td>0.652</td>
<td>0.026</td>
<td>0.0111</td>
<td>0.129</td>
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<td>0.680</td>
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<td>0.135</td>
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</tr>
<tr>
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<tr>
<td>0.700</td>
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<td>0.138</td>
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<td>0.046</td>
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<tr>
<td>0.65</td>
<td>0.027</td>
<td>0.0111</td>
<td>0.130</td>
<td>0.132</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Activities of the mature or infective larvae. The mature larvae usually are found along the edges of the filter paper, where they extend themselves in the air and wave back and forth slowly. They appear like minute hairs along the edge of the paper. If they are removed into water, they move actively as compared to the movement in the first stage. Their movement can best be described as “swimming.” They are more resistant than the younger larvae to all the influences of the environment. They are, however, less resistant than the mature larvae of other Strongylidae which are enclosed in protective sheaths.

3. The Influence of the Environment on the Eggs and Larvae.

From my experience in culturing the larvae of H. muris I learned the following facts in regard to the effect of environment.

(a) Air.

The eggs of Heligmosomum muris require a certain amount of oxygen for their development since they do not develop beyond the morula stage in liquid media of more than 0.2 cm. in depth, while the eggs hatch and the larvae develop normally in liquid media of less than 1 mm. in depth.

I am of the opinion that more air is required during the development of eggs and larvae than for resting eggs and mature larvae, since the mature larvae were found alive for a long time even when kept in water at a depth of 1 cm. or more, while the developing larvae died under the same conditions in several hours to a few days according to the stage of their development.

(b) Light.

The development of the eggs and larvae of H. muris cultured in faeces does not seem to be influenced to any extent by diffused sunlight, although darkness appears to be the most suitable condition for development. If cultures containing mature larvae are examined after they have been kept for a considerable time in darkness and compared with cultures which have been kept for a period in the light, the cultures from the darkness will show a larger
number of larvae on the edges of the filter paper and these larvae will be more active.

On the contrary, if the larvae are exposed to direct sunlight, they soon become quiet and in a short time will be found to be dead. Further the eggs will not develop and hatch when the cultures are kept in direct sunlight. This relation to light is very interesting when we consider that the normal environment for the development of the larvae, and the place where they would most probably infect their hosts, would be in the dark burrows of the rats.

(c) Moisture.

I did not carry out any definite series of experiments to determine the most favourable degree of moisture or the resistance of the larvae to desiccation. It is evident, however, that some moisture is necessary for the development of the eggs and larvae of *H. muris*. I was able to observe that the eggs and larvae develop well under the condition of moisture, in which the filter paper used for culturing was kept about as moist as the faeces are at the time of defecation. If the faeces are soaked with water, the development often failed. On the contrary, if the cultures are not kept quite moist the larvae do not develop well.

The resistance of the larvae to desiccation varies according to the degree of their development. The mature larvae are more resistant than the other stages. They were found alive for a month or more on the walls of the covered containers in which the cultures were kept. They were also alive on a glass slide which was exposed in the air for more than two days. The young larvae were killed in dried cultures and on a glass slide in several hours.

(d) Temperature.

I have no definite experimental data to show what temperature is most suitable for the development of the eggs and larvae. My experience with culturing showed that the development was more rapid during the hot summer period than during the spring and autumn when the temperature was lower.

III. METHOD OF INFECTION AND MIGRATION IN THE HOST.

After I had learned to culture infective larvae in quantity I tried two series of experiments on white rats to determine the method of infection with *H. muris*. In one series of experiments the mature larvae were fed to the rats and in the other they were brought into contact with their skin under conditions favourable for penetration.

1. Infection through the Mouth.

In this experiment seven tame rats were given great numbers of the mature larvae of *H. muris* on bread and watched carefully to make sure that they actually ingested the larvae. All of the rats were dissected at varying periods after the feedings and were found to contain only small numbers of the parasite, as summarized in Table III.
The above experiments show that when infective larvae are fed to rats only a small proportion are able to reach the intestine.

2. Infection through the Skin.

In the early experiments on infection through the skin the rats were always fastened in an aluminium form and the hair on the abdominal wall was cut very short. Then a large number of the mature larvae were put in water on the surface of the skin and the rats were kept in the shade until the water had dried. In the post-mortem examination the infestation was always found proportionate to the number of larvae applied. Later I found that the rats were infected very easily through the skin by applying the mature larvae to any part of the body. The present work was carried out by employing the simple method of application of the mature larvae to any parts of the skin of the rats after cutting the hair.

*Migratory course of the larvae in the final host.* The larvae placed on the skin of the rats penetrate very easily and quickly into the tissues and travel to the lungs, by means of blood-stream. Since the larvae were found in the trachea and reach the intestine soon after leaving the lungs it seems evident that they penetrate into the air sacs of the lungs and make their way through the trachea, oesophagus and the stomach into the intestine, following the same course as the hookworm larva. Several times I have examined very carefully the abdominal and pleural cavities of rats soon after infection through the skin, washing with normal saline, without ever finding any of the larvae.

IV. PARASITIC STAGES.

1. Outline of Parasitic Development.

   (a) General Considerations.

The larvae of *H. muris* after penetration into the host develop very rapidly. They reach sexual maturity in five to six days after infection and eggs are found in faeces after six to seven days.
From the morphological and biological viewpoint, the parasitic life of this species can be divided into three stages, viz., the third larval stage, or the first parasitic stage; the fourth larval stage, or the second parasitic stage; and the stage of the adult worm, or the third parasitic stage. These stages are separated by two moults. On account of the great complication of the parasitic development I will first give a general outline of the changes which go on, and will then describe in detail the development of certain of the organs.

(b) The First Parasitic Stage or the Third Larval Stage.

The first parasitic stage of *H. muris* begins at the moment of penetration of the mature larvae into the host and is divided from the second parasitic stage (fourth larval stage) by the second ecdysis. This stage is not separated from the second larval stage by a moult but is differentiated from it by a complete change of environment which initiates profound morphological changes. The larvae after penetrating into the host are found first in the lungs of the host in about 14 to 20 hours. Usually they remain for about 35 to 50 hours in the lungs since they are found in this location until 50 to 65 hours after infection. Exceptionally, larvae were found in the lungs of the host 72 hours after infection. Toward the end of their sojourn in the lungs the second moult begins.

The structural changes which take place during the first parasitic stage may be summarized as follows (Fig. 7, Plate VIII and Text-fig. 3 a and b):

1. The larvae grow rapidly, increasing more in width than in length.
2. The buccal-cavity becomes shorter and wider.
3. The oesophagus increases in length.
4. The cells of the intestinal wall increase in thickness and in number. They come to contain so much pigment that their nuclei cannot always be detected.
5. The excretory system becomes clearly differentiated. The cervical glands are now seen dorsally in the body-cavity at the anterior region of the intestine, and the excretory pore can be distinguished on the ventral surface in the oesophageal region.
6. The differentiation of the sexes occurs during this stage.

(c) The Second Moult.

The second moult occurs usually toward the end of the life in the lungs. The preparation for this moult begins from 35 to 40 hours after infection, and the process of moulting extends over a period of from 12 to 15 hours. The larvae at the time of moulting move very slowly and become somewhat coiled. In observing some larvae in the process of moulting, I noticed that a longitudinal rupture of the old cuticula occurred at the base of the oesophagus. Through this opening the larva gradually worked its way sideways, withdrawing the anterior end from the anterior region of the cuticula and in so doing in-vaginating the cuticula of this region like the finger of a glove. It is not
uncommon to see a larva carrying around the old skin out of which it has partly wriggled. When they cast the old cuticula they become very active and soon after start their migration toward the intestine of the host. The larvae which are found in the lungs are always in the first parasitic stage or in the moult, while in the intestine some will be found in the second parasitic stage.

The structural changes which occur during the moulting process are as follows:

(1) The buccal-cavity loses its cylindrical shape and becomes greatly reduced in size.

(2) The oesophagus extends almost up to the anterior end of the body.

(3) The characteristic cuticular expansion arises at the anterior end of the body.
(d) The Second Parasitic Stage or the Fourth Larval Stage.

The larvae in the second parasitic stage (fourth larval stage) always reach the intestine of the experimental rats soon after the casting of the old cuticula. Usually they travel from the lungs to the intestine about 50 to 60 hours after infection. Sometimes they are found in the intestine as early as 45 hours after infection, while in some cases they remain in the lungs undergoing moulting as long as 72 hours after infection. The growth of the larvae during this stage is very rapid, and they soon start the third ecdysis and reach the adult stage. It is during this stage that the sexual organs develop with remarkable rapidity as will be described in a later section.

(e) The Third Moult.

The third moult usually begins from 90 to 108 hours after infection and lasts for about 12 to 15 hours. The larvae in the moult are found somewhat coiled. The structural changes which occur during the third moult are as follows:

1. The newly formed cuticula is provided with prominent longitudinal markings in the form of ten ridges.
2. The reproductive organs become fully formed.

(f) The Third Parasitic or Adult Stage.

Shortly after completion of the third ecdysis, the larvae reach sexual maturity. The mechanism of the casting of the cuticula does not differ from that of the previous moult. The ecdysis of the female occurs a little later than that of the male. The structure of the adult worm will not be included in this paper, because it has been described in my previous paper (Yokogawa, 1920). During this stage there are two layers of the cuticula, the outer apparently representing the fourth moult, but never being shed.

2. Special Description of the Developmental Changes during the Parasitic Stages.

(a) Size, Shape and Colour.

Size. The larva, after penetration into the host, increases very rapidly in size. At the beginning of the first parasitic stage the increase in width is especially marked while the increase in length is greatest during the later stages. After the sexes are differentiated the female is almost always larger than the male. The moults of the females in the parasitic stages come a little later than those of the males. Table IV indicates the size of both sexes at the beginning of the parasitic stages.
As shown in Table IV the female is more slender in proportion to the length than the male.

**Shape.** The changes in shape which take place during the parasitic life occur in the following order. At the beginning of the first parasitic stage, the shape does not differ to any extent from that of the mature larvae in the free life. They soon increase, however, much more in width than in length, so that the shape appears quite different from that of the mature free-living larva (cf. Figs. 6 and 7, Plate VIII). During the second parasitic stage the larvae grow rapidly and many characteristic changes in shape can be noted. The anterior end is small and the cuticular expansion, which is a differentiating character of the genus Heligmosomum, makes its appearance. The length of this cuticular expansion in the second parasitic stage is 0-025 mm. to 0-037 mm., while in the next stage (adult) it measures 0-06 mm. to 0-07 mm. in length.
The shape of the posterior ends of the larvae early in the second stage begins to show the sexual differentiation which becomes so prominent later.

**Colour.** The cuticula of the larvae in the first parasitic stage is colourless while that of the adult worm is pinkish. The cuticula of the larvae in the second parasitic stage has a slightly yellowish tinge. They appear, however, at this stage as black or dark-brown spots or as curved strings on the mucous membrane of the intestine of the host, since the dense pigment of the intestinal cells shows through and gives its colour to the whole worm when examined with the naked eye. After completion of the third ecdysis, the colour of the cuticula not only increases in intensity but takes on the pinkish shade. As noted above the cuticula consists at this stage of two separate layers. These layers, the outer one of which probably represents an incomplete moult, have the same colour. The outer cuticula is much wider than the inner. The cuticula appears to have a yellowish-red colour when seen with transmitted light under the microscope, while it has a reddish or pinkish appearance by reflected light.

(b) **The Cuticula.**

The cuticula of the larva in the first parasitic stage does not differ from that of the mature free-living larva. It consists of a thin transparent membrane with closely set striations. When the larvae reach the next stage, the anterior part of the cuticle inflates and makes a peculiar cephalic area, the cuticular expansion. This cuticular expansion or the cephalic area consists of very thin cuticula without any striations. The other regions of the cuticula are relatively thick and have the transverse striations except the posterior inflation of the male which later forms the bursa. In this inflated bursal region the cuticula becomes thinner and thinner as the expansion increases.

After the completion of the third ecdysis, the cuticula shows prominent longitudinal markings in the form of ten ridges. These longitudinal ridges begin a little behind the cephalic area and run parallel to the posterior end. At this stage, the transverse striations are not continuous around the cuticula but are found only on the longitudinal ridges. During this stage, the cuticula becomes looser and looser and a new cuticula similar to that produced in moulting develops under the old. The old cuticula finally separates from the new and a relatively wide space develops between them. Especially at the posterior end of the female, the outer cuticula is widely separated from the inner and forms a peculiar sac surrounding the anus and the vulva.

The inner newly-formed cuticula has only transverse striations and not the longitudinal ridges which are so prominent on the outer cuticula. These two layers of cuticula are fused at the anterior tip, at the posterior limit of the cephalic inflation, at the anus and vulva of the female and over the whole of the bursa of the male. At all other parts of the body they are widely separated.
Heligmosomum muris Yokogawa

(c) **Digestive System.**

The mouth and buccal-cavity. The buccal-cavity of the larva in the first parasitic stage becomes shorter and wider than that of the mature, free-living larva. The anterior end of the larva at this stage becomes wider and blunter and the prominent edge of the mouth, which was recognizable in the earlier stage, disappears. With the increase in size of the anterior end the mouth expands more rapidly than the buccal-cavity, making this cavity funnel-shaped. The length of the buccal cavity of the larvae in the first parasitic stage is 0·005 mm. to 0·008 mm., while in the mature free-living larvae it had a length of 0·009 mm. to 0·019 mm.

After the completion of the second ecdysis, the buccal-cavity is almost obliterated by the forward growth of the oesophagus, only a very small space being left.

After the disappearance of the larval buccal-cavity, the mouth cavity of the adult stage is found at the anterior end of the body, surrounded by the inflated cuticula and the subcuticular layer (Text-fig. 1 b).

**Oesophagus.** The oesophagus of the larvae in the parasitic life is conoidiform and its length can be modified in an individual worm by contraction of the body. At the beginning of the first parasitic stage it measures about 0·17 mm. to 0·19 mm. in length, while it is about 0·27 mm. to 0·32 mm. long at the beginning of the next stage. After the completion of the third ecdysis, the oesophagus measures from 0·35 mm. to 0·45 mm. in length.

**Oesophageal glands.** After infection, the granular strands which represent the substance of the oesophageal glands increase in number as described by Looss (1905, pp. 88 and 89) for the hookworm, and the subventral gland-cells become hard to see, being covered with the granules, while there can be seen a large vesicular ovoid nucleus at the dorsal side of the posterior end of the oesophagus (Fig. 7, eg, Plate VIII).

**Intestine.** There are remarkable changes in structure of the intestine of the larvae soon after the beginning of parasitic life. The cells of the intestinal wall become greatly thickened, become loaded with pigmented granules, and also increase in number (Fig. 7, Plate VIII and Text-fig. 3 a and b). I found in one specimen from the lungs of an experimental rat 15 hours after infection that one side of the intestine consisted of twelve cells, while the other side was composed of fourteen cells. It is evident that the cells divide as development proceeds since a greater number of cells are found in the intestinal wall of the larvae in more advanced stages. The intestinal cells of the larvae in the parasitic life are so dark and crowded with granules that it is difficult to see the nuclei. At the anterior limit of the intestine there are found in the parasitic stages the small clear cells which are the cells of the intestinal valve, which were present in this region in the free-living larvae.

**Rectum.** The morphological changes in the rectum of the larvae during the parasitic stages of development are very great and differ in the two sexes,
since they are influenced by the development of the external sexual organs. In the male 15 hours after infection the rectum runs obliquely to the main axis of the body through a definite cell group. Later a space develops between the walls of the rectum and this cell group. This space (Fig. 9, rs, Plate IX) is clearly defined in specimens found 30 hours after infection, while it has not yet appeared in specimens within 24 hours after infection. The wall of the rectum consists of a thin membrane. The rectum of the larvae near the beginning of the first parasitic stage has a length of about 0·04 mm. while its length has increased to between 0·18 to 0·20 mm. by the end of the second parasitic stage. The space surrounding the rectum and also the rectal lumen becomes wider as the bursa develops. Toward the end of the second parasitic stage a cell situated dorsally to the rectal sphincter grows posteriorly along the dorsal side of the rectum (Fig. 15, pwc, Plate IX). At the same time a cell on the ventral side of the end of the ejaculatory duct extends posteriorly a short distance along the inside of the ventral wall of the body (Fig. 19, Plate X). These cells become thicker and from them is produced the wall of the cloaca of the adult male. With the completion of the third ecdysis, the membranous wall of the rectum breaks off from the thicker part and forms a peculiar cloaca.

The structural changes of the rectum during the development of the female in the parasitic life are not as complicated as those of the male. In the beginning of the parasitic life, the tail of the female is usually curved ventrally. The rectum runs obliquely through a cell group to the anus, and has a length of about 0·04 mm. (Fig. 22, Plate XI). The cell group surrounding the rectum grows until about the middle of the second parasitic stage and then gradually the cells seem to decrease in size and the number diminishes. At the beginning of the second parasitic stage, a cell which is situated just back of the anus projects very slightly (Figs. 27 and 30, pac, Plate XI). This projection is gradually seen to disappear as the cells of the tail degenerate. After the completion of the third ecdysis the cells of the tail region lose their definite differentiation and nuclei are no longer visible (Fig. 31, Plate XI). The rectal lumen at this stage is lined with a definite chitinous membrane, and the rectum has a length of 0·02 to 0·03 mm. The tail of the adult female is very thin, curves ventrally like a hook and is surrounded by the peculiar sac formed from the loose folds of the outer cuticula.

(d) The Excretory System.

The excretory apparatus of \textit{H. muris} was not worked out in detail but seemed to be similar to that system as described for other species of the family Strongylidae. I gained, however, some information on the development of this system during the course of my studies which will be included here. Only the excretory pore, the cervical glands, and the excretory vesicle were found. The excretory pore is situated on the ventral surface at a distance of 0·15 to 0·25 mm. from the anterior end of the body, according to the stage of development. The excretory vesicle can be seen in the anterior region of the
body extending a little in front of the excretory pore and connected with it by a short duct. The cervical glands could be very clearly distinguished in the living larvae of the parasitic stages, although they were difficult to see in the sexually mature worms. In the specimens from the experimental rats 15 to 20 hours after infection, the protoplasm of the cervical gland cells is not granular while in later stages it contains many granules. These gland cells (Fig. 7, Plate VIII and Text-fig. 3 a and b) are located in the dorsal body-cavity and extend along the dorso-lateral sides of the chyle intestine. The anterior ends of the cervical glands are narrower than their posterior ends, and they seem to be connected with the “bridge” as Looss (1905, pp. 104 and 105, Fig. 30) shows in *Ancylostoma duodenale* at the ventral-lateral side of the oesophagus.

These cells have an elongate lancet shape, and extend to about the middle of the body. They contain large transparent ovoidal nuclei, which lie in the centre of the glands. Each nucleus has a round nucleolus. As the reproductive organs extend forward in development the cervical gland cells become more difficult to see since they are crowded to the side by the anterior part of the ovary or the testis as the case may be.

3. **Sexual Differentiation and the Development of the Reproductive Organs.**

(a) General Discussion.

The post-embryonic development of the reproductive organs of nematodes is very insufficiently known. *H. muris* offers very good material for the study of these organs since they are simple in this species and the period of development is short. Therefore an especially careful study was made of the reproductive organs and a very large series of stages was examined.

The stage of development of the reproductive system does not always depend on the time elapsed after infection, but rather is correlated with the degree of growth in size. There are of course some variations in the development of this system in animals of about the same size. I will describe the average condition which has been ascertained from a very large series of specimens.

Looking in the literature on the development of the nematodes, nothing is known particularly regarding the development of the internal and the external sex-organs, while in the higher animals they develop from two different origins, one part from the genital anlage, while the other without reference to the genital primordium. Neuhaus (1903) who investigated the post-embryonal development of the *Rhabditis nigrovenosa* described that the vagina might be developed from the middle part of the genital primordium, while Leuckart (1887) described that the vagina of *Allantonema* is formed by a projection of the ventral wall. The argument of Neuhaus is based on the position of the vagina in the body and the identity of the cells.
Looss (1897 and 1911) did not add to the knowledge of this point in the development of nematodes even though he studied on the development of *Ancylostoma*, *Strongyloides* and *Rhabdonema*. Veglia (1915) who studied the life-history of the *Haemonchus contortus* added but little to the knowledge of the development of the reproductive organs. Theiler and Robertson (1915), who worked on the life-history of the wire-worm in Ostriches, mentioned briefly the development of its bursa and vulva. They described the development of the vulva as follows: "The central cell is the biggest of the lot of the female primordium; ventrally it soon splits crossways to the longitudinal axis, indicating the future vulva. This cell is also raised slightly above the adjoining ones and reaches the subcuticula. In a somewhat more advanced stage this slit is surrounded by a number of cells." Their description is inadequate and their explanation of the development of the vulva seems unreasonable. They described also very briefly the formation of the bursa. As far as it goes their descriptions agree with what has been found in *H. muris*. I studied very carefully and made clear the development of the reproductive apparatus, and knew that the testes, vas deferens, seminal vesicle, cement gland and the ejaculatory duct develop from the male primordium, while the bursa, spicules and the gubernacles are produced without reference to the genital primordium. Accordingly I shall call the former the internal sex-organs, and the latter the external sex-organs. I also found that the ovary, oviduct, seminal receptacle, uterus and ovjector (houstrix) are produced from the genital primordium, while the vulva and the vagina develop from outside the genital primordium. The second part of the ovjector is produced from the distal end of the genital primordium and the anterior part of the vagina, that is, this part corresponds to the adjoining part of the internal and external sex-organs.

*(b) The Beginning of Sexual Dimorphism.*

Sexual dimorphism has not been noted for the free-living larval stages of the Strongylidae. Looss (1911) criticized the suggestion of several authors who tried to distinguish the sexes in free-living nematode larvae by the shapes of their tails. He concluded that sexual dimorphism cannot be detected in the free-living larvae of the nematodes. Looss (1911) on the *Ancylostoma*, Theiler and Robertson (1915) on the wire-worm in Ostriches, and Veglia (1915) on the *Haemonchus contortus*, do not describe the beginning of sexual dimorphism, and so far as I know there is no literature on this subject. I think it is very interesting and important matter to learn just when and how the sexual differences appear during the parasitic life. Sexual dimorphism in *H. muris* was distinguished in very young larvae during the first parasitic stage (Text-fig. 3a and b).

Three essential points of difference between the sexes were determined at this early stage, i.e., first, the posterior migration of the genital primordium of the female; second, structural differences in caudal regions; and third, differences in the shape of the genital primordia.
Heligmosomum muris Yokogawa

In the first larval stage, the genital primordium is located at about the middle of the body. After the completion of the first moult, its position is a little behind the middle part of the body on account of the relative reduction in the length of the posterior part of the body, and the considerable increase in the length of the oesophagus (see Table II).

Table V shows the backward shifting of the position of the genital primordium during free life.

Table V.

The Position of Genital Primordium during Free Life.

<table>
<thead>
<tr>
<th>Stages in free life</th>
<th>Body (length) (mm.)</th>
<th>Body (width) (mm.)</th>
<th>Size of germ cell group (mm.)</th>
<th>Position of germ cell group (dist. from ant. end)</th>
<th>Percent. of distance from anterior end</th>
</tr>
</thead>
<tbody>
<tr>
<td>First larval stage</td>
<td>0·280</td>
<td>0·015</td>
<td>0·006 × 0·004</td>
<td>0·148</td>
<td>52·8</td>
</tr>
<tr>
<td></td>
<td>0·300</td>
<td>0·018</td>
<td>0·006 × 0·004</td>
<td>0·153</td>
<td>51·2</td>
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<td></td>
<td>0·395</td>
<td>0·018</td>
<td>0·007 × 0·004</td>
<td>0·202</td>
<td>51·3</td>
</tr>
<tr>
<td></td>
<td>0·442</td>
<td>0·022</td>
<td>0·007 × 0·004</td>
<td>0·235</td>
<td>53·2</td>
</tr>
<tr>
<td>First moult</td>
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<td>0·034</td>
<td>0·010 × 0·005</td>
<td>0·330</td>
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<tr>
<td></td>
<td>0·680</td>
<td>0·032</td>
<td>0·008 × 0·005</td>
<td>0·340</td>
<td>50·0</td>
</tr>
<tr>
<td></td>
<td>0·700</td>
<td>0·033</td>
<td>0·010 × 0·006</td>
<td>0·376</td>
<td>53·0</td>
</tr>
<tr>
<td></td>
<td>0·720</td>
<td>0·034</td>
<td>0·010 × 0·005</td>
<td>0·387</td>
<td>53·7</td>
</tr>
<tr>
<td></td>
<td>0·750</td>
<td>0·034</td>
<td>0·012 × 0·006</td>
<td>0·387</td>
<td>51·6</td>
</tr>
<tr>
<td>Second larval stage</td>
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<td>0·028</td>
<td>0·016 × 0·008</td>
<td>0·390</td>
<td>57·3</td>
</tr>
<tr>
<td>(the mature free-</td>
<td>0·715</td>
<td>0·026</td>
<td>0·016 × 0·007</td>
<td>0·420</td>
<td>58·7</td>
</tr>
<tr>
<td>living larvae)</td>
<td>0·740</td>
<td>0·026</td>
<td>0·017 × 0·008</td>
<td>0·435</td>
<td>58·7</td>
</tr>
<tr>
<td></td>
<td>0·630</td>
<td>0·025</td>
<td>0·012 × 0·008</td>
<td>0·382</td>
<td>60·6</td>
</tr>
<tr>
<td></td>
<td>0·715</td>
<td>0·025</td>
<td>0·014 × 0·008</td>
<td>0·420</td>
<td>58·7</td>
</tr>
</tbody>
</table>

As shown in Table V the genital primordium of the mature free-living larvae is situated near the middle body and no differences in its position can be noted between the sexes. After infection a remarkable differentiation of position of the genital primordium in the sexes comes about. Examining many specimens in parasitic stages, I found that the genital primordium of the female migrates from near the middle of the body toward its posterior end, while that of the male does not migrate but remains near the middle of the body. This remarkable migration of the genital primordium of the female takes place between 20 to 30 hours after infection. By 30 hours after infection the genital primordium of the female has reached a position just in front of the anus (Text-fig. 3 b). Table IV shows the progress in this migration comparing the position of the genital primordium in both sexes at various times after infection.

From Table VI it can be seen that as early as from 24 to 30 hours after infection the males and females are differentiated by the position of the genital primordium.

Finding many varying shapes of the tail of the mature free-living larvae (Text-fig. 4) I tried in vain to establish a sexual dimorphism in this character. I finally came to the conclusion that these differences were nothing but individual variation. I found, however, structural differences between the posterior ends of the sexes very early in the parasitic development. The posterior end
of the male becomes more curved than that of the female, and the arrangement of the cells in this region is different. Comparing many specimens found in the lungs of the experimental rats from 15 to 23 hours after infection, I found the following differences in the caudal regions of the sexes (cf. Figs. 8 and 22, Plates IX and XI).

1. The rectum in the male is surrounded by 10 cells excepting the cells of the rectal ligament and sphincter, while that of the female is surrounded by only five cells excepting the same cells.

Table VI.

<table>
<thead>
<tr>
<th>Age of larvae</th>
<th>Body (length) (mm.)</th>
<th>Body (width) (mm.)</th>
<th>Size of genital primordium</th>
<th>Position of gen. primordium (distance from anterior end)</th>
<th>Percent. of distance from ant. end</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 hours after infection</td>
<td>0-680</td>
<td>0-032</td>
<td>0-016 × 0-009</td>
<td>0-390</td>
<td>57-3</td>
<td>?</td>
</tr>
<tr>
<td>0-680</td>
<td>0-032</td>
<td>0-014 × 0-006</td>
<td>0-391</td>
<td>57-5</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>0-702</td>
<td>0-028</td>
<td>0-018 × 0-01</td>
<td>0-410</td>
<td>57-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-710</td>
<td>0-029</td>
<td>0-015 × 0-008</td>
<td>0-405</td>
<td>56-3</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>24 hours after infection</td>
<td>0-885</td>
<td>0-032</td>
<td>0-016 × 0-007</td>
<td>0-550</td>
<td>62-1</td>
<td>?</td>
</tr>
<tr>
<td>1-030</td>
<td>0-033</td>
<td>0-018 × 0-007</td>
<td>0-600</td>
<td>58-3</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>0-820</td>
<td>0-030</td>
<td>0-021 × 0-010</td>
<td>0-510</td>
<td>62-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-780</td>
<td>0-028</td>
<td>0-021 × 0-010</td>
<td>0-450</td>
<td>57-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-825</td>
<td>0-031</td>
<td>0-021 × 0-010</td>
<td>0-485</td>
<td>58-7</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>0-755</td>
<td>0-030</td>
<td>0-017 × 0-006</td>
<td>0-490</td>
<td>65-0</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>0-970</td>
<td>0-032</td>
<td>0-024 × 0-01</td>
<td>0-750</td>
<td>73-3</td>
<td>?</td>
<td></td>
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<tr>
<td>0-985</td>
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<td>0-028 × 0-007</td>
<td>0-865</td>
<td>88-0</td>
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<tr>
<td>0-790</td>
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<td>0-028 × 0-008</td>
<td>0-565</td>
<td>71-5</td>
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<td></td>
</tr>
<tr>
<td>1-09</td>
<td>0-035</td>
<td>0-031 × 0-01</td>
<td>0-940</td>
<td>86-2</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>30 hours after infection</td>
<td>0-825</td>
<td>0-032</td>
<td>0-017 × 0-008</td>
<td>0-421</td>
<td>51-6</td>
<td>?</td>
</tr>
<tr>
<td>0-800</td>
<td>0-030</td>
<td>0-025 × 0-01</td>
<td>0-432</td>
<td>54-0</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>0-825</td>
<td>0-031</td>
<td>0-017 × 0-011</td>
<td>0-463</td>
<td>56-7</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>0-800</td>
<td>0-036</td>
<td>0-024 × 0-01</td>
<td>0-437</td>
<td>54-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-950</td>
<td>0-035</td>
<td>0-028 × 0-01</td>
<td>0-544</td>
<td>57-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-988</td>
<td>0-032</td>
<td>0-03 × 0-011</td>
<td>0-500</td>
<td>50-6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-975</td>
<td>0-036</td>
<td>0-028 × 0-008</td>
<td>0-876</td>
<td>89-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-850</td>
<td>0-031</td>
<td>0-028 × 0-008</td>
<td>0-716</td>
<td>84-2</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>0-800</td>
<td>0-032</td>
<td>0-027 × 0-008</td>
<td>0-684</td>
<td>85-5</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>0-900</td>
<td>0-030</td>
<td>0-024 × 0-006</td>
<td>0-783</td>
<td>87-0</td>
<td>?</td>
<td></td>
</tr>
</tbody>
</table>

Text-fig. 4. Posterior ends of free-living mature larvae showing the various shapes.
The subcuticular layer of the ventral wall just in front of the anus of the male does not at this time show any cell-divisions, while in this region of the female already three or more cells can be distinguished.

In the youngest free-living stages the genital primordium apparently consists of two cells. It is a small ovoid body, measuring about 0.006 mm. by 0.004 mm. It develops gradually with the development of the body. In the mature free-living larvae the genital primordium consists of six to eight cells and has a size of 0.012 mm. to 0.017 mm. by 0.006 mm. to 0.008 mm. In this stage its shape varies and it almost always has at its anterior end a small more or less dark body as is shown in the figure (Text-fig. 2). In a few cases this unknown body was found at the posterior end of the genital primordium. After infection, the genital primordium becomes gradually transformed in size and in general appearance according to the stage of development and early shows sexual differences. In the female it appears spindle-shaped in the first parasitic stage, while in the male it is rather oblong in shape (Text-figs. 5 and 7). This difference is clearly defined 24 hours after infection. At this time the small darker body described above is found usually at the anterior part of the genital primordium of the male, while it rarely can be seen in the female.

(c) The Development of the Male Reproductive Organs.

(1) The Internal Sex-Organs.

*General description.* The term "internal sex-organs" is used to refer to the organs which develop from the genital primordium, that is, the testis, vas deferens, seminal vesicle, cement gland and ejaculatory duct. As stated in the previous section, the genital primordium of the male is situated ventrally near the middle of the body. Toward the end of the first parasitic stage it becomes separated into two parts by an extremely delicate strand of tissue. The beginning of this division can be distinguished early by a slight constriction in the middle of the genital primordium (Text-fig. 5). The division of the genital primordium usually occurs in 40 to 50 hours after infection and sometimes later. After division the posterior part develops more rapidly than the anterior one growing toward the posterior end of the body. When the young male enters the second parasitic stage the end of this posterior division of the reproductive primordium has grown back to the end of the chyle intestine. After growing to this point the anterior end of the posterior part of the genital primordium grows forward. Meanwhile, the anterior part grows anteriorly along the ventral side of the intestine. Toward the end of the second parasitic stage, and especially during the last moult, the anterior part of the reproductive body develops very rapidly, crossing the middle part of the intestine and growing forward. By the completion of the last ecdysis it reaches almost to the anterior end of the chyle intestine.
Text-fig. 5. Development of primordium of male reproductive system. a–c, First parasitic stage, 24 hours after infection; d and e, first parasitic stage, 30 hours after infection, length 0.825 mm.; f, first parasitic stage, 45 hours after infection, length 0.65 mm.; g, first parasitic stage, 35 hours after infection, length 1.13 mm.; h, first parasitic stage, 50 hours after infection, length 1.08 mm.; j, second stage, 40 hours after infection, length 1.1 mm.; k, second parasitic stage, 45 hours after infection, length 1 mm.; l, second parasitic stage, 54 hours after infection, length 1.11 mm.; m, second parasitic stage, 52 hours after infection, length 1.45 mm.; n, second parasitic stage, 60 hours after infection, length 1.75 mm.
In the beginning of the second parasitic stage, the internal reproductive system of the male shows little, if any, differentiation, and appears as a tube of clear cells, separated into two parts by a narrow strand of tissue. In the course of the second parasitic stage (Fig. 18, agp and pgp, Plate X) the cells of its posterior part become larger and clearer than those of the anterior one, and become arranged in two rows, while the cells of the anterior part remain small and are arranged irregularly. Then the narrow part becomes gradually shorter and broader. Finally all parts of the internal sex-organs become clearly differentiated.

Development of the testes. It is interesting to note that the testis in *H. muris* develops from only the anterior half of the male genital primordium and the other internal organs from its posterior half. As mentioned above, the anterior part of the genital primordium is separated from the posterior part by an extremely delicate strand of tissue, and at the beginning of the first parasitic stage the cells of this part are not yet differentiated (Fig. 17, Plate IX). During the second parasitic stage the cells of the anterior part of the genital primordium gradually become smaller and more numerous, while those of its posterior part increase in size. Toward the end of the second parasitic stage and especially during the last moult the anterior part which forms the testis develops very rapidly, crossing the chyle intestine at the middle of the body and extending anteriorly along the dorsal side of the intestine. After the completion of the third ecdysis, the testis is fully developed and spermatozoa can be distinguished at its proximal end. Its anterior end reaches nearly to the base of the oesophagus, forming a loop of various shapes (Text-fig. 6). The free anterior end of the testis is usually more blunt and broader than that of the ovary.

Development of the vas deferens. The vas deferens develops from the narrow region which is situated between the anterior and the posterior parts of the

Text-fig. 6. Variations in the anterior end of the testis of the adult male.
genital primordium (Fig. 17). At the beginning of the first parasitic stage it is represented only as an extremely fine strand of tissue. Then this tissue strand becomes a little larger, taking the shape of a small tube with one spindle-shaped cell in each side (Fig. 17). The length of this narrow central region varies greatly in different specimens. This region at the beginning of the division of the genital primordium is short, having a length of 0.02 to 0.04 mm. At the beginning of the second parasitic stage it has become much extended, measuring 0.06 to 0.0115 mm. in length, and then it shortens again, showing a tubular structure. Toward the end of the second parasitic stage it becomes much broader, with two cuboid cells on each side of its wall, where previously there was only one spindle-shaped cell (Fig. 20, vd, Plate X). These cuboidal cells are close together and are separated from the adjoining cells both anteriorly and posteriorly by definite constrictions (Fig. 20, vd). These constrictions become less clearly defined with later development. The fully developed vas deferens is narrower than the other regions of the male reproductive organs, having a length of about 0.02 to 0.03 mm. and is situated on the dorsal side of the intestine near the middle of the body.

Development of the seminal vesicle. The seminal vesicle develops from the anterior region of the posterior part of the genital primordium. At the beginning of the second parasitic stage it is not clearly differentiated. Toward the end of the second parasitic stage and especially during the last moult the region just behind the vas deferens has a wall consisting of clear cuboidal cells, while the part just beyond is lined with high columnar cells containing fine granules. The seminal vesicle (Fig. 20, sv) is produced from the region lined with the clear cuboidal cells and becomes a large tube with a wide lumen. The cells of the wall of this region decrease in thickness with the increasing of the lumen, and finally a thin membrane is produced in which it is difficult to make out the individual cells. At this time the lumen of the seminal vesicle contains a few sperms. The seminal vesicle of the adult worm is situated at the dorsal side of the intestine near the middle part of the body, and has a length of about 0.1 to 0.13 mm., and is filled with spermatozoa.

The development of the cement gland. With the formation of the seminal vesicle the region which is to form the cement gland becomes more clearly defined. This region is lined with high columnar cells which at first are all alike. Each of these cells contains a round nucleus and many fine granules. When the internal sex-organs are almost developed the cement gland becomes differentiated into two parts by the appearance of the cells of its wall (Fig. 20, cmg, Plate X). The cells of its posterior half become darker than those of its anterior half and contain a great amount of granules. In the adult worm, this gland usually is situated where the reproductive tube crosses the intestine, while in the earlier stages it is found farther back, on the ventral side of the intestine.

The development of the ejaculatory duct. The ejaculatory duct is produced from the posterior part of the genital primordium. The cells of its wall early
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become cuboidal in shape and are transparent (Figs. 12 and 13, ed, Plate IX). This part is found always along the ventral side of the intestine and its posterior end projects into the cloaca at the ventral side of the rectum.

(2) The External Sex-Organs.

General discussion. The term "external sex-organs" is used to refer to the organs connected with the reproductive processes in the male which do not develop from the genital primordium.

At the beginning of the parasitic life the number of cells in the tail and around the rectum of the male increases. As development proceeds these cells continue to increase in size and in number, and become re-arranged in a definite manner. It is from definite groups of these cells that the spicules, gubernacula and the rays of the bursa develop.

The development of the bursa. The cell group found in the posterior end of the male seems to have originated from the lateral bands and the muscle-cells, since they are located on the sides and show no relation to the ventral and the dorsal walls of the body as early as 24 hours after infection. With development these cells increase in size and number and a space is formed around the rectum (Fig. 9, rs, Plate IX). In the beginning of the first moulting the cells of the subcuticular layer at the ventral side of the tail region divide and join the other cells of this region, so that toward the end of the first parasitic stage the posterior end of the male filled up with clear cells with round nuclei (Fig. 10, Plate IX). During the moult following this stage these cells show a definite arrangement as shown in the figure (Fig. 11, Plate IX). Early the cell group which is situated just behind the rectum becomes separated and differentiated into elongate conical or spindle-shaped cells (Fig. 11, cs). The length of these cells increases as development proceeds and later they produce the spicules (Figs. 11–14, cs, and 15, s, Plate IX), while the other cells of this region later form the bursal rays.

After the completion of the second moult the space which has been formed around the rectum gradually expands from its posterior end, particularly toward the ventral and the lateral sides (Figs. 12–15 and 19, Plates IX and X). As this space expands the posterior end of the male becomes inflated into a sac lined with cells. Toward the end of the second parasitic stage the cells which line the posterior part of this bursal sac produce small finger-like projections which are the beginnings of the rays of the bursa (Fig. 16). At this time each ray consists only of the projection from a single cell. These projections already represent the number and have assumed the position of the fully developed rays. The bursa therefore consists only of the inflated posterior end of the male which is lined by cells and is separated from the true posterior end of the worm by a considerable space (Fig. 19). The rectum (Fig. 19, r) still opens at the posterior end of the bursal sac. During preparation for the third moult, the lobes of the bursa become separated into their final form and the closed bursal sac now consists only of the old cuticula. After the shedding of this skin the
bursal lobes are fully developed and entirely separated (Fig. 21, Plate X). The rays of both lateral lobes now consist of several cells and each ray has assumed its characteristic shape. The central dorsal ray usually consists of two cells, while the externo-dorsal rays are each made up of the projection from only one cell.

The development of the spicules and the gubernacula. The spicules and the gubernacula have the same origin. They are produced by the long conical or spindle-shaped cells situated just back of the base of the rectum (Figs. 11–15, es and s, Plate IX). In the beginning of the second parasitic stage these cells increase in size, while toward the end of this stage they decrease in number and produce the chitinous material of the spicules and the gubernacula. The spicules are produced at the sides of these cells and become extended very rapidly forward along the dorsal side of the intestine. They later reach a muscle cell-group which is present dorsally at a distance from the posterior end of the body. These muscle cells develop into the retractor muscles of the spicules, and come from one original cell (Fig. 19, rms, Plate X). This original cell at the beginning of the second parasitic stage is spindle-shaped and is located at the dorsal side of the intestine at a distance of about 0·22 to 0·23 mm. from the posterior end of the body (Fig. 13, rms, Plate IX). It is connected with the cells at the base of the rectum and with the subcuticular layer by tiny fibres. This spindle cell divides as the development of the sex-organs proceeds, forming two groups of muscle cells (Fig. 19, rms). These are the retractor muscles and extend anteriorly along the dorsal wall of the intestine with the further development of the sex-organs. Toward the end of the second parasitic stage, they are found at a distance of about 0·53 to 0·56 mm. from the posterior end of the body and become attached to the spicules. The spicules when first produced are transparent and colourless, while in the adult stage they have a brownish golden colour. The anterior ends of the spicules are slightly thickened and are attached to the retractor muscles, while their posterior ends are united and project from the dorsal side into the cloaca, forming a small arc.

The gubernacula are produced from the chitinous substance in the distal portion of the spicules toward the end of the second parasitic stage.

(d) The Development of the Female Reproductive Organs.

(1) The Internal Sex-Organs.

General description. The term “internal sex-organs” of the female refers to those organs which develop from the genital primordium and includes the ovary, oviduct, seminal receptacle, uterus and the ovijector.

At the beginning of sexual differentiation, the genital primordium of the female migrates from the middle of the body to its posterior end. During this time, it can be found in different individuals in every position between the middle of the body and the anal region. After reaching a position just in front of the anus (Fig. 23, gp, Plate XI) the genital primordium increases in length,
Heligmosomum muris Yokogawa growing forward (Fig. 24, gp, Plate XI). Toward the end of the first parasitic stage and especially during the second moult the female genital primordium increases considerably in length and begins to show differentiation (Text-fig. 7). At this time the subcuticular cells at the posterior portion of the body also increase in number. In the beginning of the second parasitic stage, the primordium of the ovary, seminal receptacle, uterus and the ovijector can be distinguished (Fig. 36, Plate XII). The oviduct is not differentiated from the posterior end of the ovary until the end of the second parasitic stage. During the first part of the second parasitic stage all of the internal sex-organs develop along the ventral side of the intestine, but toward the end of this stage the ovary increases very rapidly in length and crosses to the dorsal side of the intestine. As development proceeds most of the internal sex-organs grow over to the dorsal side of the intestine.

The ovary. The ovary develops from the anterior part of the genital primordium but there is no clear separation into two regions as in the development of the testes of the male. In the first part of the second parasitic stage the ovary grows forward slowly along the ventral side of the intestine (Fig. 36, o, Plate XII), while toward the end of this stage it crosses over and develops very rapidly along the dorsal side of the intestine. In the first half of the second parasitic stage (about 58 to 80 hours after infection) the size of the ovary increases from 0.08 to 0.9 mm. by 0.014 to 0.12–0.14 mm. by 0.014 to 0.015 mm., while during the last part of this stage and especially during the last moult (about 85 to 108 hours after infection) it develops from 0.14 by 0.014 mm. to 0.50–1.00 by 0.02 to 0.025 mm. In the beginning of the second parasitic stage the cells of the ovary are uniform in size. In the course of the second parasitic stage the cells in its posterior part increase in size and become arranged into two rows, while those of the anterior region are still small and closely packed (Fig. 36, o, Plate XII). During the last part of the second parasitic stage the ovary develops so rapidly that the wall of its posterior part comes to consist of flat cells, forming an empty tube. At this time the oviduct is formed from the posterior end of the ovary as will be explained in the following section. After the completion of the third ecdysis the ovary is fully developed.

The seminal receptacle. The seminal receptacle develops from the region just behind the ovary. At the beginning of the second parasitic stage this part consists of a row of several cells which are a little larger than those of the uterus (Fig. 36, sr). At the end of the second parasitic stage the walls of the seminal receptacle consist of high columnar cells (Fig. 33, sr, Plate XII) having a size of about 0.10 by 0.025 mm., while the uterus and the posterior end of the ovary are lined by flat cells. By the time spermatozoa are introduced the seminal receptacle becomes a thin walled tube lined with flat cells. Its length in the adult worm is about 0.1 mm. and it joins the uterus without any sharp demarcation.

The oviduct. The oviduct is formed chiefly from the posterior end of the ovary. In the last part of the second parasitic stage the walls of the posterior
Text-fig. 7. Development of the primordium of the female reproductive system. 

a. First parasitic stage, 30 hours, 0·9 mm.; b, first parasitic stage, 40 hours, 0·98 mm.; c, first parasitic stage, 40 hours, 1·07 mm.; d, first parasitic stage, 40 hours, 1·02 mm.; e, first parasitic stage, 45 hours, 1·07 mm.; f, first parasitic stage, 35 hours, 1·21 mm.; g, second parasitic stage, 48 hours, 1·20 mm.; h, second parasitic stage, 52 hours, 1·4 mm.; i, second parasitic stage, 52 hours, 1·49 mm.; j, second parasitic stage, 72 hours, 1·89 mm.
end of the ovary consist of flat cells and this part becomes a vacant tube, while in the beginning it consisted of large cuboidal cells, showing a more or less large, rhombic space between the seminal receptacle and the ovary itself (Figs. 32 and 33, Plate XII). When the posterior portion of the ovary forms a vacant tube as described above, two pairs of spindle-shaped or triangular cells can be seen in its wall at a distance of about 0.04 to 0.06 mm. in front of the seminal receptacle (Fig. 36, sr, Plate XII). The region extending between these two pairs of cells and the seminal receptacle forms a muscular tube, the oviduct (Fig. 33, ov). Both ends of this tube are thickened, forming sphincters. The oviduct (Figs. 34 and 35, ov) seems to be an important factor in giving a definite form to the fully developed egg-cells as they pass along it singly on their way to the seminal receptacle. The length of the oviduct of the adult worm is about 0.07-0.09 mm.

Text-fig. 8. Variations in the anterior end of the ovary of adult female.

The uterus. The uterus develops from the cell-group which is situated between the primordium of the seminal receptacle and the ovijector. In the beginning of the second parasitic stage it appears as a row of several cuboidal cells which increases in length by the division of the cells of its anterior part (Figs. 26 and 27, u, Plate XI). The cells in the posterior portion of this row gradually increase in width and their centres become vacuolated (Figs. 28 and 36, u, Plates XI and XII); the increase in the size of these vacuoles produces the lumen of the uterus. Toward the end of the second parasitic stage the uterus is fully developed and forms a tube except at the posterior part which connects with the ovijector. After the completion of the third ecdysis fully developed egg-cells are found in the uterus, and in the posterior end of the uterus the wall is thickened to form a sphincter.
The ovijector. The ovijector (pars houstrix) develops from the posterior end of the genital primordium. Toward the end of the first parasitic stage, and especially during the second moult, it can be seen as a cell-group which consists of two to three pairs of cuboidal cells. After the completion of the second moult, that is, in the beginning of the second parasitic stage, it can be seen as a tube surrounded by two rows of 7 to 8 cuboid cells, which have a size of 0.027 to 0.041 by 0.015 to 0.024 mm. (Figs. 26 and 27, oj). In the course of the first part of the second parasitic stage the ovijector increases in size, measuring 0.08 to 0.1 mm. in length and 0.03 to 0.035 mm. in width. As this tube increases in size the cells of its wall become flattened and difficult to see and the tube becomes lined with fine longitudinal filaments (Fig. 28, oj, Plate XI). Its distal portion at this stage protrudes into the lumen of the vagina like a plug (Fig. 28, poj). Toward the end of the second parasitic stage the ovijector increases in length but becomes somewhat constricted and longitudinal folds appear along its inside (Fig. 29, oj). At this time a pair of the large cells can be seen at the sides of the posterior end of the ovijector (Fig. 30). These cells gradually become difficult to see, producing some granules. The structures formed from these cells seem to be a fixation apparatus to hold the ovijector in that place, since the posterior end of the ovijector is always found on the ventral side of the intestine, while all other parts of the internal sex-organs remove from the ventral to the dorsal side of the intestine. Therefore the ovijector of the adult worm is found to be more or less twisted, crossing the posterior part of the intestine. It has a well-developed wall, and has a size of 0.1 to 0.13 mm. by 0.025 to 0.03 mm. in living specimens. The wall is thickened at the beginning of the ovijector, forming a sphincter, and its posterior end projects into the vagina like a plug, forming the second part of the ovijector (pars ejectrix of the ovijector). Accordingly the second part of the ovijector (Figs. 28–31, poj) is composed of the distal end of the ovijector and the anterior portion of the vagina. The former is inside and shows longitudinal folds, while the latter is outside and is transversely striated.

(2) The External Sex-Organs.

General description. The term “external sex-organs” refers to those organs which have to do with reproduction in the female but do not develop from the genital primordium. They include the posterior part of the ovijector, the vagina, and the vulva.

At the beginning of parasitic life there are many cells in the tail region of the female. These cells gradually increase in size and in number with development. The majority of these cells are not associated in any demonstrable way with the formation of the external sex-organs. In the course of the second parasitic stage, they gradually decrease in number and in size. Only the cells which are situated on the ventral side just in front of the anus are associated with the formation of the external sex-organs. During the second moult these cells increase in number and come into contact with the genital primordium.
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(Fig. 24, Plate XI). At the completion of the second moult they invaginate and push their way into the genital primordium, dividing it into two parts (Fig. 25, eso). The posterior portion of the genital primordium becomes very distinctly separated from its main part by this invagination. It gradually decreases in size with the development of the external sex-organs and finally disappears (Figs. 25–27, pdr). The invaginated cells increase in size and number and fuse with the cells of the distal end of the ovijector (Fig. 26). At this time, the cells which form the external sex-organs become arranged in a definite manner and the vagina and vulva become differentiated (Fig. 27).

The vulva. Seven to eight cells situated at the entrance of the invagination are high columnar or conical in shape and form a funnel-shaped opening (Figs. 27 and 28, v). With the development of the body, these cells gradually become larger and show hook-like projections from their protoplasm (Figs. 28 and 29, v). The cells with projections are usually three to four in number on each side of the opening. During the last moult these cells become cuboidal in shape and lose their projections (Fig. 30). At this time there is no opening through the cuticula into the vulva. Just before the last moult is completed the vulva opens through the newly formed cuticula. The new formed skin becomes loose, showing small folds at the entrance to the vulva. After completion of the third ecdysis, the vulva opens directly toward the outside (Fig. 31, v, Plate XI). The new formed skin becomes much looser and extends posteriorly, forming a peculiar sac at the posterior end of the female.

The vagina. The vagina develops from the invaginated cells mentioned above. When the vagina first becomes differentiated from the vulva it consists of two to three pairs of cuboid cells which connect with the posterior end of the ovijector (Fig. 27, vg). In the course of the second parasitic stage the vagina develops gradually, forming a tube, and its anterior end extends a little upward along the distal portion of the ovijector, forming the second part of the ovijector (Fig. 28, vg and pjo). Toward the end of the second parasitic stage, the vagina develops rapidly. The cells in its wall increase in size and are very irregular in shape (Fig. 29, vg). They then become flattened and difficult to see, undergoing a granular degeneration. Finally the wall of the vagina appears to be formed of cuticular substance and is transversely striated (Figs. 30 and 31, vg). These striations appear first in the second part of the ovijector and then develop along the whole wall of the vagina. Until the completion of the third ecdysis, the vagina and the vulva are clearly distinguished, while in the adult stage they are not separated. Accordingly, the vagina of the adult worms is much longer than that of those in the fourth parasitic stage.

V. ADULT.

While studying the life-history of *H. muris*, I found some points of adult structure which were overlooked in my previous paper (1920). I will here make them clear.

1. As noted above, the cuticula of the adult worm consists of two separate
layers, the inner one of which has not been observed before. The outer one probably represents an incomplete moult, and is much wider than the inner one. The inner cuticula is thin and has only transverse striations and not the longitudinal ridges which are so prominent on the outer cuticula. These two layers of cuticula are fused at the anterior tip, at the posterior limit of the cephalic inflation, at the anus, vulva and the tail of the female and over the whole of the bursa of the male.

2. The oviduct of the adult female was not distinguished in my first study. However, by the study of the life-history it is made clear, measuring about 0.07 to 0.09 mm. in length.

3. The structure of the second part of the ovijector (pars ejectrix of the ovijector) was not clearly understood before. Now I understand its structure by the study of its post-embryonal development.

VI. SUMMARY.

1. *Heligmosomum muris* proved to be very favourable material for the study of nematode development, since it will develop perfectly normally in culture rats, infection is easily carried out and since sexual maturity is reached in 7–10 days after infection.

2. The post-embryonal development of *H. muris* is divided into five stages, two free and three parasitic, with three moults. There is only one moult during free life, the second and third stages being separated by change of habitat brought about by entrance into the host. Sexual maturity is attained soon after the completion of the third moult. The mature worm has two cuticular layers, the outer of which is separated by a space from the inner. This outer cuticula is probably the beginning of a fourth moult which is never completed.

3. Under favourable conditions the eggs hatch in about 20 to 24 hours after being passed with the faeces.

4. The first two stages of post-embryonal development, which are passed in free life, are separated by a relatively long moult during which the larva changes from the rhabditiform type to the filariform type. During this period there is a rapid division of the cells lining the intestine, which frees masses of these cells into the lumen and leaves the intestine of the filariform larva lined with flattened cells.

5. The infective stage is not enclosed in a sheath and tends to work its way out of the culture onto the glass or along the edges of the filter paper. At this stage it is impossible to distinguish the sexes.

6. Infection of the rat can be accomplished both by way of the mouth or through the skin although the latter method is by far the most effective. The larvae reach the lungs about 14 to 20 hours after penetration through the skin. They remain in the lungs until about 35 to 65 hours after infection. The majority of them reach the intestine 50 to 65 hours after infection, although in a few they were found as early as 45 hours.
7. In the lungs the larvae increase rapidly in size and moult just before they migrate to the intestine. Early in the development in the lungs the sexes can be distinguished by: (1) the migration toward the posterior end of the genital primordium of the female, (2) structural differences in the caudal region, and (3) differences in shape of the genital primordium.

8. After reaching the intestine the larvae grow rapidly and enter into the third moult from 96 to 108 hours after infection. In the fourth larval stage between the second and third moults growth and differentiation are most marked. It is during this stage that the differentiation of the organs of the reproductive system occurs.

9. Shortly after the completion of the third moult sexual maturity is reached and later the cuticula separates into two layers.

10. During the course of development the changes in size and shape and in the character of the cuticula were traced step by step and the differentiation of the digestive and excretory systems were followed as completely as the material would permit. However it was in following the details of the development of the reproductive organs that the investigation was most fully carried out.

11. In the male reproductive system the testes, vas deferens, seminal vesicle, cement gland and ejaculatory duct arise by differentiations of the genital primordium and are therefore called internal sex-organs, while the bursa and the spicules which are not developed from the genital primordium are known as the external sex-organs.

12. Toward the end of the third larval stage (first parasitic stage) the genital primordium of the male becomes separated into two parts by an extremely delicate strand of tissue. The anterior half of this genital primordium grows forward up to the oesophageal region and forms the testes, the narrow strand connecting the two parts develops into the vas deferens, and the posterior part grows backward to the posterior end, becomes tubular and forms the seminal vesicle, cement gland and ejaculatory duct.

13. The bursa is formed from the walls of the posterior end of the male which become very much inflated, and the spicules develop from secretions of a group of spindle-shaped cells which are early differentiated in the posterior region.

14. In the development of the female reproductive system the ovary, oviduct, seminal receptacle, uterus and the anterior part of the ovijector arise from the differentiation of the genital primordium and are therefore called internal sex-organs, while the vulva, vagina and posterior part of the ovijector arise from invagination and differentiation of subcuticular cells of the posterior end and are therefore called external reproductive organs.

15. After the genital primordium has migrated backward to a position on the ventral side just in front of the anus, it elongates very greatly and grows forward. The anterior part remains as a solid mass of cells and differentiates into the ovary. The rest of the primordium becomes tubular and differentiates into the oviduct, seminal receptacle, uterus and ovijector.
16. A group of cells just in front of the rectum and just over the posterior part of the genital primordium increases in number, invaginates, becomes differentiated into a tube which joins with the posterior part of the genital primordium. This tube differentiates into the vulva and vagina. Where it joins the posterior end of the internal reproductive organs there is an overlapping so that the posterior end of the ovijector has a double origin.

REFERENCES.


Veglia, F. (1915). The Anatomy and Life History of the *Haemonchus contortus* (Rud.). *3rd and 4th Reports of the Director of Veterinary Research, Department of Agriculture,* Union of South Africa, pp. 349-47, 22 pls.


DESCRIPTION OF PLATES VII—XII.

All figures were drawn with a camera lucida from living material.

ABBREVIATIONS USED.

agp, anterior part of genital primordium of male; *be,* buccal-cavity; *bs,* bursal space; *cg,* cervical gland cell; *cg,* cervical glands; *cmg,* cement gland; *cs,* cells which form spicules; *dl,* dorsal lobe of bursa; *e,* egg cell; *ed,* ejaculatory duct; *eg,* cells of esophageal glands; *ep,* excretory pore; *ev,* excretory vesicle; *eso,* beginning of external sex-organs; *gpp,* genital primordium; *iv,* cells of intestinal valve; *lb,* lateral band; *n,* nerve ring; *neg,* nuclei of cervical gland; *oj,* opening of ejaculatory duct; *oj,* ovijector; *ov,* oviduct; *oxy,* oviduct; *pv,* posterior-anal cell; *ppa,* pulvillus post-analis; *poj,* posterior division of ovijector; *peo,* posterior end of ovary; *pdr,* posterior degenerating region of genital primordium; *ppp,* posterior part of genital primordium of male; *puc,* posterior wall of cloaca; *r,* rectum; *rc,* cells of rectal ligament or sphincter; *rm,* rectal membrane; *rms,* retractor muscles of spicules; *rs,* space surrounding rectum; *s,* spicules; *sr,* seminal receptacle; *sv,* seminal vesicle; *t,* testis; *u,* uterus; *vd,* vas deferens; *vy,* vagina.

PLATE VII.

Fig. 1. Rhabditiform larva just after hatching, 15 hours after culturing in liquid medium.

Fig. 2. Rhabditiform larva, partly developed, two days after culturing in liquid medium.

Fig. 3. Full grown rhabditiform larva, just before first moult, with cells of the intestinal wall beginning to divide.

Fig. 4. Larva preparing for the first moult, division and degeneration of outer layer of intestinal cells complete.
PLATE VIII.

Fig. 5. Filariform larva, just before casting of the cuticula in the first moult.
Fig. 6. Mature filariform larva in infective stage.
Fig. 7. Larva in the first parasitic stage, 23 hours after infection in the lung of experimental rat.

PLATE IX.

Figs. 8–16 of Plate IX and Figs. 19 and 21 of Plate X show the development of the external sex-organs—bursa, spicules, etc.—of the male.

Fig. 8. Posterior end of male in the first parasitic stage 23 hours after infection.
Fig. 9. Posterior end of male in the first parasitic stage 30 hours after infection.
Fig. 10. Posterior end of male in the first parasitic stage 40 hours after infection.
Fig. 11. Posterior end of male during the second moult 48 hours after infection.
Fig. 12. Posterior end of male in the beginning of the second parasitic stage, 62 hours after infection.
Fig. 13. Posterior end of male in the second parasitic stage 70 hours after infection.
Fig. 14. Posterior end of male in the second parasitic stage 80 hours after infection.
Fig. 15. Posterior end of male in the second parasitic stage 90 hours after infection.

Figs. 8–15 represent optical sections through the middle of the posterior end of the males.

Fig. 16. Surface view of posterior end of male in the second parasitic stage 90 hours after infection.
Fig. 17. A portion of the body of a male, 60 hours after infection, showing the genital primordium divided into two distinct parts.

PLATE X.

Fig. 18. Male in the second parasitic stage 72 hours after infection.
Fig. 19. Posterior region of male in the second parasitic stage, 96 hours after infection.
Fig. 20. A portion of the body of a male, 108 hours after infection, showing the development of the vas deferens, seminal vesicle and cement glands.
Fig. 21. Posterior end of male at the beginning of third parasitic stage 120 hours after infection.

PLATE XI.

Figs. 22–31 show the development of the external reproductive organs of the female.

Fig. 22. Posterior end of female in the first parasitic stage 23 hours after infection.
Fig. 23. Posterior end of female in the first parasitic stage 35 hours after infection.
Fig. 24. Posterior end of female during the second moult 48 hours after infection.
Fig. 25. Posterior end of female at beginning of the second parasitic stage 55 hours after infection.
Fig. 26. Posterior end of female in the second parasitic stage 60 hours after infection.
Fig. 27. Posterior end of female in the second parasitic stage 70 hours after infection.
Fig. 28. Posterior end of female in the second parasitic stage 76 hours after infection.
Fig. 29. Posterior end of female in the second parasitic stage 85 hours after infection.
Fig. 30. Posterior end of female during the third moult 100 hours after infection.
Fig. 31. Posterior end of female at the beginning of the third parasitic stage 120 hours after infection.

PLATE XII.

Fig. 32. A small portion of the body of a female in the second parasitic stage 82 hours after infection showing part of the ovary and seminal receptacle.
Fig. 33. A portion of the body of a female in the second parasitic stage 120 hours after infection showing the formation of the oviduct.
Fig. 34. A portion of the body of a female in the second parasitic stage 120 hours after infection showing the oviduct more advanced in development than in Fig. 33.
Fig. 35. A portion of the body of a female in the adult condition 140 hours after infection showing the whole reproductive system at this stage.
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ON THE LARVAL DEVELOPMENT OF *DACNUSA AREOLARIS* NEES (BRACONIDAE), A PARASITE OF PHYTOMYZINAE (DIPTERA), WITH A NOTE ON CERTAIN CHALCID PARASITES OF PHYTOMYZIDS.

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(With 5 Text-figures.)

INTRODUCTION.

In the summer of 1921, the Wild Angelica (*Angelica campestris*) in the neighbourhood of Cambridge was infested by a leaf-mining fly, *Phytomyza angelicae* Zett. At the end of May and beginning of June, about 60 per cent. of the larvae collected were found to contain an endoparasitic Braconid, *Dacnusa areolaris* Nees. No other internal parasite was present, a fact which simplified working out the development of this form.

I must express my best thanks to Prof. J. Stanley Gardiner, who gave me facilities for carrying out the work in the Zoological Laboratory at Cambridge; and my obligations to Messrs J. E. Collin and G. T. Lyle, who kindly identified examples of host and parasite respectively.

NOTE ON THE BIOLOGY OF THE HOST.

The larva of *Phytomyza angelicae* mines into the leaves of *Angelica campestris*, forming irregular discoloured blisters on their surface. Usually each mine contains a single larva, but occasionally, when the blisters become confluent, two or three larvae may be found together. When fully fed, the larva leaves the leaf by a slit on the undersurface of the blister, and falls to the ground where it pupates. The imagos usually emerge 19–20 days later, but the pupal period may be prolonged up to 25 days.

In captivity, pairing and oviposition took place a few hours after emergence, and the flies lived only one or two days.

THE DEVELOPMENT OF THE PARASITE.

The Phytomyzid is liable to attack by *D. areolaris* only in the earliest larval stages, when less than 0·5 mm. in length, and older larvae seem to be immune.

When introduced into a tube with suitable material, the female Braconid eagerly explored the upper surface of the leaf until she located a suitable host.
under the epidermis. The actual insertion of the egg apparently presented some difficulties, and the ovipositor was half inserted and withdrawn many times. Occasionally two parasites attacked the same larva, and jostled and thrust each other aside in their efforts to oviposit. If the blister was cut open, the Braconid would sometimes slip inside it, but in such cases she ignored the object of her search which lay exposed before her, and ran out again immediately to probe for it through the epidermis.

Larvae which had already been parasitised were never attacked, and in the considerable quantity of material examined two parasites were never found in the same host. Epiparasitism\(^1\) (double infestation) does not seem to occur in this species.

The egg, when first laid, is an oval body somewhat pointed at the anterior end (Fig. 1); but, as segmentation proceeds, it increases in size and becomes more spherical. It lies freely in the haemocoele, generally in the posterior third of the body, but occasionally may be found near the head. The material available was insufficient for the study of the embryology of the parasite. The earliest larval stage, which appeared in every larva found parasitised in the field, is that shown in Fig. 2. The larva, transparent and vermiform, lies slightly curved to the ventral side, in the cavity of a hollow sphere of flattened cells, the "trophic membrane," which completely envelopes it. The body consists of thirteen segments, each of which from the second to the twelfth bears dorsally a few short spines. The thirteenth segment, which is blunt and rounded, is provided with a semicircle of stouter setae, arranged fanwise round the anus. The larva is able to move about within the trophic membrane. The cells of the latter, though polygonal in surface view, are flattened and crescentic in section, and towards the end of embryonic development their nuclei appear in various stages of degeneration. This cellular sphere is enclosed in a fine structureless membrane.

In this condition the parasite remains until the host larva is fully fed and falls from the leaf. Examples examined twelve hours after this event show some increase in the size of the parasite, which half fills the cavity of the membrane. The latter becomes attenuated and is pierced by the dorsal and caudal spines. It ultimately ruptures in the region of the mouth of the parasite, which now begins to feed, and the mesenteron, filled with ingested matter, is clearly visible through the transparent tissues.

About 36 hours after the host has formed its puparium, the parasite throws off the trophic membrane, and with it the first larval skin, both of which soon disappear among the surrounding histolysed tissues.

In the succeeding stages the parasite larva is a semi-transparent, smooth, apodous form, slightly curved to the ventral side, and without appendages. As it feeds it increases rapidly in size, and, about a week after the first moult, having demolished the viscera of the Phytomyzid, it comes forth from the empty skin and lies free within the puparium.

\(^1\) The writer first suggested and defined this term (1922) in Proc. Camb. Philos. Soc. xxi. 27.
The fully-fed larva is creamy, white and opaque, with a cuticle studded with minute scales or spines. The mandibles are small, blunt and unidentate.

_Dacnusa areolaris_ Nees. Fig. 1. The egg at oviposition. ×350. Fig. 2. The larva shortly before rupture of the trophic membrane. ×100. Fig. 3. The full grown larva. ×30. Fig. 4. Buccal armature of the full grown larva. ×350.

Young _Eulophinid_ larva. Fig. 5. ×150.

The labrum, which projects prominently, bears two pairs of papillae. Three similar processes occur on each maxilla, and the labrum is likewise furnished
Dacnusa areolaris Nees, etc.

with a pair. There are now nine pairs of open spiracles on segments 2, 4–11, and, in addition, a closed spiracular trunk on segment 3 (Fig. 3).

The internal structure approximates closely to the type of hymenopterous larvae in general, and calls for no particular comment.

_Dacnusa areolaris_ undergoes metamorphosis within the host's puparium, and no cocoon is woven. The period of pupation is usually about two weeks. The development of the parasite is however very closely correlated with that of the host, as may be seen by the accompanying table:

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<td>&quot; 10</td>
<td>July 1</td>
<td>July 1</td>
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All the puparia collected on one day were set apart, and the emergences of hosts and parasites noted separately. It will be seen that the development of the parasite from the first moult to the emergence of the imago is one or two days longer than the puparial period of the host. In the first collection, where the host’s emergence was delayed for five days, the emergence of the parasite was correspondingly deferred.

The imagos lived for five or six days in captivity, when fed on sugar and water. It is possible that this is a further correlation between parasite and host, for, as previously remarked, the life of the latter is short, and oviposition takes place soon after emergence. In parasitised populations, the flies thus emerge and oviposit a day or two before the Braconids appear. Hence there will be a sufficiency of hosts by the time the parasites are ready to deposit their eggs.

**GENERAL REMARKS.**

The genus _Dacnusa_ was named by Haliday in 1839, although certain of the species now included in it were described earlier by Nees von Esenbeck. Marshall (1885) in his Monograph describes 29 forms, but he remarks that some of these are ill-defined, and that their systematic position requires further investigation. The species whose hosts are known have all been reared from Phytomyzid or Agromyzid larvae.

Other Braconidae whose life cycles have been described include a few species which are endoparasitic in dipterous larvae.

Keilin and Picado (1913) published an account of the larval forms of _Diachasma crawfordi_, parasitic on the fruit fly _Anastrepha striata_ Shin.

Pluvinel (1914) observed the development of _Adelura gahani_, a parasite of a Phytomyzid.

Alston (1920), in a detailed bionomical study, described the larva of _Alysia manducator_ Panz., a parasite of the blow-fly.

The larva of _D. areolaris_, with its trophic membrane, much resembles that of the Chalcid, _Smicra clavipes_, figured by Henneguy (1892), and endoparasitic
in *Stratiomys strigosa*. In comparing the present species with the Braconidae-Flessili ventres, such as *Aphidius*, in which a trophic membrane is also formed round the embryo, there appears to be a tendency to lengthen embryonic at the expense of larval development. Thus in *Aphidius*, the membrane is cast off early, and the candate larva moults twice before assuming the typical grub-shaped form; whereas in *D. areolaris* the larva begins to ingest food while still within the membrane, and after throwing off the latter and the first larval skin together, it at once appears in the typical form. Fig. 2 shows the only stage found in a large number of Phytomyzid larvae of all ages which were examined; and this suggests that, as the parasite oviposits when the host is very young, development before the metamorphosis of the latter does not proceed beyond a certain limit, although there is some increase in the size of the embryo, proportionate to the growth of the host.

This adaptation may be correlated with the very complete metamorphosis of the host. In the early stages, the only nourishment taken is that which transfuses through the trophic membrane, but when the host forms its puparium, the parasite begins to ingest the surrounding tissues, and the mid-gut is filled with food. The mandibles of the hymenopterous parasites are better adapted for prehension than for mastication; and *Aphidius* and others break down the host’s tissues by a kind of “external digestion.” But if parasites of holometabolous insects can in any way delay growth until the metamorphosis of the host takes place, the organs of the latter will of themselves disintegrate and undergo profound physical and chemical changes, providing what is presumably a suitable pabulum for the further development of the parasite. Unfortunately the literature of insect parasitism throws little light on this subject, but it has been suggested by me elsewhere (1921) that the early form of *Charips*, a Cynipid endoparasite of *Aphidius*, may be a kind of resting stage.

It is obvious that it would be to the advantage of the parasite that the host should attain the full size before death or metamorphosis, in order that plenty of food should be available for its own subsequent development. The ectoparasitic Chalcids mentioned below probably rely partly on bacterial action for the disintegration of their food, and begin to feed immediately after hatching, no matter what the size of the host may be. As a result, there is considerable diversity of size of the imagos, and this may be suggested as the cause of the occasional disability of the parasite to complete its transformation.

This diversity of size, owing to variability of nutrition, may lead to far-reaching results.

Keilin (1915) observed that the size of the imagos of *Pollenia rudis* Fab. is determined by the dimensions of the host, or by its accidental death before the parasite larva is full fed; and he points out that if the difference in size should make mating impossible between large and small forms, distinct races might arise within the species. He cites the observations of Pantel, according to which the Tachinid, *Meigenia floralis*, typically a parasite of *Crioceris*...
Dacnusa areolaris Nees, etc.

(Coleoptera), occasionally attacks another and larger larva of the same group (? Timarcha). The flies bred from the latter host are so much larger than the type, that they have been described as a variety, Meigenia floralis var. major. Inversely, examples of Thrixion halidaynum, reared from a small phasmid, are smaller than the type, which is parasitic upon a larger species of the same genus.

This segregation could become permanent only if one form is structurally or physiologically compelled to oviposit in a host resembling that from which it was itself reared. Otherwise its offspring would revert to the grand-parental type.

In the Phytomyza + Dacnusa complex, the correlation between the cessation of growth and metamorphosis of the host, and the inauguration of post-embryonic development of the parasite, insures not only that the latter shall be provided with sufficient food to carry it through its transformation, but also that the imago shall approximate to the mean size of the race.

NOTE ON CHALCID PARASITES OF PHYTOMYZA ANGELICA.E.

At the beginning of August, collections of Phytomyza angelicae showed that while there was still considerable infestation by D. areolaris, 80 per cent. of the fly larvae had been parasitised or epiparasitised, as the case might be, by certain ectoparasitic Chalcids. Dr Luigi Massi of Genoa kindly examined examples of the three forms obtained, and referred them to the genera Chrysocharis, Eulojphus or Hemitarsus of the sub-family Eulophinae, some of which are known to be parasites of Phytomyzinae.

The larvae feed externally upon the host, and are of the usual type of Chalcid larva. The body is 13 segmented, and the head is furnished with two tactile processes. At first there are four pairs of open spiracles, but when fully fed there are nine, namely on segments 2–10 (Fig. 5).

As development proceeds, the larvae increase in size, but do not change materially in form. The host dies soon after the parasite has begun to feed, and in five or six days only the empty skin is left, together with the calcospherites, or crystalloid concretions of certain cells of the fat body. These Chalcids weave no cocoon, but undergo metamorphosis within the blister on the leaf. The pupal period is about four weeks.

SUMMARY.

1. Dacnusa areolaris Nees is a parasite of the fly Phytomyza angelicae Zett.
2. The egg is laid and development takes place within the body of the host.
3. A membrane of trophic cells is formed, within which the embryonic and first larval stages are passed.
4. The further growth and development of the parasite are delayed until after metamorphosis of the host, probably to ensure sufficient food in the later stages.
5. The length of the larval and pupal life of the parasite is intimately correlated with the puparial period of the host.

6. It is suggested that this is an adaptation to ensure that an adequate supply of host larvae shall be hatched by the time that the parasites are ready to oviposit.

7. Certain Chalcididae of the genera *Eulophus* and *Chrysocharis* are ectoparasites of *Ph. angelicae*, and a summary of observations on their life cycle is given.

REFERENCES.


NOTES BEARING ON DUFOUR, VON SIEBOLD AND GOODSIR, WHOSE PORTRAITS APPEAR IN PARASITOLOGY, XIV, No. 2.

PORTRAIT-PLATES XV—XVII.

(Continuing the series begun in Vol. XIII.)

BY GEORGE H. F. NUTTALL, F.R.S.

(From the Molteno Institute for Research in Parasitology.)

Jean-Marie-Léon Dufour.

1780–1865.

(Portrait-plate XV.)

Léon Dufour was born 11 April, 1780, at Saint Sever (Landes) and died there 18 April, 1865. He was a medical man and a naturalist. He took his M.D. at Montpellier in 1806. From 1806 to 1814 he served in the army, then turned his attention to entomology and botany, but in 1823 he took part in the Spanish Campaign as an army doctor. He distinguished himself especially through his work on the anatomy and physiology of arthropods and upon the habits and metamorphosis of insects, but he also wrote on botany, agriculture and meteorology. He was elected to the Académie des Sciences and was the first Frenchman upon whom that body conferred the Cuvier Prize (1861). During 1811–1864 he published 232 papers on entomology, the relation of insects to plant diseases, parasites of insects, parasitism, Protozoa and Helminths.

His parasitological papers relate to Ascaris lumbricoides, Hippobosca equina (1825) and H. camelina n.sp. (1858), Ornithomyia biloba n.sp. (1827–1845), parasitic insect larvae (1828), Gregarina ovata n.g., n.sp. (described as a "worm" in the insect’s gut, 1828), Nycteribia (1831), Pteroptes vespertilionis n.sp. (1832, a mite), Nematodes and Gregarines parasitic in Orthoptera and Hymenoptera (1836–7), Cecidomyia spp. parasitic on briars, various gall-producing insects (1837–64), Ceratopogon (1845), parasitism (1851), flea cocoons (1861).

For Biography see La Grande Encyclopédie (Paris), xv. 14; Vapereau’s Dictionnaire des Contemporains (Paris, 1865), p. 562, from which sources I have drawn; Albertus (1888), Un médecin naturaliste en province, Léon Dufour (Paris), Gaz. Méd. de Paris (1888), 7 s. v. 157 et seq.; Caffe’s Nécrologie in Journ. d. conn. méd. prat. (Paris, 1865), xxxii. 205, also "Un Savant," etc. written by his sons (1884–6), Gaz. d. Hôp. Paris, LVII–LIX (very lengthy,

**Carl Theodor Ernst von Siebold.**

1804–1885.

*Portrait-plate XVI.*

Carl von Siebold was born 16 February, 1804, at Würzburg, and died 7 April, 1885, in Munich. He belonged to a family of whose members a number were distinguished medical men. He matriculated at Berlin as a medical student in 1823, studied also in Göttingen (1824–27) and in 1828 took his M.D. in Berlin, where he fell under the influence of Rudolphi and Ehrenberg. He practised medicine for some years at Heilsberg, East Prussia, whence he proceeded to Danzig where he spent six years (1834–40) and devoted much of his time to zoological studies and the collection of Insects and Helminths. In 1836 he discovered ciliated epithelium in man when examining an extirpated nasal polyp. He studied the biology of a variety of animals, including Gregarines and Helminths. In 1840 he was called to Erlangen as Professor of Zoology, Comparative Anatomy and Veterinary Medicine. In the article "Parasites" in R. Wagner’s *Handwörterbuch* (1844) he writes of Cestodes and Cystici being stages in the life-history of one animal as exemplified in the case of *Taenia crassicollis* of the cat and *Cysticercus fasciolaris* of rodents. In the first part of his *Lehrbuch* (1845) he established the class *Protozoa* which he distinguished as unicellular animals; this book was subsequently translated into French (1849) and English (1854). In 1845–49 he was Professor of Zoology, Comparative Anatomy, Physiology, and Special Physiology at Freiburg, where, with Albert von Kölliker, he founded the *Zeitschrift für wissenschaftliche Zoologie* (1848). He succeeded Purkinje as Professor of Physiology and Director of the Physiological Institute in Breslau but remained there only a few years (1850–53) before he finally attained a suitable goal by proceeding to Munich. Here he taught Physiology and Comparative Anatomy as professor until in 1856 his chair of zoology was established. Whilst in Breslau, following the initial lead of Küchenmeister into the field of experimental helminthology, von Siebold, beginning in 1852, carried out extensive feeding experiments which showed that *Cysticercus* and *Echinococcus* gave rise to Cestodes in animals fed with them; his work on the subject was finally brought together in his publication *Ueber die Bandwürmer und Blasenwürmer* (Munich, 1854), wherein he cites Haubner and Leuckart as having proved the converse,
namely, that Cestodes give rise to *Echinococcus* and *Cysticercus*. In Munich he published a number of papers on parthenogenesis and general zoological subjects but retained his interest in parasitology, at times reverting to matters of which he had treated long before: *Syngamus trachealis* (1835, 1867), *Nematodes* in insects (1842–58), *Mermis, Gordius*, etc. (1845, 1856), Cestodes, *Echinorhynchus* and the parasitic insects *Ornithobia* (1845) and *Liptotena* (1845, 1850), *Pentastomum* (1856). His laboratory facilities in Munich were poor, this doubtless leading to his devoting much time to museum work which checked his original scientific activities and in the opinion of his biographer, Ehlers, proved the truth of Schiödt's criticism of Claparède that "Les musées pèsent lourdement sur la science.”

The work of von Siebold, whilst not highly original, was marked by pains-taking industry, minute care and sound critical judgment. He laid stress on the need of studying the living animal and in this respect his work recalls that of Spallanzani, Réaumur or de Geer. Among his pupils were Theodore Bilharz, Ferdinand Cohn, Ernst Ehlers and Elie Metchnikoff, to mention but a few. He was the recipient of many honours which will be found enumerated in the *Almanach d. baier. Akad. Wiss.* 1884, p. 130.


**John Goodsir.**

1814–1867.

(Portrait-plate XVII.)

John Goodsir, the anatomist, was born 20 March, 1814, at Anstruther, Fifeshire, and died 6 March, 1867, of spinal disease in Edinburgh, where he lies buried beside his friend Edward Forbes (1815–54) in the Dean Cemetery. He studied at St Andrews and matriculated at Edinburgh (1830) where he became a Licentiate of the Royal College of Surgeons in 1835, Curator of the Museum of the College (1841) and afterwards (1844) Demonstrator of Anatomy. He became Professor of Anatomy at Edinburgh (1846–1867) and was elected Fellow of the Royal Society.

The value of his original work was widely recognized, a striking tribute being that of Rudolf Virchow who dedicated the first edition of his *Cellular Pathology* (1859) to Goodsir "as one of the earliest and most acute observers of cell-life both physiological and pathological.” Goodsir wrote on the develop-

1 A granite obelisk erected on the spot was engraved with a curved line symbolic of "the law of the vital force.”
THE LIBRARY
OF THE
UNIVERSITY OF ILLINOIS
CARL VON SIEBOLD

1804—1885
THE LIBRARY
OF THE
UNIVERSITY OF ILLINOIS
ment of teeth (1839) and for a period worked as a dentist; he also wrote on embryology and zoology.

His discovery of *Sarcina ventriculi* (1842) aroused much interest in the medical world; he founded the genus and species on material obtained from a case of pyrosis or "waterbrash" lasting nearly two months in a youth of 19. He practically cured the case by altered diet, exercise, and creosote per os which checked the fermentation in the stomach. He wrote but few papers on helminthology (*Distomum hepaticum*, *Gymnorchynchus horridus* Goodsir (1841) and various Cestodes (1844)), and studied potato blight (1846).


(1844). "Of the anatomy and development of the Cystic Entozoa," a paper read at the York Meeting of Brit. Assoc. and reprinted (1868) in *Memoirs* (v. supra), pp. 476–503 with pls.; this paper is of historical interest since it refers to his meeting with Johannes Müller, Kölliker, etc. His papers on general medical subjects are listed in *Index Catal. Libr. Surg.-Gen.* Washington, 2nd s. vi. 365, and his botanical work is referred to by Balfour (1866–7, v. supra).
A CONTRIBUTION TO THE KNOWLEDGE OF THE
HIPPOBOSCIDAE (DIPTERA PUPIPARA).

By G. F. FERRIS, M.A. AND F. R. COLE, M.A.,
Stanford University, California.

(With 20 Text-figures.)

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INTRODUCTION.

The approach to the study of the ectoparasitic flies of the pupiparous family Hippoboscidae has heretofore been almost entirely by way of the conventional methods of the Dipterists. In other words, descriptions have been based almost exclusively upon pinned specimens. Consequently these descriptions have, for the most part, considered only the conventional subjects of colour, wing venation, size, shape, form of the claws and a few of the more obvious chaetotactic characters. A few descriptions have been based upon alcoholic specimens and these descriptions are decidedly better than those based upon pinned specimens, although they still leave something to be desired.

It is probable that the greater part of the specimens of Hippoboscids in collections are of the pinned type and it is the belief of the present writers that however satisfactory such specimens may be in the case of representatives of the other families of Diptera they are not adequate for the proper study of the Pupipara. In nearly all the members of this group the abdomen is so soft that it becomes greatly shrivelled in pinned specimens and practically none
of its characters can definitely be determined. A review of the existing descriptions shows that in almost all cases this portion of the body has been passed over with little or no mention. Yet an examination of the material available to us has shown that the abdomen frequently presents characters that we believe to be of very great usefulness.

We are consequently presenting this paper more as a contribution to the methodology of the study of the Hippoboscidae than for any other reason, although in its inception it was intended merely for the description of a few new and little known species. We are taking advantage of the opportunity to figure and give notes upon all the species that are available to us for study.

**Technique.**

In the course of its development the study of practically all the ectoparasitic groups of insects has passed through certain fairly well-defined stages. Beginning with pinned material, even in those groups the members of which are the most fragile, it has progressed through various types of inadequate preparations resulting finally in the development of a specialized technique for the production of microscopic preparations in the making of which a considerable degree of care and skill is necessary.

In the preparation of slide mounts of Hippoboscids we have utilized the following procedure. In the case of winged specimens the wings are detached and mounted directly. The body is boiled in a 10 per cent. solution of caustic potash until the contents are entirely liquefied. It is then transferred to water, judiciously placed slits are made in it and the contents carefully pressed out. In the case of these insects this is a bit difficult because of the highly developed network of tracheae within the body. The specimen is then transferred to 95 per cent. alcohol for a few minutes, then to carbol-xylene and then mounted in balsam. In most cases it is desirable to support the cover-glass on bits of broken glass in order to avoid distortion of the specimen.

There are certain very definite disadvantages in this method. It is rather difficult to avoid the accidental removal of more or less of the vestiture of setae and some distortion of the body is almost inevitable. The development of a certain amount of skill on the part of the preparator, however, will reduce these disadvantages to a minimum. We cannot consider that objections to the method based solely upon the necessity for acquiring this skill and the time consumed are valid. The histologist does not complain of the time spent in acquiring the skill in technique that is necessary for the accomplishment of his aims.

A further objection that the colours of the specimen are lost by this method of preparation is in part valid. However, what we have seen has not led us to accord any special respect to colour as a taxonomic criterion in this group and in any case colour notes can be taken before the specimen is prepared. The general colour, whether light or dark, can be determined well enough from the mounted specimens as can the colour of setae.
Hippoboscidae (Diptera Pupipara)

Figures.

Very few illustrations have been employed in describing members of this family and such as have been given have usually been of but little value to the systematist. In fact the majority of the existing figures can only be described as crude. A most gratifying exception to this rule is that of the figures given by Massonat (1909) in an extended paper on the family. Yet even these figures, although they are probably the best that have been given by any author, are not as detailed and careful as is desirable, too little attention having been given, for instance, to the chaetotaxy, which in some genera is a matter of considerable importance.

The figures that we are presenting have for the most part been made from carefully corrected camera lucida sketches. We have endeavoured to put into them all the structures visible in the specimens, but this ideal cannot entirely be realized, it being impossible to show some of the pleural structures of the thorax. We have endeavoured also to attain a degree of accuracy that will at least come within the probable range of variation. All the figures have been checked over by both authors.

In the case of figures that are divided, the left half represents the dorsal aspect, the right half the ventral aspect. We believe that the many advantages of this type of figure far outweigh any possible disadvantages and are sufficient to overcome any possible objections on the ground that the figures are not artistic.

Acknowledgments.

The material upon which this paper is based has come from various sources. We are especially indebted to Mr E. P. Van Duzee, Curator of Entomology of the California Academy of Sciences, for placing at our disposal the Hippoboscidae in the collection of that institution. The Department of Entomology of the University of California, through Prof. S. B. Freeborn, has loaned material of the genus Lipoptena. Certain specimens were taken some years ago by the senior author from skins in the collections of the United States National Museum and the Field Columbian Museum and acknowledgments are due to the authorities of these institutions for this privilege. Finally, to Major E. E. Austen, of the British Museum, we are indebted for specimens of Lipoptena cervi and Ornithomyia lagopodis.

Genus Lipoptena Nitzsch.

The material of this genus that is before us represents five species, one of which is apparently new. In addition to notes on specific characters we are enabled to add also some information concerning the larvae.

Specific characters.

It appears from our material that excellent specific characters are to be found in the genitalia of the males, which can be seen to advantage only in
cleared specimens; in the chaetotaxy of the head and thorax; in the form of the first abdominal sternite and in the form and arrangement of the tergal plates of the abdomen. There is evidently a certain amount of variation in the chaetotaxy but not enough to be especially disturbing. The presence or absence of an apical seta or setae on certain of the tibiae is evidently a valuable specific character in some instances.

The volant individuals, that is those taken before the wings have been dropped, differ so markedly from those that have dropped the wings and become distended by full feeding that at first difficulty was experienced in correlating the two forms. The abdomen in the volant individuals is so small and contracted that the distribution of the setae can not be determined with accuracy. The tergal plates are not entirely defined, the diverging dorsal lines seen in *L. depressa*, for instance, not appearing at all. Reference to the chaetotaxy of the head and thorax, however, has been sufficient to permit the definite placing of all examples of this sort that we have seen.

**Sexual dimorphism.**

In pinned specimens of the ordinary type it is very difficult to distinguish between the sexes, in fact most authors appear not to have attempted any such distinction. It seems to have been supposed by some that the males retain the wings. Massonat (1909, p. 59) has pointed out that this is a mistake and we are entirely in accord with his views, for, in our material of *L. depressa* and *L. subulata*, both sexes appear without the wings. It is probable that this mistake has arisen simply from a failure to distinguish the sexes. Correlated with this error, some authors appear to have thought that the slender-bodied volant individuals must be males. For example, one such individual received, through the kindness of Major Austen and by him labelled as a male, is in reality a female.

It is true that in the male the abdomen appears never to attain the size that it does in the female, but otherwise there is little but the presence of the genitalia by which to distinguish them, at least in all the species we have examined except *L. cervi*. We have not seen the male of this species, but according to Massonat there is a decided difference in the form and arrangement of the abdominal plates. The external genitalia of the males at the most consist of a pair of small, ventral processes, and in some of the species even these are lacking. The internal structures show very plainly in cleared specimens and permit no possibility of error as to the sex.

**Larvae.**

Larvae of two species, *L. depressa* and *L. mazamae*, were found within the bodies of females. The number of specimens (one of each species) is not sufficient to permit any extensive study. Nevertheless, certain interesting facts are revealed.
In both of these larvae the posterior portion of the body is heavily chitinized. There are no distinct spiracles, the place of these being taken by a large number of small, pore-like openings which communicate with tracheal branches, the arrangement being somewhat like that of the polyneustic lobes described by Newstead as occurring in the larva of *Glossina*. In the larva of *L. depressa* (Fig. 2 A) these openings are very numerous, are arranged in two general series and appear to occur on both the dorsal and ventral sides of the body. In that of *L. depressa* they appear to be very few and confined to one side of the body, but our single specimen is in too poor condition for study to permit a definite determination of the condition.

*Lipoptena depressa* (Say).

Figs. 1, 2 B, 2 D, 2 F.


Previous Records. Originally described by Say from *Cervus virginianus*, without indication of locality. This is probably some sub-species of the deer now known as *Odocoileus americanus*, which ranges throughout a large part of North America east of the Rocky Mountains. Recorded by Coquillet from "black tailed deer" (*Odocoileus columbianus*) from Humboldt County, California.

Speiser has placed *L. mazamae* Rondani as a synonym of this, but, as will be pointed out below, we regard this as an error.

Specimens Examined. Numerous examples from sub-species of *O. columbianus* from the following localities in California: Humboldt County; Gualala and Laytonville, Mendocino County. Also from "deer" at Deer Park, British Columbia, Canada, and specimens taken in flight at Sobre Vista and on Mt Wilson and from unstated locality in California.

Notes. The original description of Say contains little of value except the statements that there are "two impressed lines from the base to the margin beyond the middle" of the dorsum of the abdomen and that the venter of the abdomen has an "arquated series of spines near base." These characters will permit the positive separation of the species from *L. subulata* Coq. but not from more closely related forms such as *L. mazamae* Rondani. We present the following notes on the species, indicating the most salient characters.

The sexes are very similar except for the characters associated directly with the genitalia, and we are figuring the female only (Fig. 1).

Head above almost destitute of setae. There is some variation, some specimens showing but a single pair of large setae on the front, others showing two pairs and a few smaller setae, but in no case are these setae to be described as numerous. The front (as in most Hippoboscids) shows a median area or frontal vitta which presents a roughened appearance and the posterior margin of which bounds a semicircular area about the ocelli. The form and extent
of these areas appear to afford specific characters. In *L. depressa* the frontal vitta is rather short and broad and the ocellar area correspondingly larger. The ventral side of the head bears a few small setae.

*Thorax* with a row of large but slender pre-alar setae; with an irregular series of smaller setae across the mesonotum; with a group of three post-alars and a group of three or four pre-scutellars on each side; with a median pair of scutellars. On the ventral side both meso- and meta-sterna are thickly beset with short, stout setae. The legs present no special characters except that the anterior tibiae are without an apical seta on the inner margin and that the claws are equal (Fig. 2 D).

*Wings* (Fig. 2 B) weakly veined as is characteristic of the genus, vein *R*$_{4+5}$ (the third vein of Dipterists) with a stigma-like expansion at the margin.

*Abdomen* with a conspicuous pair of diverging lines on the dorsum, these lines forming an inverted V and attaining the lateral margins slightly behind

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Fig. 1. *Lipoptena depressa* (Say), female.
the middle. The spaces between these lines and the lateral margins tend to be more or less chitinized and these areas are beset with numerous setae. Behind these lines the dorsum is membranous except for two small, transverse, pre-apical sclerites, and bears but a few scattered setae.

On the ventral side the basal sternite is quite heavily chitinized and is divided by a deep posterior emargination into two pronounced lobes the tips of which are free. The entire sternite is beset with numerous stout setae. The remainder of the venter is quite thickly beset with small, slender setae.

Genitalia of the male (Fig. 2 F) without external processes, the position of these (b) indicated merely by two or three small setae. We are not prepared to discuss the homologies of the internal parts—to do so would necessitate a separate study for which we scarcely have the material—and must content ourselves with a general description. They consist of a pair of stout, chitinized

Fig. 2. Lipoptena mazamae Rondani: A, posterior extremity of larva showing spiracular openings; E, genitalia of male. Lipoptena depressa (Say): B, wing; D, anterior tarsus; F, genitalia of male. Lipoptena subulata Coq.: C, anterior tarsus. Lipoptena traguli n. sp.: G, genitalia of male.
processes (a) attaching basally to a large apodeme or basal plate. Between these two processes is a ring-shaped piece (c) which extends forward on to the basal plate and terminates distally in a small point. Within this ring-like piece are some ill-defined structures that probably represent the true penis.

_Lipoptena mazamae_ Rondani.

_Figs. 2 A, 2 E._


**Previous Records.** Known only from the original records, from _Cervus mexicanus_, Central America.

**Specimens Examined.** Three males and two females from skin of a deer, _Mazama_ sp., Yacuiba, Bolivia, in the collection of the Field Columbian Museum.

**Notes.** Speiser (ref. cited) has placed this species as a synonym of _L. depressa_, but if our identification of the species be correct, this is decidedly in error, the genitalia of the males being so different that the two certainly cannot be the same species.

In general appearance _L. mazamae_ is very similar to _L. depressa_, so much so, in fact, that the figure given for the female of the latter will apply almost equally well for the former. There are slight differences in the arrangement of the setae, but no more than might be included within the possible range of variation. The presence of a stout apical seta on the inner margin of the anterior tibia will permit the separation of _mazamae_, however. The genitalia of the latter (Fig. 2 _E_) are very different, being not only relatively but actually smaller than in _depressa_ and having the inner ring-like piece (c) bluntly rounded at the tip.

_Lipoptena traguli_ n. sp.

_Figs. 2 G, 3._

**Specimens Examined.** Five individuals, the holotype, a female, and one female paratype from _Tragulus subrufus_ Lingga Id., China Sea; the allotype and one male paratype from _T. rufus_ Tsangku Id.; and one female paratype from _T. rubeus_ Pulo Bintang, Rhio Archipelago. All the specimens are from skins in the National Museum and a paratype will be deposited in the collection of that institution.

The hosts are members of the family _Tragulidae_, the “mouse deer.”

**Female** (Fig. 3). Length (on slide) 2:75 mm. General colour, pale brown or yellowish.

_Head_ with narrow, elongate frontal vitta; the ocellar area much reduced; the front almost destitute of setae, those which may be present rather small; ventral side with but few setae.

_Thorax_ dorsally with but few setae; pre-alars slender; two pairs of slender pre-scutellars and two pairs of scutellars, the outer pair of the latter small.
Hippoboscidae (*Diptera Pupipara*)

Sternum thickly beset with small, stout setae. Anterior and middle tibiae with a single, stout inner apical seta; the posterior tibiae with two or three such setae; claws of each pair of equal size. Wings and halteres broken off in all the specimens examined.

*Abdomen* with the dorsum marked by two diagonal lines which diverge from the base to the lateral margins well toward the apex, the base of each of the lateral areas thus delimited with a more or less circular, more heavily chitinized area, the whole sparingly beset with rather large setae. Remainder of the dorsum practically destitute of setae and membranous except for a small pre-apical plate. Basal sternite but slightly emarginate, thickly beset with small, stout setae and with a few longer setae. Remainder of the venter with numerous small, slender setae.

**Male.** Length (on slide) 2.25 mm. In general very closely resembling the female. *Genitalia* (Fig. 2 G) with a pair of short external lobes (*b*) which are beset with small setae; internally with the inner ring-like piece (*c*) very sharply pointed at the apex.

![Figure 3: Lipoptera traqui n. sp., female.](image-url)
Notes. While in general this species quite closely approaches *L. depressa* it differs in numerous small details including the presence of a stout apical seta on the anterior tarsi and in the character of the genitalia.

*Lipoptena subulata* Coquillet.
Figs. 2 C, 4.


Fig. 4. *Lipoptena subulata* Coq., female.

Previous Records. From "deer," Woodstock, New Hampshire, U.S.A.
Specimens Examined. Males and females from *Odocoileus columbianus*, Humboldt and Mendocino Counties; from a locality not stated; and specimens taken in flight on Mt Wilson; all from California.
Notes. The original description contains nothing of value in aiding to identify the species except the statement that there is “a stout black spine at the apex of the inner side of the front tibiae,” which is sufficient to distinguish the species from *L. depressa*. We are simply assuming that our specimens represent this species.

The species presents a wealth of structural characters by which it is distinguished from any others that we have examined.

*Head* beset dorsally with numerous stout setae along the orbits and ventrally with numerous slender setae.

*Thorax* dorsally with a row of short, stout, but sharply pointed pre-alar setae and with numerous small setae on the mesonotum; with a group of three post-alars and three pre-scutellars on each side and with four scutellars.

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*Fig. 5. Lipoptena cervi* (L.), female.
Ventrally there are two rows of moderately large setae across the mesosternum and a single row across the metasternum. Wings as in *L. depressa*. Legs quite stout; anterior tibiae with a strong, inner apical seta; tarsi (Fig. 2 C) with one claw much smaller than the other.

**Abdomen** dorsally without the pair of diverging lines seen in *depressa* but with a pair of basal plates which bear a row of slender setae along the posterior margin. Remainder of the dorsum membranous and with quite numerous setae, except for a pair of small, pre-apical chitinized plates which bear three or four long setae. Basal sternite, rounded posteriorly, not emarginate as in *depressa*, and quite small, bearing a row of small setae. Remainder of the venter with numerous setae, those in the sub-marginal regions larger than the others.

**Male.** In general appearance closely resembling the female. External genitalia merely a pair of small protuberances bearing small setae. The internal genitalia in the two males available are not in condition to figure but appear to resemble those of *L. traguli*.

*Lipoptena cervi* (Linnaeus).

**Fig. 5.**


Through the kindness of Major Austen we have received specimens of the female of this species and are figuring it for comparison with the others here described. Massonat (ref. cited) has given a detailed description of the species, accompanied by figures which are quite satisfactory except that the details of the vestiture of setae are not indicated with sufficient clearness. According to this author there is a much greater difference between the sexes than is present in the other species here included.

**Genus Allobosca** Speiser.

The peculiar species that is the sole representative of this genus is regarded by Speiser as representing also a distinct sub-family.

*Allobosca crassipes* Speiser.

**Figs. 6, 7.**

1899. *Allobosca crassipes* Speiser, *Wien Ent. Zeitung*, xxviii. 199; Fig.

**Previous Records.** From *Propithecus diadema* and *Lepilemur mustelinus*, Madagascar. The hosts are lemurs.

**Specimens Examined.** Five females and one male from skin of *Propithecus edwardsi*, Madagascar, in the United States National Museum.

**Notes.** The original description of this species was accompanied only by a figure of the wing and we are taking advantage of the opportunity to
present figures and to add some notes. These are especially desirable as the species is one of considerable interest.

Speiser seems to have been in doubt as to the identity of the sexes, but as he suggested, the female (Fig. 6) has the apex of the abdomen quite deeply bilobed, while in the male (Fig. 7 B) it is smoothly rounded. The abdomen of the male bears a large, oval genital plate which is almost destitute of setae. In other respects it is quite as in the female. The genitalia of the male are entirely internal and it has not been possible to make much out of them from the single specimen available.
The clypeal region (Fig. 7 C) has the median portion separated by a pair of deep incisions from the lateral parts. The front tarsi (Fig. 7 D) differ from the others in the presence of short, almost spatulate setae on the first four segments. The wing (Fig. 7 A) was figured by Speiser, but for the sake of completeness we are figuring it again.

Genus *Melophagus* Linnaeus.

An apology may be deemed advisable for discussing this well-known genus but our attention has been attracted to it by the discovery of an apparently new form, and in connection with the description of this some general notes may not be out of place. Furthermore, as far as we have been able to determine, there exists no illustration of any species of the genus that is of any particular value from a systematic standpoint. The crude figures that adorn the pages of most of our textbooks of parasitology, at least, can scarcely be regarded as pre-occupying the field, for at the best they show little more than the general characters of the genus.

Massonat (1909) has discussed at some length the question of the homology of the small projection at each posterior angle of the thorax which some authors seem to have regarded as a haltere. He arrives at the conclusion that these structures are in reality vestigial wings and with this conclusion we are entirely in accord. In fact their position is such that they cannot be halteres unless a most remarkable shifting of the position of these organs has taken place. They are to be regarded as almost the irreducible minimum of wing vestiges. No trace of the halteres can be found.
**Melophagus ovinus ovinus** Linnaeus.

Figs. 8, 9 A–D.

**Previous Records.** A cosmopolitan species on domestic sheep. Speiser has described a "variety," *M. ovinus ferus*, from "Steinbok" in the Caucasus.

**Specimens Examined.** From domestic sheep in various parts of the United States.

**Notes.** The accompanying figures will form a basis for the comparison of this species with other closely related forms such as that which we are describing below. The differences will be pointed out in connection with the description of this form.

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**Melophagus ovinus montanus** new sub-species.

Figs. 9 E, 10.

**Specimens Examined.** Males only, from "mountain sheep," probably either *Ovis dalli* or *O. canadensis*, on the Alaska-Yukon boundary. The specimens, which have been in the Stanford Collection for some years, were received from the late Dr C. Gordon Hewitt.
Fig. 9. *Melophagus ovinus ovinus* L.: A, wing vestige; B, portion of anterior tarsus; C, tip of antenna; D, genitalia of male. *Melophagus ovinus montanus* n. ssp.: E, tip of antenna.

Fig. 10. *Melophagus ovinus montanus* n. ssp., male.
Male (Fig. 10). Differing from the male of *M. ovinus ovinus* (Fig. 8) in the following particulars especially. Setae everywhere tending to be larger and more numerous, the difference in size being especially conspicuous on the dorsum of the thorax; abdomen above without a bare apical space as in the male of *ovinus ovinus*; scutellum apparently lacking and no scutellar setae present, while in the typical form the scutellum, although very small, is distinct and bears a cluster of apical setae.

There appears to be a slight difference in the antennae, the typical form (Fig. 9 C) having the apical setae much longer than they are in *montanus* (Fig. 9 E). The wing vestiges, genitalia and claws seem to be the same as in the typical form (Fig. 9 A, B, D).

Notes. While these two forms are certainly closely related we cannot but regard them as worthy of distinction. The differences are small, but upon a direct comparison of specimens are sufficiently evident. A sufficient amount of material of the typical form has been examined without evidence of variation to reduce the chance that *montanus* is simply a variant.

A comparison with *M. ovinus fera* Speiser is not possible, for the description of this form consists merely of the statement that “sie ist etwas schlanker, etwas heller und etwas kleiner” than the typical form.

Genus *Olfersia* Wiedemann.

*Olfersia americana* (Leach).

Figs. 11, 12.


Previous Records. A widely distributed and often recorded species, infesting chiefly owls, in North America. Massonat (1909) has recorded the species from *Platalea leucorodia* in France, but, if we may rely upon his figures, his specimens represent an entirely distinct species.

Specimens Examined. Numerous examples from California and from Washington, District Columbia, from various species of owls.

Major Austen has very kindly compared one of our specimens with the type of this species in the British Museum and has confirmed this determination.

Notes. The accompanying figure of the female (Fig. 11) should make the recognition of this species relatively easy. It is usually a reddish-brown species, some specimens being paler than others. The antennae are comparatively small and are strongly bristled at the apex. The frontalia is quite large and the orbits are wide. The section of horizontally striated membrane down the centre of the dorsum of the abdomen is quite noticeable. The chaetotaxy and form of the male are essentially the same as in the female. The male genitalia are characteristic, consisting mainly of two slender, pointed side pieces, possibly corresponding to the superior forceps, and a long central piece which is curved at the base, rather heavily chitinized and pointed. This may be
Fig. 11. *Olfersia americana* (Leach), female, from "barn owl," *Aluco pratineola*, San Bernardino, California.

Fig. 12. *Olfersia americana* (Leach), wing. Lettering of venation according to Comstock-Needham system.
Hippoboscidae (Diptera Pupipara)

the aedeagus as there is a chitinized piece at the base, evidently the apodeme of the penis.

The hind tibiae suggest those of *Pseudolfersia spinifera*, described below, but the small tubercles are in a single row and the circular structures near them are slightly different in appearance. The posterior tarsi are toothed on the margins, but not so strongly as in *P. spinifera* and the teeth do not extend to the apex of the last segment. The outer tooth of the claws is very slender, the middle tooth distinctly wider and with a blunt, rounded tip. The arrangement of the fine setulae on the wing membranes is like that figured for *P. spinifera*, but there are not so many in the basal part of the wing and none at all in the anal region.

Our interpretation of the wing venation (Fig. 12) is in accord with the Comstock-Needham system of nomenclature. Massonat (1909) who has attempted to apply this system seems not entirely to have understood it and has arrived at results which are impossible under its application. His \( R_3 \) is in reality \( R_{4+5} \), his \( R_2 \) is \( R_{2+3} \).

**Genus Pseudolfersia** Coquillet.

*Pseudolfersia spinifera* (Leach).

Figs. 13, 14.


**Previous Records.** A tropicopolitan species occurring on a wide range of hosts including especially sea birds. Austen (ref. cited) gives a list of the species from which it has been taken and also a list of the names that have been applied to it.

**Specimens Examined.** A paratype male of *Pseudolfersia diomedeae* Coq., from *Diomedea irrorata*, Galapagos Ids.; a single male from *Fregata aquila*, Cape San Lucas, Lower California; three males from “king vulture,” Belize, British Honduras. All of these specimens from the Stanford University collection.

**Notes.** We are entirely convinced that the specimens that we have examined represent but a single species—*P. spinifera* (Leach). Major Austen has kindly compared one of our specimens from “king vulture” with the type of *vulturis* in the British Museum and has identified it as this species. Our specimen from frigate bird agrees exactly with the very good description of *spinifera* given by Speiser and as this species is known to occur characteristically on the frigate bird, we do not doubt the correctness of our determination. The distribution of the species is remarkable, but it should be borne in mind
that certain of its hosts are wide ranging forms, both as species and as individuals.

We present the following notes on the morphology of the species.

*Head* with no strong fronto-orbital bristles.

*Thorax* with the mesonotum almost bare, the scutellum with a marginal row of very small setae. Sternum practically entirely bare. The tibiae and

**Fig. 13.** *Pseudolfersia spinifera* (Leach), male. From paratype of *P. diomedeae* Coq.
tarsi show what are probably good specific characters in the arrangement of the setae and in the presence of small, tooth-like projections on the tarsi. The hind tarsi (Fig. 14 B) have a row of these teeth along each ventral margin of each segment; on the front tarsi there are but two of these teeth near the base of the last segment. The hind tibiae have, in addition to setae and small spines, a large number of small circular structures near their apices (Fig. 14 C) which are probably sensory pits.

Wings (Fig. 14 A) with the whole membrane, back to the basal cross veins, densely beset with microscopic setulae which cannot be shown in the figure; the stippled portion in the figure is so densely beset with these setulae as to appear gray, the remaining portion is yellowish and has only a thin covering of setulae.

Abdomen with the basal, chitinized portion of the dorsum extensive. The median portion of the dorsum bears a broad area of fine, dense striations and the posterior fourth of the dorsum is chitinized. The ventral side is thickly beset with fine setae.

The genitalia of the male show a pair of quite long, tapering external processes and internally a pair of pointed structures, probably the superior forceps.

The specimens from "king vulture" differ very slightly from the others, having the anal area of the wing entirely bare of setulae and the cell 2M (second basal) noticeably wider and shorter. However, the latter character is somewhat variable in the specimens examined.
Genus Ornithomyia Latreille.

Ornithomyia avicularia (Linnaeus).

Figs. 15, 16.

1909. Ornithomyia avicularia (L.), Massonat, Ann. de l'Université de Lyon, N.S. (1), xxviii. 271-78; Pl. 4, figs. 33-34.


Previous Records. A widely distributed European and North American species on many passerine and raptorial birds.

Specimens Examined. Two females, one from Keyport, Washington, without indication of host, and one from Sayornis sayi, Pacific Grove, California,
both in the Stanford University collection; one male from *Cyanocitta stelleri*, Upper Alsea River Valley, Oregon, in the collection of the junior author. This last specimen has previously been recorded by Cole (ref. cited) as *O. anchineuria* Speiser.

**Notes.** There have been numerous descriptions of this species but only that given by Massonat (ref. cited) is sufficiently precise and accompanied by such figures as to make identification definitely possible. Our specimens agree very closely with the figures given by this author, but as there are certain chaetotactic details which he has not included we are figuring it again.

Some of the chaetotactic characters are possibly generic and some are undoubtedly specific. There appears to be some slight variation in the size and arrangement of the bristles. The abdomen is densely beset with small setae (Fig. 15) and bears numerous large setae, the position of which is apparently constant. In addition to the basal plate there are on the dorsum

three small median plates (the anterior-most of which is partially concealed beneath the basal plate in the figure) and a pair of larger plates near the apex.

The figure of the abdomen of the female given by Massonat indicates that the apex of the abdomen is quite deeply bilobed, while in our specimens there is a median lobe. This lobe, however, is membranous and is probably more or less retractile, and we are not inclined to regard this difference as important. The abdomen of the male differs from that of the female chiefly in not being lobed at the apex and in having a complete, transverse, pre-apical plate instead of the paired plates of the female. The genitalia show no external processes; the internal structures consist of a pair of slender, pointed, lateral processes and a pointed median process.

The wing venation is characteristic, $R_1$ (first vein) ending considerably before the radio-medial (first) cross-vein. The fine setulae are confined to a definite area as indicated in the figure. The costa is covered with short, bristly setae, especially dense beyond the end of $R_1$. 

![Fig. 16. Ornithomyia avicularia (L.): A, wing; B, portion of anterior tarsus.](image-url)
Genus *Stilbometopa* Coquillet.

*Stilbometopa impressa* (Bigot).

Figs. 17, 18.


**Previous Records.** Originally recorded by Bigot from California and recorded by Speiser from a specimen from California.

**Specimens Examined.** A female taken from "valley quail," *Lophortyx californica*, California, in the collection of the California Academy of Sciences; one female from Mecca, California, without indication of host, in the same collection; one broken specimen of undeterminable sex from "quail," Mount Hamilton, California, in the Stanford University collection; one female, without indication of host, from San Diego, California, June, 1921.

**Notes.** Speiser (ref. cited) gives a very good re-description of this species from the type. It is an easily recognizable form of very peculiar character. It is interesting to note that the type of the genus which is the only other included species, *S. fulvifrons* (Walker), also infests gallinaceous birds, Austen having reported it from *Ortyx virginiana*, *Geotrygon sylvatica* and *G. montana*, among other hosts.

The colour of the body is evidently to some extent variable, some specimens being yellowish brown, others blackish. The setae on the legs and antennae are blackish, those on the frons and mesonotum yellowish. The face, clypeus and underside of the head are yellowish. There are few setae on the mesonotum and these are placed in definite areas as indicated (Fig. 17).

The antennae in Fig. 17 are pointing downward, their shape is better shown in Fig. 18 B.

The prothoracic spiracles are very large and heavily chitinized. The scutellum has a distinctive shape, being very broad and almost emarginate posteriorly. On each side of the thorax, just under the vestigial squamae, is a remarkable, heavily chitinized, hammer-shaped projection, that is very distinctive of the species.

The abdomen has a dense covering of short setae borne upon small tubercles, most of these setae being short and heavy. The basal, chitinized portion of the dorsum is large and the remainder is membranous except for a pair of plates near the apex. The ventral side is entirely membranous except for a pair of plates on the anterior margin of the vulva.

The ungues (Fig. 18 C) have the outer tooth much longer than the second; the few setae on the last tarsal segment are strong, a blunt seta near the base of the pulvilli being especially noticeable.

The wings (Fig. 18 A) are entirely destitute of the microscopic setulae that are present in all the other bird-infesting forms known to us. The veins near the base and anterior margin of the wing are heavy and black and there is a thick anal cross-vein (*Cu*₂).
Fig. 17. *Stilbometopa impressa* (Bigot), female.

Fig. 18. *Stilbometopa impressa* (Bigot): A, wing, lettering in accordance with the Comstock-Needham system; B, head; C, portion of tarsus.
Genus *Ornithoica* Rondani.

*Ornithoica promiscua* n. sp.

Figs. 19, 20.

Specimens Examined. Holotype, a female, in the collection of the California Academy of Sciences, taken from *Pipilo crissalis carolae*, Castle Hot Springs, Lake County, California, Sept. 23, 1920 (J. Maillard). Three female paratypes all from California; one from "fox sparrow," San Francisco, Dec. 5, 1919 (L. Little), in the same collection; one from *Regulus calendula*, Pacific Grove, Jan. 25, 1904; one from *Falco sparverius*, King's River; the last two in the Stanford University collection.

Fig. 19. *Ornithoica promiscua* n. sp., female. From the holotype.
Female (Fig. 19). Length (on slide) 2.5 mm. A yellowish brown species, the thorax darker than the legs.

*Head* with the frons probably almost parallel-sided in perfect specimens but in the holotype curving in slightly. Frontal orbits with at least three strong setae and several smaller setae. Ocellar setae small. Ventral side with a row of slender setae more or less paralleling the orbits.

*Thorax* with several short, stout, black setae on the humeral callosities and on the margin in front of the wing. Mesonotum with numerous small, pale setae, all with distinct pustulations about the base. There is a single long seta just behind the humeral callosity, two in front of the wing and one just behind the wing. Scutellum with small, pale, pustulated setae on the disc and with four long, black setae. Halteres rather small and delicate.

On the ventral side both meso- and meta-sternum are beset with numerous fine setae, mingled with a few that are small and stout.

*Wings* (Fig. 20A) with a well-defined anal cell; \( R_{2+3} \) distinctly curved toward the costa and bristly to the tip; \( m-cu \) cross-vein broken, the upper part obsolete; vein above cell \( 2M \), broken near the middle; distal half of the wing covered with microscopic setulae as shown in the figure.

![Fig. 20. Ornithoica promiscua n. sp.: A, wing; B, anterior tarsus.](image)

*Legs* comparatively strong, the anterior femora noticeably thickened; setae arranged in a rather definite fashion, as shown in the figure. Claws (Fig. 20 B) rather slender.

*Abdomen* above with a chitinized basal plate extending from side to side, with four quite large plates occupying the median half and with a small plate on each side of the anal region, the basal plate and the succeeding four with numerous small setae and the para-anal plates or their immediate region with two long setae. Lateral margins with a number of small, stout setae which are borne on tubercles. On the ventral side there is a median region beset with small setae, a region along the anterior, lateral margin with numerous setae on tubercles and a smaller region of these near the genital opening. Near the genital opening there is also a pair of small plates bearing several slender setae.

*Notes.* One other species of this genus, *O. confluens* (Say), has been recorded from North America, and, as far as we can learn, all references to this species have been based upon Say's description. We regard *confluens* as unrecognizable from this description and are therefore describing our species as new. *O. confluens* was taken from *Ardea candidissima*.

Austen (1903) states that *O. vicina* (Walker) may be a synonym of *confluens*, that *O. beccariina* Rondani from Amboina, on *Ardea alba*, is identical with...
O. *exilis* (Walker) from New Guinea, and that this too is probably the same as *vicina*.

Our species is certainly very close to *O. turdi* (Latreille) (Massonat, 1909) from Europe. Certain differences are evident on the basis of Massonat's description and figures, but it is not at all improbable that these differences might disappear upon a comparison of specimens.

Two specimens from "California russet-backed thrush," *Hylocichla ustulata*, without indication of locality, differ somewhat from the typical *promiscua*, the setae being in general fewer and smaller, approaching more closely *turdi*, but our material is not in the best of condition, and for the present we place them provisionally with *promiscua*.

REFERENCES.


THE STRUCTURE AND SYSTEMATIC POSITION OF *STRONGYLUS POLYGYRUS*.

BY C. L. BOULENGER, M.A., D.Sc.

*(From the Zoological Department, University of Birmingham.)*

(With 4 Text-figures.)

Dujardin (1845) was the first to give a satisfactory account of the small Strongylid worms inhabiting the intestinal tract of field-mice and voles. His descriptions of the various species were careful and for the most part accurate, they were, unfortunately, not accompanied by figures, and incomplete in that the characters of the bursal rays of the males were not given, an omission which has led to considerable confusion, since the modern classification of the Trichostrongylidae is based largely on the structure of the male bursa.

Of the four worms described by Dujardin, *Strongylus costellatus* has been taken as the type of the genus *Heligmosomum* Railliet and Henry (1909), to which genus his other species *S. minutus*, *S. laevis* and *S. polygyrus* were also assigned. On the authority of Travassos (1914) *S. polygyrus* has since been removed to his genus *Viannaia* by Hall (1916) who, however, remarks that the available descriptions are not sufficiently detailed to warrant the change.

I have recently been fortunate in obtaining a number of small Strongylids from the intestine of *Microtus (Arvicola) agrestis* L. from the neighbourhood of Birmingham, which I am convinced belong to the same species as that described by Dujardin as *S. polygyrus*, their study has enabled me to clear up a number of misconceptions with regard to this form.

Von Linstow (1878, 1879 and 1882) is the only authority since Dujardin who has attempted to reinvestigate the species; his account contains descriptions and figures of the male bursa, but in certain important characters the worm studied by him seems to deviate so much from the original specific diagnosis that Hall (1916) felt justified in concluding that a different form had been dealt with by the German helminthologist, and in removing it to a new genus *Heligmosomoides* under the designation *H. linstowi* Hall. Travassos (1921) accepts Hall's conclusions and in his recent monograph on the Trichostrongylidae we find *S. polygyrus* Dujardin listed as *Viannaia polygyra* and *S. polygyrus* von Linstow as *Heligmosomoides linstowi*. 
My investigations of the new material have led me to a quite opposite conclusion, and I am convinced that Dujardin and von Linstow have dealt with the same species. The specimens before me agree in all important characters with *S. polygyrus* as described by the French author; at the same time it is quite easy to reconcile my account of the male bursa with von Linstow's description and figures. The confusion has been due largely to the latter's mistake with regard to the position of the vulva of the female, but partly also to a misunderstanding of Dujardin's original description by some recent authors.

Dujardin's specific diagnosis of *S. polygyrus* clearly shows that one of the important characters of the species is the shape of the female tail, the latter being narrow, conical, truncated posteriorly and terminated by a slender, transparent spike 0.02 mm. in length; the anus is given as 0.075 mm. from the posterior extremity. The shape of the tail is therefore very similar to that found in species of the genus *Nematodirus*.

Hall (1916) has evidently misunderstood Dujardin's account, in his diagnosis he gives "Tail 20 μ long, thin, conical, diaphanous, truncated, and terminating abruptly in a narrow point. Anus 75 μ from the tip of the tail," further commenting on the fact that Dujardin evidently does not regard the length of the tail and the distance from the anus to the tip of the tail as the same thing. Dujardin's actual words are "queue amincie, conique, tronquée, et terminée brusquement par une pointe grêle, diaphane, longue de 0 mm., 02; —anus à 0 mm., 075 de l’extrémité." The measurement 0.02 mm. here clearly refers to the length of the terminal spike, not to that of the tail.

It is evident therefore that in the shape of the female tail, with its truncate extremity and terminal spike, we have an important character which distinguishes *S. polygyrus* from *S. costellatus*, the type species of the genus *Heligmosomum*. The form examined by von Linstow clearly possessed this character, the anus being described as 0.098 mm. from the end of the tail and definite reference made to a slender, terminal spike, 0.016 mm. long.

Von Linstow described the vulva as 0.24 mm. from the head end, one of the chief characters used by Hall and Travassos in separating *S. polygyrus* v. Linst. from *S. polygyrus* Duj.; this statement I think, however, should be regarded as a clerical error, *Kopfende* having been accidentally substituted for *Schwanzende*. The distance 0.24 mm. from the head end would place the vulva anterior to the termination of the oesophagus, an almost impossible situation in this group of Nematodes; von Linstow's description of the ovejector (1882) is, moreover, incompatible with such a forward position for the vulva.

The specimens obtained by me from the Birmingham voles agree with those described by Dujardin and von Linstow in so far as the shape of the tail and the position of the anus are concerned. The vulva was found to be situated 0.24–0.35 mm. from the posterior extremity.

Dujardin's account contains little useful information with regard to the
male organs, the spicules are, however, given as 0·58 mm. long, this measurement agreeing well with von Linstow’s (0·54 mm.) and that observed by me in the new material (0·5–0·58 mm.).

Von Linstow’s description and figures of the male bursa have given rise to much discussion; according to his account the bursa of *S. polygyrus* has no separate dorsal lobe and no median dorsal ray, the latter being replaced by two rays with separate origins; between these and behind the cloaca are shown seven pairs of small ray-like structures ending in papillae. The bursal characters as figured by von Linstow differ so much from those of other species now assigned to the genera *Heligmosomum* and *Viannaia* as to justify Hall’s transference of the worm to a new genus *Heligmosomoides*.

Travassos (1921) accepts the systematic position assigned to the species by Hall, but adopts a somewhat different interpretation of von Linstow’s figures of the bursa; he prefers to regard the two dorsal rays as representing the externo-dorsal rays of other species, and the true dorsal rays as being replaced by the seven pairs of small post-cloacal rays.

My material has shown that Travassos’ interpretation is the more correct. In the Birmingham specimens the male bursae bear a striking resemblance, both in shape and in the general arrangement of the ventral and lateral rays, to those figured by von Linstow. When completely spread out and viewed from the dorsal or ventral aspect (text-fig. 3) the bursa is seen to be of considerable width with an almost straight dorsal margin, without a separate dorsal lobe or marked median incision. There are two long, slender externo-dorsal rays with separate origins, and, between them, a very small median dorsal ray branching dichotomously into four delicate branches. A genital cone is well developed, bearing one or two pairs of small, but highly retractile papillae on each side of the cloaca.

In an incompletely extended bursa, as shown in text-fig. 4, the two externo-dorsal rays appear much closer together and the genital cone covers and hides the greater part of the dorsal ray, revealing only the tips of the four branches which, together with the cloacal papillae, give the appearance of a series of small rays in the neighbourhood of the genital opening.

The peculiarities of von Linstow’s figures of the bursa of *S. polygyrus* are thus readily explained; comparison of these figures (1878, Fig. 21 and 1879, Fig. 26) with my own (text-figs. 3, 4) will show conclusively that the form described here is specifically identical with that studied by the German authority.

The facts stated above make it clear that there is no justification for the separation of *S. polygyrus* Duj. and *S. polygyrus* v. Linst. It is evident from the structure of the female tail and from the peculiarities of the male bursa that the species cannot be referred to either of the genera *Viannaia* or *Heligmosomum*; Hall’s genus *Heligmosomoides* must therefore be retained, the worm, however, figuring as *H. polygyrus* (Duj.) instead of *H. linstowi* Hall.
A revised diagnosis of the genus *Heligmosomoides* is given below, together with a new specific diagnosis of *H. polygyrus* based on the material before me:

**HELIGMOSOMOIDES** Hall, 1916.

*Generic diagnosis.* Heligmosominae: Body commonly coiled in a spiral, with transverse and longitudinal striae. Male with long, filiform spicules. Bursa without separate dorsal lobe or middorsal incision. Dorsal ray very short with four small branches. The extero-dorsal rays are long and slender, with separate origins. The lateral rays arise from a common trunk and are divergent. Ventro-ventral and latero-ventral rays divergent with a common origin. Prebursal papillae long. Female with a truncated posterior extremity bearing a slender caudal spike. Vulva situated posteriorly.

Type species. *H. polygyrus* (Dujardin, 1845).

*Heligmosomoides polygyrus* (Dujardin, 1845).

*Synonyms:* Strongylus polygyrus Dujardin, 1845.  
von Linstow, 1878.  
*Heligmosomum polygyrum* Railliet and Henry, 1909.  
*Viannaia polygyra* Hall, 1916.  
*Heligmosomoides linstowi* Hall, 1916.

Small worms, reddish in colour when alive, and usually coiled in a spiral. The head, 0.04–0.05 mm. wide, is provided with a cuticular expansion, usually asymmetrical and with conspicuous transverse striae (text-fig. 2). Head papillae appear to be present, but their number and arrangement could not be definitely ascertained.

The cuticle of the body is striated both longitudinally and transversely, there are, moreover, 18–20 longitudinal crests. The transverse striae are very fine and most apparent along these crests and in the neighbourhood of the vulva of the female.

The oesophagus is short, 0.48–0.6 mm. in length. There is a small buccal cavity with chitinous walls.

**Male:** 4.5–5.5 mm. long, with a maximum thickness of 0.07–0.09 mm. in the posterior part of the body.

The bursa has a length of 0.17–0.18 mm. and a breadth of 0.3–0.33 mm. The dorsal edge of the bursa is almost straight (text-fig. 3), there is no separate dorsal lobe or median dorsal incision separating the lateral lobes. The dorsal ray is slender and very short, branching dichotomously into four small rays, the total length of dorsal ray and its branches measures 0.025–0.035 mm. In the neighbourhood of the dorsal ray the bursa is frequently transversely folded, so as to give the appearance of an accessory bursal membrane. The extero-dorsal rays are long and slender, they have
Strongylus polygyrus

separate origins and extend almost to the margin of the bursa. The lateral rays arise from a common trunk and are divergent; the postero-lateral ray is

Fig. 1. Heligmosomoides polygyrus. Posterior extremity of female, lateral view. × about 220.

Fig. 2. Heligmosomoides polygyrus. Head, with cuticular expansion. × about 650.

only slightly thicker than the externo-dorsal and runs almost parallel with it, the medio-lateral and externo-lateral rays are considerably thicker than the
Fig. 4. *Heligmosomoides polygyrus*. Male bursa, incompletely spread out; the dorsal region is folded longitudinally so as to approximate the externo-dorsal rays, the genital cone covers the greater part of the dorsal ray. × about 350.

EXPLANATION OF LETTERING.

an., anus; bu., buccal cavity; cu., cuticular expansion of head; d.r., dorsal ray; e.d., externo-dorsal ray; e.l., externo-lateral ray; g.c., genital cone; int., intestine; l.v., latero-ventral ray; m.l., medio-lateral ray; oe., oesophagus; p.l., postero-lateral ray; p.p., prebursal papilla; ra., vagina; v.v., ventro-ventral ray; vu., vulva.
postero-lateral. The ventral rays arise from a common trunk and are markedly divergent; the latero-ventral is very thick, being quite twice as broad as the other bursal rays, the tip of the ventro-ventral ray is directed towards the anterior border of the bursa. Prebursal papillae are well developed and are long and slender.

The simple, filiform spicules are 0·5–0·58 mm. long, their distal portions are united. No gubernaculum could be traced.

**Female:** 6·2–10 mm. long. The greatest width of the body is in the posterior third, where it measures 0·09–0·12 mm.; the width in the middle of the body is 0·06–0·08 mm.

The anus is 0·067–0·1 mm. from the posterior extremity; the tail is conical, truncated posteriorly, and bearing a slender, pointed terminal spike which measures 0·012–0·018 mm. in length (text-fig. 1).

The vulva is a conspicuous opening, situated 0·24–0·35 mm. from the tip of the tail. The female organs are single; the ovejector is well developed and similar in shape to that described by von Linstow (1882), the combined length of the vagina and ovejector (exclusive of anterior nonmuscular portion) is 0·45–0·55 mm.

The eggs are 0·62–0·78 mm. long by 0·035–0·045 mm. wide, they are in the morula stage when laid.

The systematic position of *S. laevis* Dujardin remains to be considered. Stossich (1899) has suggested that this is a synonym of *S. polygyrus*. According to Dujardin the two worms are very closely allied and only to be distinguished with difficulty. He specially notes that the caudal extremity of the female is similar in the two species, *S. laevis* also having the extremity truncate and bearing a terminal spike. In the absence of any description of the male bursa we have only the female characters to help us in assigning a position to this worm among the genera of Heligmosominae; these strongly suggest that *S. laevis* should be placed alongside *S. polygyrus* in the genus *Heligmosomoides*.

Seurat (1915) has published a description of a worm from *Dipodillus campestris* in Algeria which he considers to be *S. laevis*; his account shows that in this form the bursa is divided into distinct dorsal and lateral lobes, and possesses a long dorsal ray, the branches of which extend to the bursal margin; the female tail is conical with a pointed termination. If these characters are correctly interpreted, I think there can be little doubt that Dujardin's species was not dealt with.

**REFERENCES.**


A PARASITIC COPEPOD BELONGING TO THE GENUS *MEDESICASTE* (KRØYER), AND ITS RELATION TO THE TUMOURS IT PRODUCES ON THE FISH, *TRIGLA GURNARDUS*.

By W. HAROLD LEIGH-SHARPE, M.Sc. (Lond.).

(With 7 Text-figures.)

Habitat and record. The genus *Medesicaste* (Krøyer), belonging to the family Chondracanthidae, is usually considered to be represented by but one British species, *Medesicaste asellinum* (Linn.)\(^1\), whose characters are diagnosed as very variable. It is open to question whether the additional appendicular details given herein and the presence of the conjunctive tubes (*vide infra*) are sufficient to warrant the erection of a new species.

The following observations were made upon eight specimens of *Medesicaste*♀ taken from various *Trigla gurnardus* caught at Lowestoft in August, 1918. They were, without exception, upon the gills, upon which they cause characteristic tumours.

Body. The outline of the animal is best seen from Figs. 1 and 2. The cephalothorax consists of a subglobose anterior end or head, bearing the antennules and antennae, and a considerably elongated slender neck at the base of which are situated the mouth and the other cephalic and thoracic appendages, which are thus widely separated from the antennae.

The head is 1-4 mm. long, bulbous, trilobate, rounded in front, with each side expanded into a hemispherical lobe. The neck, which has its posterior portion enlarged, is 1-8 mm. long. Both head and neck as far as the mouth are embedded in the tumour which is formed upon the gill-filaments of the host.

The trunk is square in outline, 2-4 mm. long, slightly wider than long. It is divided by a deep transverse constriction into two almost equal parts; the anterior constituting the thorax bearing appendages, carrying two pairs of obtuse processes ventrally and a small pair dorsally; the posterior, the larger, the genital segment, the antero-lateral corners of which extend outwards into angular projections. The lateral margins of this posterior division are arcuate, converging distally, and forming two small postero-lateral lobes between which is a small medio-ventral V-shaped depression or sinus.

\(^1\) A full list of references and synonyms is given by Scott and Scott (1913). *The British Parasitic Copepoda*, The Ray Society, London. The only fairly good figure is that in this work, Plate LII, fig. 6.
The two ventral anterior thoracic processes curve ventrally and frequently meet. Each process bears a pair of pronounced, fleshy appendages, also curved, and always meeting, so that most specimens appear to have a fleshy loop at this point caused by the interlocking of the apical "digits."

**Fig. 1.** *Medesicaste*. Ventral aspect. a. abdomen; Ov. ovisacs; Th.Ap. two pairs of ventral thoracic appendages.

**Fig. 2.** *Medesicaste*. Dorsal aspect. Th.Ap. one pair of dorsal thoracic appendages.

The abdomen is very small, bi-articulated, the posterior segment being the larger, and enclosed between the postero-lateral lobes. There are no abdominal appendages.
The *ovisacs* are 4.4 mm. long, longer than the trunk, of a dark brown colour, and show externally six rows of ova, 31 in a row.

The total length of the preserved animal is about 10 mm. A fully developed female is found embedded in a relatively small tumour, has a larger trunk, bears two ovisacs, and has no trace of conjunctive tubes.

The **appendages**, all paired, none of them setigerous, are, in order, as follows (Fig. 3): The *antennules* are minute, two-jointed, the joints not making any angle with one another. The terminal joint is rounded. They are situated dorsally and are barely half the size of the antennae. The *antennae* (Figs. 3 and 4) are ventral, two-jointed, short and stout. The terminal joint, which is much the longer, terminates in a powerful, incurved, sharp-pointed claw. The *mandibles* are falciform as is usual in this family, instead of styliform as in the Lernaeopodidae, broad at the proximal end, and tapering to a more or less attenuated distal extremity. Both margins appear minutely serrated under a low magnification, but in reality the teeth, which resemble prickles,
are set at right angles to the undersurface of the lamina, and are marginal only at the apex, while they form a loop and do not approach the margin at the base. Towards the convex edge of the lamina the number of teeth is twenty; along the concave edge they are more numerous.

There follow two pairs of appendages of uncertain homology, variously styled *maxillae* or *maxillipedes*. They are both on the same plan, two-jointed, there being in each case a stout basal joint, with a bulbous distal joint, terminating in a long straight style. The anterior pair is distinguishable by having

![Diagram](image)

**Fig. 4. Medesicaste.** The anterior end, with the antennae (A2) in the erect position.

![Diagram](image)

**Fig. 5. Medesicaste.** The posterior end. a. abdomen; m. muscle; V. vulvae; S. spermathecae; C.T. conjunctive tubes.

the inner margin of the style strongly serrated (Fig. 3, 1 max.). There are six large, stout spines away from the apex which interlock in a scissor-like manner with those of its partner. The *thoracic appendages* have already been described (v. supra); their surface is covered with numerous very minute prickles.

The most striking feature, now for the first time described, is the presence of the **conjunctive tubes**. These occur in three of the specimens, which are immature, bear no ovisacs and are embedded in relatively large tumours.
These tubes are paired, for although only one is preserved entire in each of the specimens, the broken end of the second is present in the best specimen (Fig. 6). The tubes are attached to the vulvae of the female and communicate directly with the spermathecae, the ovisacs issuing forth dorsally at another aperture. The reproductive system is on the same general plan as I have described for *Lernaeopoda* (*Parasitology*, VIII. 269 and Fig. 6), the ovaries occupying the arcuate lateral portions of the genital segment.

A most remarkable phenomenon is that the other end of the tube, which is long, free, flexible and sinuous, enters the upper portion of the same tumour as that in which the head and neck of the female are embedded, where it bifurcates; hence the term "conjunctive" (Fig. 6). The tube is one third the width of a gill filament, and about 12 mm. long.

The tumour produced by the parasite is structureless and is to be regarded as a hypertrophied gill filament. It may be irregular, but is usually clavate,
with or without a constricted neck, about seven times the breadth of a normal gill filament. No doubt the hypertrophy was occasioned by the penetration of the free-swimming nauplius larva into the tissues of the gill filament.

At the proximal bifurcation of the conjunctive tube, deep within the tumour, are the faint traces of what appear to be the remains of the cuticle of a dead and disintegrated male, the only certain detail of which is the rounded dorsum (Fig. 7). If this be proved, the tubes might be definitely called conjugation tubes, exhibiting an occurrence wholly unique. During the past four years I have examined some hundreds of gurnards without finding Medesicaste, neither has that genus been taken at the laboratory of the Marine Biological Association, Plymouth, though specially sought at my request.

![Fig. 7. Medesicaste. An enlarged view of the entrance and bifurcation of a conjunctive tube (C.T.) in the tumour (T). ♂ remains of male animal.](image)

**SUMMARY.**

A tumour among the gill filaments of *Trigla gurnardus* bears a female *Medesicaste* with the head and neck buried in its apex, and a male *Medesicaste* completely embedded in its base. The two sexes of the parasite are connected by a conjugation tube, *external* to the tumour, down which tube the spermatophores presumably pass. The appendages of the copepod are described.

My thanks are due to Michael G. L. Perkins for furnishing me with the specimens, and to Miss Edith C. Humphreys for drawings of the specimens and preparations which are all mounted unstained in Farrant's medium.
PARASITISM AND SYMBIOSIS.

(A REVIEW.)

Parasitology, owing to its economic importance, has received very great attention during the last 50 years and several good text-books have been devoted to this subject. In almost all recently published books, however, stress has been laid chiefly upon the economic side of the problem: medical, veterinary, agricultural, etc., but the scope of Parasitology is by no means restricted to the problems economically connected with man. We know now that there is hardly any group in the animal or vegetable kingdom which does not harbour parasites. The latter themselves do not escape this rule and are often parasitised in their turn. Moreover, the interrelations between living organisms are not restricted to parasitism only; there are, in fact, all shades of transition between parasitism on the one hand, and commensalism and symbiosis on the other. These interrelations have never been adequately dealt with in text-books of biology or parasitology and all the information concerning them is scattered in a great number of papers dealing with various biological or parasitological problems. This gap has been recently filled by a small volume issued by Prof. M. Caullery [of the "Laboratoire d'Evolution des Étres Organisés de la Sorbonne, Paris] under the title Le Parasitisme et la Symbiose in which the author has incorporated a series of lectures delivered by him on this subject during the academic year 1919–1920. The book deals with the most interesting cases of commensalism, parasitism, and symbiosis and with various important cases of transition between these three great ethological groups.

In discussing parasitic adaptation, the author has examined not only the classical cases of parasitism found in the text-books, but he has also included many interesting examples of other parasitic groups such as Crustacea, Polychaeta and especially Mollusca which show a remarkable series of transitions between ecto- and endo-parasitic life. The author summarises also the results of investigations on several helminths, i.e. Schistosoma, Dibothriocephalus and Ascaris, whose life-histories have been successfully worked out during the last few years.

Special prominence is given to a series of problems of general parasitology: (1) parasitic adaptations, (2) the influence of the parasite upon the host, especially parasitic castration, (3) reaction of the host (phagocytic reaction and elaboration of antiferments, formation of galls, etc.). The last part of the book deals with symbiosis. In the chapters discussing symbiosis in plants, the author lays special stress on the very important work of Noël Bernard upon the symbiosis existing between the Orchidae and Fungi. In dealing with symbiosis in animals, apart from classical examples, attention is given to symbiosis between Insects and yeasts and the intracellular symbionts in Cephalopoda. Portier's views on the dualism of a living cell and the relation between the symbionts and mitochondria are submitted to a searching criticism. Prof. Caullery concludes that there is complete lack of evidence in support of this theory as we know now that mitochondria are characterised by a very low resistance while the symbionts cultivated by Portier appear to be highly resistant. The author finally refers to recent work on the aseptic breeding of various animals, and especially Insects. This short review only faintly indicates the various problems of general parasitology dealt with by the author.

The book will serve as a well of information for teachers who will find in it ample illustrations of all kinds of interrelations between living organisms; it should stimulate original research and enable workers in different fields of parasitology to coordinate the results of their investigation with those already obtained and generalised in this most interesting volume.

D. KEILIN.

ON THE MORPHOLOGY AND LIFE HISTORY OF A MYXOSPORIDIAN, LEPTOTHECA OHLMACHERI, PARASITIC IN RANA CLAMITANS AND R. PIPIENS¹.

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(With Plates XIII—XX, containing Figs. 1—111.)

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INTRODUCTION.

In 1893 Ohlmacher observed spores of a Myxosporidian in the tubules of kidneys of Bufo lentiginosus. Whinery (1893) followed Ohlmacher with a further study on the Protozoan in the same host species. Both investigators obtained their material from De Kalb County, Illinois, U.S.A. They did not notice the vegetative form, and confined themselves solely to the descriptions of the spore. Gurley (1893), according to Whinery, named it Chloromyxum ohlmacheri after examining Ohlmacher's preparations. The same author (Gurley, 1894) summarized the results obtained by Ohlmacher and Whinery, and recorded the Myxosporidian as Chloromyxum (Sphaerospora) ohlmacheri. Thélohan (1895) gave a new specific name, Leptotheca ranae, to a Myxosporidian found in the kidneys of Rana esculenta and R. temporaria without giving any description or figure except its habitat². Labbé (1899) placed the first-named species in the genus Leptotheca, and thought that it was identical with L. ranae.

The description and figures given by the first two American investigators show clearly that the shell-valves of the spore are finely striated, which fact had never been seen in any other species of the genus Leptotheca. Moreover, it is exceptional to find one that infects an amphibian host, because all

¹ Contributions from the Zoological Laboratory of the University of Illinois, No. 201.
² Thélohan does not mention the locality. Judging from the other species described in the paper, the host animals were most probably collected in France.
the other species of the genus were found in marine fish. In the monograph on Myxosporidia, I (Kudo, 1920a) have, therefore, placed the species provisionally in the new genus *Wardia* on the basis of these peculiar characters which fit better to that genus than to *Leptotheca*.

Recently I have observed a Myxosporidian which, I believe, is identical with *L. ohlmacheri*, in the kidneys of *R. clamitans* and *R. pipiens*. The vegetative forms as well as spores at various stages of development were so abundant in numbers and the conditions were so favourable that I have undertaken an extensive study on the morphology and the development of the organism. The study is without significance in view of the circumstance that none of the Myxosporidia parasitic in batrachian hosts, which are listed in my monograph (Kudo, 1920a), has been examined in detail by the previous authors. It has been found that *L. ohlmacheri* possesses all the essential features characteristic of the genus *Leptotheca*, except that the spore membrane is striated and it occurs in amphibian hosts. The interesting character of the spore is that it contains two uninucleate sporoplasms which are independent and remain separated from each other. The development of the trophozoites, especially the process of gemmation occurring in the present species, stands sharply in contrast with those of other species observed recently by Georgévitch (1916, 1917 and 1917a), Davis (1916), Erdmann (1917) and Stempell (1919). Thus the study has revealed more or less interesting facts of general importance concerning this group of Protozoa.

The purpose of this paper is to present the observed facts, their interrelations, and the necessary reference to other disporous or mictosporous Myxosporidia. The discussion concerning spore formation in two vastly different groups of Myxosporidia, one infecting the tissues of the host and producing large cysts and the other living in the organ cavities of the host, was entirely omitted because of the recent appearance of papers (Erdmann, 1917, 1917a, and Stempell, 1919) and also of my belief that what is at present known of different species of Myxosporidia is yet too small for this purpose.

**MATERIAL AND METHODS.**

The *Rana clamitans* Latreille studied by me were collected in small creeks in Spring Valley, New York, during the summer of 1920. The *R. pipiens* Schreber were bought from a Chicago supply store and their locality is unknown, except that they were collected from various places in Iowa, Minnesota and Wisconsin. Further consideration upon the occurrence and the relation between the Myxosporidian and the host animals will be given later.

After studying the external characters, each frog was etherized and dissected. Smears of portions of the liver, spleen, blood, gall bladder, muscle, kidney, urinary bladder, reproductive organ and wall of the digestive tract, were studied in physiological solution. The majority of *R. clamitans* have shown several protozoan blood parasites in the circulatory system. In no
case, however, was the gall bladder found to harbour any Myxosporidia. The same has been true with other organs except the kidney. The observations on fresh smears were further confirmed in stained smears and sections.

*L. ohlmacheri* was only found in the kidneys. Isolated spores were also found in the ureters, cloaca and urinary bladder of the host whose kidneys were heavily infected.

In order to get accurate knowledge of the organisms in the fresh state, large numbers of hanging drop preparations were made by mixing fresh spores of the Myxosporidian with the fluids taken from the stomach, small and large intestines and the urine of *R. pipiens*, physiological solution, and several diluted solutions of pepsin hydrochloric acid, lecithin and sodium glycocholate.

At the same time, many smears were made on slides from the kidneys, infected as well as normal. As my experience taught, in preparing the smears, it is very advantageous to have an extremely thin and a more or less thick region of film on each slide. In the former part, the number and division of the nuclei of trophozoites can be distinctly seen, while in the latter portion the general appearance, shape and size of the trophozoites can be clearly recognized.

The rest of the organ was fixed *in toto*, and sectioned. The serial sections varied from 2 to 5µ in thickness.

The fixatives employed are Schaudinn’s fluid, Flemming’s weak solution and Bouin’s mixture, as used in my previous studies except the last named one (Kudo, 1916, 1917, 1920a and 1921). The first mentioned fixative proved to be most satisfactory. The methods of staining are the same as I have used before, *i.e.* Heidenhain’s iron haemotxylin, Delafield’s haematoxylin and Giemsa’s stain. The advantage of mounting Giemsa-stained smears or sections in cedar oil for immersion, after dehydrating by acetone and acetone-xylol of different proportions, is noticeable compared with those mounted in the neutral Canada balsam or any other media. According to my experience, typical Giemsa staining in sections as well as smears, has been preserved perfectly in cedar oil for more than three years while those in neutral balsam have hardly lasted over six months.

As to the extrusion of the polar filament, I have recently discussed this elsewhere (Kudo, 1921a). It was easily induced by means of potassium hydrate or mechanical pressure. Moreover, a weak solution of sodium glycocholate gave good results in five to ten minutes in hanging drop preparations.

The gastric as well as intestinal fluids were collected by means of fine capillaries from the digestive tract after the frog was dissected. As the manipulation was done in November and December, the quantity of fluids obtained were usually so small as to suffice only for two or three preparations.

For artificial infection *per os*, small pieces of infected kidney or of a sterilized cork which was made porous previously by means of a needle, and immersed in an emulsion of fresh spores in physiological solution, were fed to the frog.
THE AMOEGBULA.

When fresh spores are brought into contact with the gastric fluid of the frog either in a hanging drop preparation or in the host’s body, the following changes take place. The two sporoplasms increase gradually in size, and seem to fuse into a single mass along the surface of contact (Fig. 1). The amoebula shows very slow amoeboid movements, and appears to press the polar capsules against the anterior margin of the spore membrane (Figs. 1–3), which results in the extrusion of the polar filaments through the foramina. This condition was noticed only in gastric fluid or weak pepsin hydrochloric acid. In all other cases where the extrusion of polar filaments took place under the influence of reagents, such as potassium hydrate, lecithin or sodium glycocolate, the extrusion was more quickly brought about than in the case mentioned above, and no visible change occurred in the sporoplasms.

A similar movement of the amoebula inside the spore membrane prior to its emergence, seems to have been noticed by Auerbach (1910) in the case of Myxidium bergense.

After some time, the shell-valves become separated first at the anterior half (Figs. 4, 5) and then along the entire sutural plane (Figs. 6, 7). The amoebula was frequently seen making its way through this opening to the outside of the spore. The movements are very slow, yet can distinctly be followed in hanging drop preparations. When kept at 20° to 25° C., numerous amoebulae and empty spore membranes were noticed in the preparations after 6 to 24 hours. Similar changes to those stated above, were further noticed in spores treated with a mixture of gastric fluid and bile, and with the fluid taken from the duodenum. But no change was visible in the spores mixed with the fluids taken from the large intestine and with the urine. On the other hand, the action of weak pepsin hydrochloric acid upon the spores was similar to that of gastric fluid. The physiological solution and solutions of lecithin and sodium glycocolate did not cause any further change than the extrusion of the polar filaments.

The liberated amoebula moves about very slowly. Its nuclei cannot distinctly be made out in the fresh state, although not infrequently they were seen as faintly outlined bodies (Figs. 3, 5). The cytoplasm does not show any distinct differentiation either in the fresh state or when stained. In the fresh state, it appears to be composed of homogeneous substance with three or more spherical granules of variable size. Figs. 8 and 11 show most probably one and the same amoebula. In this preparation, five spores were mixed with a drop of the gastric fluid after separating them from the others by means of serial dilution. The amoebula shown in Fig. 11 was moving about sluggishly after leaving the spore membrane. The cover glass was fixed and stained. Judging from its position upon the stained preparation, the amoebula shown in Fig. 8 is, in all probability, identical with that shown in Fig. 11.
The nuclei in the amoebulae could only be studied in stained preparations. Staining shows that the nuclei of the two sporoplasms remain independent in the amoebula after the fusion of the cytoplasm, although they are found lying side by side (Figs. 4, 7).

Further observation on the amoebula could not be made either in hanging drop preparations owing to bacterial contamination, or in the animal body, although the sectioned stomach and intestine of four host animals which had been fed with the infected kidneys or cork, were carefully studied. In one amoebula shown in Fig. 9, which has been seen in a hanging drop preparation after 22 hours, the two nuclei were lying together, indicating that in the next stage a complete fusion of the two nuclei might occur.

Apparently, stages nearest to this were observed in the space between the glomerulus and Bowman’s capsule of Malpighian bodies and in the uriniferous tubule below the Malpighian body of infected kidneys of *R. pipiens*. Quite frequently stages such as shown in Figs. 12–17 were observed in these parts of the kidney, although none were found to be in the epithelial cells of the tubule or in the surrounding connective tissue of the kidney. I consider the stage shown in Fig. 12 as that in which the nuclei of the amoebula are completing the fusion and those shown in Figs. 13–17 as those in which stages of schizogony of the young uninucleate amoebulae are represented. Since I have not seen the schizogony in the fresh state, I cannot state definitely that it occurs. Judging from the number of the parasites found, I am, however, inclined to think that schizogony also takes place beside the process of gemmation which occurs at a later stage of development.

The young uninucleate form is rounded with deeply staining reticulated cytoplasm and a large nucleus. At first the nucleus seems to be rich in the nuclear sap and to be surrounded by a somewhat indistinct membrane. The chromatic substance is collected in one place in the form of a karyosome (Figs. 17, 18). Owing to the variable shapes it assumes (Figs. 18, 19), the young parasites seem to undergo amoeboid movements. The cytoplasm is uniformly reticulated, often showing more or less large vacuoles (Fig. 19). As the body grows, the nucleus undergoes changes. The nuclear membrane becomes now distinctly visible and the karyosome more compact. The achromatic network, on which chromatin granules are scattered, becomes clearly observable (Figs. 19, 20).

The germination of the myxosporidian spores has undoubtedly been one of the important objects of study to investigators. I shall quote some records on the subject in species of similar habitat. Auerbach (1910) figured the separation of the shell-valves of a spore of *Myxidium bergense* which had been introduced into the intestine of one of its hosts, *Gadus virens*, and further stated that when spores were taken from the stomach and mixed with a drop of the host’s bile on a slide, the polar filaments became extruded, and that “nach 20 Min. bis ½ Stunde kann man auch leere Schalen finden, ein Zeichen, dass die Amöboidkeime ausgekrochen sind.” Davis (1916) observed that
the shell-valves of the spores of *Sinuolinea dimorpha* became separated from each other along the sutural line, and that when spores were subjected to the action of the fluid taken from the pyloric caeca of the host fish, the amoebulae emerged "in five to fifteen minutes" through the openings. Georgévitch (1917a) stated that he observed a similar separation of valves in the spore of *Ceratomyxa coris*. On the other hand, Erdmann (1912) describes an apparently abnormal occurrence in a spore of *Chloromyxum leydigi*. By adding to the spores placed on a slide first gastric fluid and then the bile of the host fish, she noticed the liberated binucleate amoebulae in the mixture, and stated that "die Sporenwände reissen (Fig. 8) nicht in der Nahtlinie, sondern in einer um 90° zu ihr geneigten Ebene." The mentioned figure does not contain any of the four polar capsules, and may be interpreted as a case of separation of the valves along the sutural plane.

Concerning the nuclear changes prior to and after the emergence of the amoebula, there are two views. According to Auerbach (1910, view 1) and Stempell (1919), the two nuclei of the sporoplasm fuse into one prior to the emergence of the amoebula. According to Auerbach (1910, view 2), Erdmann (1912 and 1917) and Georgévitch (1917 and 1917a), however, the fusion of the two nuclei takes place after the emergence of the amoebula. The nuclear changes in the present species agree with the latter view.

Whether or not there is an intracellular stage in a Myxosporidian, the trophozoites of which live in the organ cavity of the host animal, has been the subject of study of several writers. The fact that Myxosporidia living in organ cavities of the host, may invade the tissues under certain conditions, was demonstrated recently by two examples. Debaisieux (1918) noted that *Myxidium lieberkühni* Büttschli which is a well-known parasite in the urinary bladder of *Lucius lucius*, invades not only the lumina of the ureters and uriniferous tubules, but also the glomerulus of the Malpighian body of the kidney of the host and develops into large cysts. I have also seen a similar case in *Mitraspora elongata* parasitic in the kidney of *Lepomis cyanellus* (Kudo, 1920a). Several investigators suggested the possible occurrence of an early intracellular stage in the Myxosporidia occurring in the organ cavities of the host animals.

Not in many cases was this stage actually recognized. Regarding the young intracellular stages of *Chloromyxum protei* parasitic in the uriniferous tubule of *Proteus anguinus*, Joseph (1907) makes the following statement: "Ich fand ausgesprochene Jugendstadien anfangs regelmaessig nur in den Zellen der flimmernden Anfangsteile der Nierenkanälchen, also in den Nephrostomial- und den Nebenkanälchen im Sinne der Nomenklatur von Felix." Auerbach (1910) observed in *M. bergense* and *Zschokkella hildae* that the uninucleate amoebula enters into the epithelial cell of the gall bladder or of the proximal portion of the gall duct of the host fish, although its significance was unknown.

Examination of a large number of sections of kidneys infected by *Leptotheca*
ohlmacheri to a varying degree, has failed to reveal any evidence which might prove the occurrence of an intracellular stage.

THE TROPHOZOITE.

The nucleus in the young uninucleate form undergoes division. Its karyosome becomes larger and less dense (Fig. 21). It divides into two equal parts. The daughter karyosomes move toward the opposite ends of the nucleus. Frequently a deeply staining chromatic strand exists between them for some time (Fig. 23). At first, the reticulated achromatic network does not show much change, but later it becomes more densely accumulated at the equatorial plane together with the chromatin granules (Fig. 23). The nucleus finally divides into two along this plane and the two daughter nuclei may remain attached to each other (Fig. 24). At the end of the division, the two nuclei seem to be similar in structure. Changes in appearance, however, take place soon afterward. One nucleus (a) shows a large karyosome which is usually attached to the nuclear membrane and a very fine achromatic network, thus resembling in appearance that of the uninucleate form before division. The other nucleus (b), however, shows a decrease in the size of the karyosome compared with the former and chromatin granules become scattered over the coarse achromatic network (Fig. 25). The former (a), as its later history indicates, is the vegetative nucleus, the latter (b) is generative. The two nuclei show a striking difference especially when stained with Giemsa's solution: the vegetative nucleus assumes a light pink colour with a light bluish karyosome, while the chromatin granules scattered on the achromatic network and the karyosome of the generative nucleus stain a deep red.

The generative nucleus undergoes further division without any resting period. The division takes place in a similar manner as was stated above for the first division, preceded by the division of the karyosome and chromatic network (Figs. 26–28). Fig. 29 shows two views of a trophozoite in which the two daughter generative nuclei have just completed the division. In well-made smears, the distance between these two nuclei is much smaller than that between the vegetative nucleus and either one of them. Figs. 30, 31 and 41–43 show typical trinucleate trophozoites.

In all known cases, the earliest phase of the trophic life is the uninucleate form. Concerning the formation of the binucleate form, there are two views. According to Auerbach (1910) in the development of *M. bergense*, two uninucleate forms come in contact and one of the copulants casts off a part of the nuclear substance, thus forming a binucleate form with nuclei dissimilar in size. On the other hand, Davis (1916), Georgévitch (1917a), Erdmann (1917) and others, maintain that the nucleus of the uninucleate form produces those of the binucleate form by division. Of these latter authors, Georgévitch thinks that the division is unequal and forms a smaller generative and a larger vegetative nucleus. Davis, however, observed equal division so that the daughter nuclei were of “equal size and similar appearance.” In *Leptotheca*
ohlmacheri, the two nuclei, as before stated, are usually of similar size, but assume soon afterward strikingly different aspects.

Formation of trinucleate trophozoites similar to that described above, was observed in Sinuolinea dimorpha (Davis, 1916), and Ceratomyxa coris (Georgévitch, 1917a). Stempell (1919), however, believes that the trinucleate trophozoite of Ceratomyxa (Leptotheca) coris is formed directly from a uninucleate form by a heteropolar nuclear division. This controversy, arising from the observations of Georgévitch and Stempell on apparently one and the same species, is beyond my understanding. But in L. ohlmacheri, I have not seen a single instance which might suggest the occurrence of the process described by Stempell in the above-mentioned species.

Concerning the relative number of trophozoites with one, two and three nuclei respectively in the infected host organs, Georgévitch (1917a) and Stempell (1919) noticed that the number of trinucleate trophozoites of Ceratomyxa coris, were much greater than any other stages. In this connection, Georgévitch states that “ce stade assez fréquent dans nos préparations indique un stade de repos.” On the other hand, Stempell attributes the reason to the above quoted heteropolar division of the nucleus of the uninucleate form.

In L. ohlmacheri, the number of trinucleate trophozoites is far greater than that of uninucleate or binucleate forms. In my opinion, this is due to the following two reasons. In the first place, this stage is most probably one of growth. As was noted in every one of the infected kidneys, the body of the trophozoites at this stage varies greatly in size which is reasonably explained by regarding it as a growth stage. Secondly, as will be stated later, young trinucleate trophozoites are constantly produced by the process of gemmation in the later stage.

The karyosome in the vegetative nucleus of a trinucleate trophozoite is frequently divided into two (Fig. 33), one of which remains in the vegetative nucleus. This gives rise to another generative nucleus (Fig. 34) which divides into two (Figs. 35, 36). At the same time, these become surrounded by more deeply staining cytoplasm, and separated from the main part of the cytoplasm by a clear space. One of the nuclei, becomes the vegetative nucleus, while the other divides once more, thus forming a trinucleate form, with one vegetative and two generative nuclei. The trinucleate body is extruded from the mother trophozoite. I have repeatedly seen gemmation in hanging drop preparations, the time needed for the completion of the process varying from 30 minutes to 3 hours at 20° to 25° C. The extrusion may in some cases be much delayed (Figs. 39, 40). The liberated trinucleate stage is essentially the same in the nature of the nucleus with that of the mother trophozoite, mainly differing in size of the body (Figs. 37–40). The body of the gemma now grows and repeats the same process. One gemma is formed in a trophozoite at one time. Whether or not one individual undergoes repeated gemmation cannot definitely be stated. But judging from the large number of the trinucleate stage present in every case, I am inclined to think
that the gemmae are formed repeatedly one after another in one individual. Frequently one of the two generative nuclei, instead of developing into a spore, forms a gemma (Fig. 38). This appears to occur near the end of repeated gemmation. In such cases, after the liberation of the gemma, the mother trophozoite contains one spore and the vegetative nucleus (Figs. 38, 86).

Davis (1916 and 1917) observed the formation of several gemmules in the polysporous trophozoites of *Sinuolinea dimorpha* and *S. capsularis*. In regard to the gemmation of the first named species, Davis writes as follows:

Occasionally a degenerative cell is seen, in which division of the nucleus is not followed by a corresponding division of the cytoplasm. Successive nuclear divisions follow in rapid succession until eight nuclei are formed, all enclosed in a common cytoplasmic mass (Figs. 67 to 71). Meanwhile, the entire structure increases considerably in size, forming a very characteristic rounded body, sharply marked off from the surrounding endoplasm (Figs. 59, 60, 69 and 70). These bodies are probably homologous with the pansporoblasts but have a very different history from the ordinary structures of that name. They are, in reality, similar to the gemmules formed by many species of Protozoa.

Although many other authors record the occurrence of plasmotomy in various species of Myxosporidia (Kudo, 1920a), the discussion of which is beyond the scope of the present paper, the above quoted observation of Davis deserves consideration here. Yet the two types of gemmation differ in several respects. In the first place, the character of the sporulation in the trophozoites differs in each case—the one is polysporous (*S. dimorpha*) and the other disporous (*L. ohlmacheri*). In the disporous form of *S. dimorpha*, Davis did not see the process of gemmation. Secondly, the nature of the nuclei in the gemma and the number of gemmae formed at one time differ in the two forms. In this connection, it may be interesting to note that although the authors did interpret their observations in different ways, a study of their figures reveals cases in which gemmation similar to that of the present species may occur. For examples, the trophozoite of *Ceratomyxa (Leptotheca) coris* which Stempell (1919) showed in his Figs. 54 and 116, contains a sharply outlined body which the said author interpreted as “Vierergruppe.” One may say, especially after studying Fig. 116, that this appears to be a trinucleate gemma similar to that of the present species, since the nucleus located near the lower edge of the body seems to be that of a schizont of *Nosema marionis* which had been included in the cytoplasm of the gemma. Again, the disporous trophozoites of *S. dimorpha* shown by Davis (1916) in his Figs. 29 and 31, may represent stages of a gemmation similar to the above. Comparative study of the three species will probably lead to interesting results.

This process of gemmation is of great significance to the parasite. As the Myxosporidian lives in the lumina of the uriniferous tubules of the host, possibly absorbing the waste matter secreted by the host cells, it is advantageous that its body should remain small so as not to disturb the function of the host organ. By schizogony and gemmation, the number of the parasites will be greatly increased thereby maintaining the species
without greatly impeding the function of the host organ. Thus the process of gemmation may be interpreted as an adaptation of the parasite to its habitat.

The cytoplasm of the young trophozoite is poorly differentiated. Up to the trinucleate stage, the ectoplasm is hardly distinguishable from the finely reticulated endoplasm. When fixed, the differentiation becomes less visible than in the fresh state. Although there is no difficulty in distinguishing young trophozoites from leucocytes in stained preparations (Figs. 109–111), it is frequently a hard task to separate them in hanging drop preparations, as both form pseudopodia of similar shape, and the nucleus is usually invisible (Figs. 105–108).

THE SPORE FORMATION.

The trinucleate trophozoite, as was stated before, contains the vegetative nucleus and two generative nuclei, each of which becomes a spore by future development (Figs. 41–43). Rarely the vegetative nucleus is seen dividing at later stages (Fig. 48). This division produces two daughter nuclei, one of which remains as the vegetative nucleus of the trophozoite, while the other produces a gemma (Figs. 39, 48, 52). A single vegetative nucleus is always observable in the trophozoites at later stages of development (Figs. 49–60, 71, 81–86).

The division of the generative nuclei is similar to that described before in the case of the primitive generative nucleus. It is preceded by the elongation of the nucleus in the shape of a spindle and the division of the karyosome. The chromatin granules scattered on the network become separated into two groups, each condensing near the end of the spindle (Fig. 44). After the complete separation of the karyosomes, the separated chromatin granules surround each of the former (Figs. 45, 48, 49) and the daughter nuclei finally result.

Since the divisions of the two generative nuclei may or may not take place simultaneously, there are seen trophozoites, the number of whose nuclei vary from five to thirteen (Figs. 45–54). I shall describe the divisions of one of the generative nuclei, as those of the other are essentially the same.

The generative nucleus (Figs. 41–43) divides once and forms two daughter nuclei, one for the spore membrane and the other for the sporoplasms and the polar capsules (Figs. 45–47). The former divides again forming two nuclei,

1 Young as well as sporulating trophozoites were abundantly present in the tubules of the kidneys of every infected host animal. At the time when Ohlmacher and Whinery studied the Myxosporidian, very little was known regarding the Myxosporidia possessing in their life cycle small trophozoites and living in the organ cavities of the host. On the other hand, Myxosporidia that produced large cysts in the host tissue or that developed into large vegetative forms such as *Sphaeromyxa* (*Cystodiscus*) *immersa*, were comparatively well known. It seems probable that these authors searched for large cysts or trophozoites, and overlooked the relatively small vegetative forms as stated here. In this connection, Ohlmacher states that "it is probable that, in this case, the parasite did not reach its adult condition in its batrachian host; but here only passed one stage of its evolution; that is, the spore stage." The inaccuracy of this statement is obvious.
each becoming the nucleus of the valve cell, and occupies the outermost position in the group (Figs. 38, 50–55). The latter also divides into two nuclei, each one being destined for the nuclei of sporoplasts and polar capsules respectively (Figs. 50, 51). The third division follows quickly. Finally we have two nuclei for the capsulogenous cells and two for sporoplasts, which become arranged in a space between valve cells (Figs. 53–55). These last mentioned two divisions seem to take place in quick succession so that before the complete separation of the daughter halves, the next division becomes partly completed. The result is that stages such as are shown in Fig. 38 (in which the two sporoplasts and the two capsulogenous cells are still connected at one point) were seen quite frequently. This circumstance, I believe, is well fitted for the purpose of development of normal spores, allowing time for the valve cells to surround and include the other four cells inside the mass. The nuclear changes stated above are always seen under normal conditions.

Various abnormalities are, however, frequently noted. In the first place, as already stated, one of the generative nuclei in the trinucleate stage, may not form a spore at all, but it may produce a gemma (Fig. 38), or it may not divide whilst the sister nucleus divides repeatedly and develops into a spore (Fig. 85). In this case, the trophozoite is monosporous. Again one of the valve cells and one of the capsulogenous cells, may divide once more, in which case a spore with three shell-valves and as many polar capsules, is produced (Figs. 84, 101).

Since the trinucleate trophozoites and their nuclei differ more or less greatly in size, the later stages show similar difference in size. But, since the generative nuclei in one and the same trophozoite appear to undergo divisions with more or less accurate mathematical ratio, the daughter nuclei of corresponding stages in each of the developing stages are similar in size to each other. Indeed in the great majority of cases two spores developed in one trophozoite are of approximately the same dimensions (Figs. 53–60, 81–83, 87–89). Two trophozoites shown in Figs. 82 and 83 were seen lying side by side in a smear. While the size of the spores differs considerably in these trophozoites, the two daughter spores in each trophozoite are of similar size.

The valve cells which are usually located laterally in relation to the other four cells (Fig. 53), become elongated, and surround the other four cells completely in the form of a deeply staining narrow band (Figs. 54, 55, 57–74). During the formation, the nuclear substances break up into small fragments and transform with the cytoplasm of the cell into a typical substance, the nature of which I have studied elsewhere (Kudo, 1921a). In the formation of the spore membrane of *L. ohlmacheri*, there is no indication that glycogen constitutes a part of the spore membrane as was maintained by Erdmann (1917) in the case of *Chloromyxum leydigii*.

As to the formation of the polar capsules and the polar filaments, there have been large numbers of contributions by authors such as Thélohan (1895), Doflein (1898), Auerbach (1910), Awerinzew (1909), Davis (1916), George-
A Myxosporidian

vitch (1917a) and Erdmann (1917) since the first work by Bütschli (1881) on the subject. Of the recent workers, Erdmann (1917) states that the polar filaments of C. leydigi are composed of glycogen and plastin. That this was not the case in the several species of Myxosporidia which I had studied, was reported in one of my papers (Kudo, 1921a). The first indication of the formation of the structure under consideration, is recognized when a deeply staining club-shaped mass (Fig. 53) appears in the capsulogenous cell. The nature of this substance is not clear, but it is certainly not glycogen or a similar substance. On the other hand, since the nucleus is not only closely applied to the said mass, but directly connected with it, it is most probably composed of chromatin substance and nuclear sap and of the transformed cytoplasm of the cell. The mass grows larger; one end becomes much larger than the other (Fig. 53). At the same time, the cytoplasm shrinks and a vacuole is formed in which the deeply staining substances accumulate. As the mass becomes still larger, it bends to one side, assuming a retort shape, the nucleus feeding its chromatin granules constantly into the growing mass (Figs. 61–63, 65). The fine extremity of the mass becomes coiled around the other rounded end (Figs. 60, 66–69). When the spiral of the filament is completed, there differentiates from the outer region of the mass a sac-like structure which surrounds the polar filament (Figs. 60, 66–69). This is the polar capsule. Although its length is a moderate one compared with that of several other species I have studied (Kudo, 1917, 1918 and 1920a) the polar filament is comparatively thick. The nucleus of the capsulogenous cell seems, therefore, to be used up entirely in most cases for the formation of the polar capsule and polar filament. In mature spores, no remnant of the nucleus of the capsulogenous cell, is usually seen (Figs. 78, 87–90, 94, 96–99, 101–103), though occasionally this is not the case (Fig. 81).

During these changes, the sporoplasms, each containing a single nucleus, remain separated from each other and without any noticeable change (Figs. 53–77, 81, 87–90, 94, 96, 97). This is certainly a peculiar feature of the species. Even in the completely developed spores, the two sporoplasms remain independent.

During the formation of spores, the vegetative nucleus of the trophozoite remains unchanged in its external appearance. It is, in almost all cases, found on one side of the body and usually between the developing spores (Figs. 53–60, 81–85, 89). Contrary to the observations made by other authors on several species of Myxosporidia, the vegetative nucleus of the trophozoite of L. ohlmacheri, instead of undergoing degeneration, grows and increases in size as the trophozoite grows and the spore formation proceeds (Figs. 52, 60, 81–83). This demonstrates that it controls the vital trophic function of the vegetative form during the entire period of its existence. A similar state has been observed in S. dimorpha (after Davis) and C. coris (after Stempell). The necessity of having a functioning vegetative nucleus is also well understood, if one considers the fact that the fully grown trophozoite containing two mature spores undergoes active formation of pseudopodia (Fig. 89).
There is a little difficulty in distinguishing young stages from the leukocytes in the fresh state. The larger trophozoites can easily be distinguished from any other objects found in the preparations even in unstained conditions. An unmistakable character of a living trophozoite is the presence of four polar capsules under formation, even when no nucleus can be detected in the body. The cytoplasm of larger trophozoites is clearly differentiated into two parts, the ectoplasm and the endoplasm. When a drop of emulsion of an infected kidney in physiological solution is made into a hanging drop preparation, large trophozoites will be seen to produce active pseudopodia from localized parts or from the entire surface of the body (Fig. 89). The pseudopodia are those that have commonly been seen in several species of the family Ceratomyxidae. They are of a long conical form and end in points. When the trophozoites are kept in the preparation for from 3 to 8 hours, the pseudopodia are withdrawn, and lobose ones may be seen sluggishly formed in their place. Frequently even in freshly-made emulsion, rounded trophozoites without any pseudopodia are encountered (Fig. 87). The ectoplasm is especially visible at the place where the pseudopodia are formed, they are entirely composed of the ectoplasm. In rounded forms, the differentiation is poor (Fig. 87) and the ectoplasm forms a rather tough homogeneous hyaline layer around the body. The endoplasm is finely reticulated, and in places greatly vacuolated. The trophozoite contains spherical bodies of variable size (Figs. 87–89), which are of fatty nature.

MORPHOLOGY OF THE SPORE.

The mature spores are, as a rule, rounded elliptic with the longest axis standing at right angles to the sutural plane (Figs. 87–98). The form varies, however, considerably. Frequently the breadth is reduced to that of the sutural diameter so that the spore approaches a sphere in the front view. In all cases, the anterior tip is more or less drawn out due to the thickening of the spore membrane at that point. The posterior margin is always rounded except the sutural ridge which stands out prominently at its middle portion. In the anterior end view, the spore is elliptic (Fig. 87), while in profile, it is almost circular with a slightly pointed anterior end (Fig. 99). The spore membrane is moderately thick, and is composed of two valves of usually equal size and form. The anterior tip of each valve is thickened and forms a small rounded highly refractive body (Figs. 87, 92, 96–98). The sutural ridge is straight and prominent. The spore membrane is marked with numerous striae, the pattern of which can be easily studied on an empty spore. Although the striation is apparently irregular in many spores, the following pattern is recognized in most cases: A few striae, three to seven, run parallel to the sutural line, while all the others form somewhat similar angles with the sutural line (Figs. 91, 93, 95). Frequently the latter striae on each valve centre

1 The definitions of the terms used here, are given in one of my papers (Kudo, 1920a).
in two points near the ridge and midway between the anterior and posterior ends. Along the lateral surface, the majority of the striae run parallel to each other, although not infrequently the regularity is broken by short longitudinal striae near the posterior end (Fig. 100). These striae appear as fine spinous projections around the entire margin of the spore in its optical section (Figs. 90, 92, 94). The number of striae on each valve varies from 25 to 35.

Each spore contains two polar capsules in its anterior half. They are spherical in shape, and are in most cases of equal dimensions in one spore. The wall is very thin and the coiled polar filament can distinctly be seen in vivo. The polar filament is spirally coiled usually from four to five times, though rarely up to eight times. It always leaves a considerable space between it and the wall of the polar capsule. The coiled condition of the filament gives further evidence in support of my observations made on other species (Kudo, 1920a). There is no central axial portion around which the filament might be coiled as was supposed by some authors such as Awerinzew (1909) and Stempell (1919) on the same structure in other species.

The polar filament penetrates through the capsule at its anterior part, and is connected with its foramen at the anterior tip of the spore membrane and outside of the thickened portion (Figs. 73, 75, 88, 96). The continuity of the polar filament with the capsule and further with the foramen in the spore membrane, has long been known and many authors referred to the structure mainly showing the relation in the front view of the spore as shown in my Figs. 73, 75 and 88. Joseph (1907) saw the foramina of spores of *Chloromyxum protei*, and gives two excellent microphotographs.

In section preparations I have frequently noticed such parts of spores as are shown in Figs. 79 and 80. These are tangential sections of the spore membrane through the anterior tip. That this is the case is easily seen by the presence of a more deeply stained area on both sides of the sutural line, which corresponds to the anterior thickening in the shell-valves, by the direction of the striae, and further by tracing the rest of the spores in the adjacent sections. These foramina are very small openings situated on either side of the sutural plane and almost equidistant to it.

The extrusion of the polar filaments was easily induced (see p. 223).

The most striking feature of the spore which commands especial attention is its sporoplasms. Ohlmacher who only studied stained sections made from the infected kidneys of *Bufo lentiginosus* which were fixed with absolute alcohol, stated that "in the case in which this substance (protoplasmic mass) was less in amount, a well defined segmentation was frequently noted; the line of division extending through the middle of the mass, each half of the divided mass enveloping a pole corpuscle (polar capsule) in the form of a well defined crescent." I have paid little attention to this statement because the kidneys were fixed with absolute alcohol which might have caused the "segmentation" in Ohlmacher's preparations, and also because Whinery, studying fresh spores under Ohlmacher's direction, did not emphasize this appearance.
In the spores of several species of Myxosporidia, which I have studied up to the present (Kudo, 1920a, 1921b), I have always seen a single binucleate or occasionally uninucleate sporoplasm in each individual.

On examining the spores of *L. ohlmacheri*, I was surprised to find that each spore contained always two sporoplasms. Even in a fresh condition, one can distinctly recognize two sporoplasms in every spore, young as well as mature, clearly separated from each other (Figs. 55, 87, 90, 94, 96, 97). They are rounded and distinctly contoured. They may be of unequal form in one spore, yet their volume appears to be approximately the same. In some spores, they may be small and remain as rounded masses in the posterior portion of the spore (Figs. 87, 97), while in others they may be comparatively large and occupy more than half the cavity, extending anteriorly around the polar capsules (Figs. 88, 90). In the fresh state, the sporoplasms are of uniformly homogeneous hyaline cytoplasm destitute of the usual fine granulation found in the sporoplasm of spores of other species. They, however, contain small refractive globules of variable size and number (usually from three to five) which are of fatty nature. The nucleus can ordinarily not be observed in the unstained state. Another strange feature of the sporoplasms is their strong affinity for nuclear stains. When the spores in smears are fixed and stained by any of the stains used, the sporoplasms often withstand the decoloration, and remain deeply stained, in which case the detection of their nuclei is impossible.

As far as I am aware, Erdmann (1917) is the only investigator who, dealing with *Chloromyxum leydigii*, has concluded that “die junge einkernige Chloromyxidie ist aus einer Anlage entstanden, die zwei Zellen und zwei Kerne in der Sporenhülle hatte.” She has figured three stained spores of the species, which, however, do not illustrate the statement distinctly. Aside from Erdmann’s observation Thélohan (1895) mentioned that in the spore of *Ceratomyxa sphaerulosa* the “protoplasm n’occupeant qu’une des valves, dans l’autre valve, on voit d’ordinaire de petit amas d’une substance très pâle(x).” Whether this substance represented the second sporoplasm or not remains unsettled.

Although the size of two spores formed in one trophozoite is usually similar, spores from different individuals may vary considerably. Measurements of a large number of spores gave the following average dimensions: Fresh spores: sutural diameter and thickness 9-5-12 μ, breadth 13-14.5 μ, diameter of polar capsules 3-4.5 μ, length of extruded polar filaments 42-62 μ. Stained spores: sutural diameter and thickness 8-10 μ, breadth 9-12 μ, diameter of polar capsules 3-4 μ.

These dimensions differ greatly from those of Ohlmacher’s form, since this author records the sutural diameter and breadth of spores in sectioned preparations as 6 μ and 8 μ respectively. It is, however, understood that the section preparations always give much smaller dimensions of spores or trophozoites compared with those of stained smears or particularly of fresh
preparations (Kudo, 1922). I am inclined to think that the spores measured by Ohlmacher underwent a strong shrinkage. Yet it is difficult to understand that Whinery, who, notwithstanding his having studied fresh spores, records exactly the same dimensions as Ohlmacher, although he remarks that the size varied to some extent.

In view of the size of the spores, and their general appearance, striae and occurrence, etc., I am unable to regard the form I have herein described as a new species.

As is the case with every species of Myxosporidia, malformation of spores is frequently noticed. Two or three spores, each with three shell-valves and as many polar capsules, are always seen in every smear preparation. Extremely small spores are also not infrequently noticed (Figs. 83, 98).

THE MODE OF INFECTION.

Regarding the mode of infection with *L. ohlmacheri*, Ohlmacher states: “as to the origin of the myxosporidian infection, we can only conjecture that it must have occurred by way of the cloaca to the bladder, eventually lodging in the kidneys.” This amounts to a mere supposition, and consequently has received little attention from recent investigators.

Joseph (1907), working on *Chloromyxum protei*, parasitic in the lumen and epithelium of the uriniferous tubule of the kidney of *Proteus anguinus*, came to the following conclusion:

Fragt man sich nach dem Infektionsmodus, so ist es nach den Befunden an anderen Myxosporidien und den lokalcn physiologischen Verhältnissen in der Niere am wenigsten wahrscheinlich, dass ein befallenes Tier mit den in ihm entstandenen Sporen sich selbst von neuen infiziert, das Naheliegendste ist doch sicher die Annahme einer Übertragung auf ein anderes Individuum auf dem Wege des Wassers. Der Umstand, dass die jüngsten Infektionsstadien sich meist in den Harnkanälenabschnitten fanden, die unmittelbar mit dem Gewebe in Verbindung stehen, liess die Vermutung in mir aufkommen, ob nicht dies der Weg ist, den die Keime, die Darmwand durchsetzend, nehmen und veranlasste meine Fütterungs- und peritonealen Infektionsversuche.

In the course-of his study on *S. dimorpha*, parasitic in the urinary bladder and Wolffian ducts of *C. regalis*, Davis (1916) noticed that “spores when placed on the slide without previous exposure to sea water, and mixed with a drop of fluid from the pyloric caeca of the host, usually germinated within five to fifteen minutes,” and concluded that “it appears probable, therefore, that the free spores, when taken into the intestine of the host, germinate, and the sporozoites, as free amoebulae, actively make their way into the urinary bladder.”

According to the results obtained by Auerbach and Erdmann, the spores of *M. bergense*, *Z. hildae* or *C. leydigi*, germinate in the duodenum of the new host fish. The liberated amoebulae pass through the bile duct, and reach the gall bladder where they grow into trophozoites and undergo sporulation.

In the case of *L. ohlmacheri*, the circumstances are similar to those of
C. protei, but differ from the latter forms. Ohlmacher’s view is untenable because myxosporidian spores have no power of locomotion and have never been seen or been made to germinate in water outside of the host.

As previously stated, a series of experiments was carried out to discover the way in which infection occurs. An emulsion of fresh spores in physiological solution was prepared. Small pieces of a sterilized cork, made porous by means of a fine needle, were immersed in the emulsion, and introduced into the oesophagus of several specimens of R. p. p. Small pieces of infected kidneys were also fed in a similar manner. At various intervals of time, the animals were dissected. The cork was found in the pylorus even after 48 hours, and in two cases it was seen to have reached the large intestine. The corks, after being taken out, were cut into smaller pieces on many slides and studied. Four intestines with the introduced corks were fixed and sectioned.

Hanging drop preparations were made by mixing fresh spores with the fluids taken from various parts of the digestive tract. Further changes than the extrusion of polar filaments and occasional separation of the shell-valves were noticed only in the preparations with the gastric fluid with or without bile or fluid from the duodenum. In these preparations which have been absolutely free from mechanical pressure, the amoebulae were frequently noticed making their way through the opening of the spore membrane. Similar changes were noticed in the smears of corks taken from the pylorus. From these experiments, we may conclude that the amoebula emerges in the pylorus or duodenum of the new host.

It may seem probable that the liberated amoebulae pass through the alimentary canal into the cloaca where the ureters open, as was thought by Davis in S. dimorpha, and make their way up the ureters and further into the uriniferous tubule of the kidney. However, this supposition does not seem to hold, as the young stages only occur in or near the Malpighian bodies.

I therefore concur with Joseph’s opinion that the liberated amoebulae penetrate through the wall of the digestive tract, appear in the coelomic fluid, and finally reach the nephrostome through which they further make their way into the lumen of the uriniferous tubule of the host’s kidney. Judging from the conditions observed in sections of infected kidney, this seems to be the most common way for amoebulae to reach their final seat of infection. Frequently the space between Bowman’s capsule and the glomerulus of the Malpighian body is greatly enlarged, and filled with young as well as sporulating trophozoites, while the uriniferous tubule originating from this body, contains no parasites. This may be due to the amoebulae which have entered the blood stream and which, after reaching the capillary in the glomerulus, have traversed its wall and become free in the said space where active multiplication takes place.

The mature spores pass down the uriniferous tubules and ureters, and escape outward through the anus. When they are swallowed by a new host, the fresh infection follows.
THE RELATION BETWEEN THE HOST AND THE MYXOSPORIDIAN.

*L. ohlmacheri* has been observed in the following three North American batrachian host species: *B. lentiginosus* (Illinois, after Ohlmacher and Whinery), *R. clamitans* (New York, after Kudo) and *R. pipiens* (locality unknown, after Kudo).

As was mentioned on p. 121, Thelohan gave no description of his *L. ranae* which would permit of two species of *Leptotheca* similar in habitat being differentiated. *L. ranae* is probably identical with *L. ohlmacheri* because some species of Ranidae such as *R. temporaria* and *R. agilis*, occur both in America and Europe. Ohlmacher kept his infected toad with two dozen frogs¹, which he did not examine. In my opinion these frogs were also infected by the Myxosporidian, and acted as a source of infection for *Bufo lentiginosus*. I further think that if examination of various species of frogs from different localities is carried out, the Protozoon may be found to be far less specific and more cosmopolitan.

Fourteen specimens of *R. clamitans* were examined from July 31 to September 5, 1920. They measured from 3 to 10 cm. in length. Of these, eight were males and six females. The examination showed that three males and three females were infected. Twenty-four specimens of *R. pipiens* were examined between November 12 and December 6, 1920. They measured from 8 to 10 cm. long. Of these fifteen were males and the remaining nine females. The examination showed that seven males and three females were infected. The percentage of infection in the two host species *R. clamitans* and *R. pipiens* are 43 and 42 respectively.

Although *R. pipiens* were almost of similar size, a great variation in the size of *R. clamitans* was noticed. One individual, which was only 3 cm. long, had both its kidneys heavily infected, young as well as sporulating trophozoites being present in large numbers. On the other hand, three individuals, 10 cm. in length, were found to be free from the infection. The infected animals varied from 5 to 9 cm. in length. This probably indicates that the infection occurs at any stage of the development of the host. Further, the infection was recognized only in five specimens collected from a short stretch of creek “a” between August 3 and 17. The other individual mentioned above was obtained on August 25 from creek “b” where two other uninfected larger specimens were also collected.

Concerning the effect of the infection upon the host animal, I have not seen any serious pathological change which might cause the death of the host. As to the possible effect of the parasites upon the toad, Ohlmacher made the following statement:

As to the pathogenic rôle of the Myxosporidia in this case, there can scarcely be a doubt but that they were the direct factors in the production of the pathological changes leading to the death of the host. In fact, the tubules of both kidneys were filled with the parasites;

¹ No specific name is given.
and it is evident that the mere mechanical effect of this foreign material in the tubules must have led to an obstruction of the secretory functions of the organ, and as a remote result, to the ascites and general oedema. Undoubtedly, the presence of large numbers of bacteria in the already overburdened kidney was potent in hastening the fatal termination; but these vegetable microorganisms must be regarded as the elements of a secondary infection. It is not difficult to conceive that the diseased kidney, with its damaged secretory function, would furnish an inviting focus for bacterial invasion; and particularly since the route from the exterior is such a direct one in these animals in which the urinary secretions empty into a cloaca.

Whinery (1913) collected "about a dozen" *B. lentiginosus*, and kept them in a sink. The toads died in confinement within three weeks. Two males and five females were examined, one male and four females being found to be infected. About the condition of the toads, he remarked as follows:

Before death no external change in appearance of the animals was noticed, with the exception of a distension of the abdomen in some cases. Opening the abdominal wall, some increase in the amount of peritoneal fluid was usually noticed, but never a large amount in the toad examined and described by Dr Ohlmacher. The abdominal viscera showed signs of congestion. The kidneys were enlarged and in a congested state. The intestines were usually distended with gas.

In *R. clamitans* and *R. pipiens* infected by *L. ohlmacheri*, I could not recognize any difference either in external appearance or in activity between uninfected and infected animals. No marked difference in the quantity of coelomic fluid in healthy and infected animals was noticed. The internal organs of different individuals were carefully compared, but no definite data could be obtained regarding the possible effect of the infection upon the organs.

The kidneys of infected frogs seemed to be slightly larger than those of the uninfected, and they were usually greatly congested. In the case of *R. clamitans*, this could not be explained merely as a result of the Myxosporidian infection, because trypanosomes were found in almost every host in far greater number in the blood vessels of the kidneys than in those of other organs. The congested condition of the kidneys observed in some of the infected *R. pipiens* was, however, probably due to the Myxosporidian infection, because few trypanosomes were present in this species.

The examination of sections revealed but little disturbance in the secretory function of the kidney, since the parasites do not seem to invade the tissue, but simply lie in the lumina of the uriniferous tubules probably absorbing the fluid waste matter secreted by the host cells. A parasite will be tolerated for a long time provided that it does not greatly harm its host.

**SUMMARY.**

1. *Rana clamitans* of New York and *R. pipiens* from the middle part of the United States, were found to be infected by a Myxosporidian, apparently identical with *Leptotheca ohlmacheri* (Gurley) Labbé, found by Ohlmacher in the kidney of *Bufo lentiginosus*.
2. The Myxosporidian was found only in the space between Bowman's capsule and the glomerulus of the Malpighian body and in the uriniferous tubules of the kidneys of the host, no other organ being infected.

3. The mature spore contains two independent uninucleate sporoplasms which fuse into one prior to the germination in the posterior region of the stomach or duodenum of a new host.

4. The germination of the spore was observed in hanging drop preparations with the digestive fluid.

5. The youngest stage found in the lumen of the tubule of the kidney, was the uninucleate form.

6. The trophozoites multiply actively by a process of gemmation and probably also by a schizogony of the uninucleate forms.

7. The trophozoites are, as a rule, disporous and the spores develop independently of each other.

8. The vegetative nucleus persists throughout the entire trophic life of the individual.

9. The Myxosporidian does not exercise any fatal effect upon the host.

10. Infection takes place through the mouth. The liberated binucleate amoebulae probably penetrate through the wall of the small intestine, reach the coelom and are carried to the uriniferous tubules through the nephrostomes or blood vessels.

REFERENCES.


R. Kudo


— (1917). Contributions, etc. II. Myxobolus toyamai n.sp., a new Myxosporidian parasite of Cyprinus carpio L. Journ. Parasit. iii. 163–170, Pls. I–II.


EXPLANATION OF PLATES XIII—XX.

All the figures, except Figs. 104 to 111, represent various stages in the development of Leptotheca ohlmacheri. The figures were drawn with the aid of Abbé's drawing apparatus at the level of the base of the microscope, with the combinations of Zeiss' compensation oculars 8 and 12 and homogeneous oil immersion objective 2 mm., thus obtaining magnifications of about 1500 and 2350 diameters respectively. Figs. 1 to 86, 88 to 95 and 101 to 111, were magnified 2350, and the rest 1500 diameters. The abbreviations used in the explanation are as follows: B., Bouin fixation; C., from the kidneys of Rana clamitans; D., staining with Delafeld's haematoxylin; F., 4 per cent. formal fixation; G., staining with Giemsa's solution, followed by acetone dehydration and mounted in cedar oil; H., staining with Heidenhain's iron haematoxylin; P., from the kidneys of Rana pipiens; S., section preparations; Sa., Schaudinn’s fixation; Sm., smear preparations; Tcs., thickly made smears; Tns., thin smears.

PLATE XIII.

Fig. 1. A spore treated for 30 minutes with the gastric fluid of the frog in a hanging drop preparation, showing the fusion of the two sporoplasms. P.Tns.Sa.G.

Figs. 2, 3. Two views of a spore which was kept for 2 hours in the stomach fluid of the frog animal (P), drawn from a fresh hanging drop preparation. The two sporoplasms fused into one mass and are seen pressing the polar capsules against the anterior margin of the spore membrane. P.

Fig. 4. Another spore treated like the last one for 6 hours, showing the binucleate amoebula emerging through the opening made by the separation of the shell-valves. The empty polar capsules lost their spherical form under the pressure of the amoebula. The greater portion of the filaments are not shown. P.Tns.Sa.G.
Fig. 5. A spore treated for 16 hours with a weak pepsin hydrochloric acid in a hanging drop preparation. The amoebula is seen escaping through the opening. The emergence did not take place further than this condition although it was observed for 40 minutes. P.

Fig. 6. A spore under a similar treatment to those shown in Figs. 2-6, for 6 hours. P.Tns.B.H.

Fig. 7. A later stage than the one shown in Fig. 4. P.Tns.Su.H.

Fig. 8. A liberated amoebula lying beside the empty spore membrane, after being treated with the gastric fluid for 16 hours. P.Tns.Su.H.

Fig. 9. An amoebula observed in a hanging drop preparation of spores and stomach fluid after 22 hours. The fusion of the two nuclei is seen taking place. P.Sm.Su.G.

Figs. 10, 11. Fresh amoebalae from the same preparation which contained the amoebula shown in Fig. 8. P.

Fig. 12. An amoebula whose nuclei are undergoing fusion. P.Tns.Su.G.

Figs. 13-16. Four stages of schizogony in the tubules of kidney. P.S.Su.G.

Figs. 17, 18. Young trophozoites from the uriniferous tubules of the frog's kidney. P.S.Su.G.

Fig. 19. A young trophozoite. P.S.Su.G.

Fig. 20. A young trophozoite. P.Tns.B.D.

Fig. 21. A fully grown uninnucleate trophozoite. P.Tns.Su.G.


PLATE XIV.

Fig. 26. A binucleate trophozoite, with its vegetative and the dividing generative nucleus, attached to the epithelial cell of the tubule of the frog's kidney. C.S.F.D.

Figs. 27, 28. Trophozoites, one of the nuclei (the generative nucleus) having just completed its division producing two generative nuclei. P.Tns.Su.G.

Fig. 29. Two views of a trinucleate trophozoite attached to the epithelial cells of the tubule of the frog's kidney. The generative nucleus has just completed its division C.S.Sa.D.

Fig. 30. A typical trinucleate trophozoite. C.S.Sa.D.

Fig. 31. A trinucleate trophozoite. P.Tns.Sa.G.

Fig. 32. A trinucleate trophozoite. P.Tns.B.H.

Fig. 33. A trinucleate trophozoite, the vegetative nucleus undergoing further division. P.Tns.Sa.D.

Fig. 34. A tetrnucleate trophozoite. P.Tns.Sa.D.

Fig. 35. A similar stage. The nucleus on the right of the vegetative nucleus is surrounded by a clear space, and dividing to form a gemma. P.Tns.Sa.G.

Fig. 36. A similar stage. P.Tns.Sa.D.

Fig. 37. A trophozoite with a gemma. Besides the vegetative nucleus which is seen below and two generative nuclei on the left, an island of cytoplasm with similar nuclei, a gemma, is observable. C.Tns.Sa.G.

Fig. 38. A trophozoite with a gemma at the left half, the cytoplasm being spread out in an extremely thinly made smear. In the right half, six generative nuclei beginning to form a spore, below which a large vegetative nucleus is noticeable. C.Tns.Sa.G.

PLATE XV.

Fig. 39. A more developed trophozoite with a fully grown gemma. P.Tns.Sa.G.

Fig. 40. A trophozoite with a fully grown gemma. P.Tns.Sa.G.

Figs. 41-43. Liberated gemmæ, each with a vegetative nucleus and two generative nuclei. Fig. 41, C.S.Sa.G.; Fig. 42, P.Tcs.Sa.D.; Fig. 43, P.Tcs.Sa.D.

Fig. 44. A trophozoite showing the dividing generative nuclei. P.Tns.Su.G.

Figs. 45-47. Trophozoites, each with a vegetative nucleus and four generative nuclei. Fig. 45, P.Tns.Su.G.; Fig. 46, C.S.Sa.D.; Fig. 47, P.Tcs.Sa.D.

Fig. 48. A trophozoite. The vegetative nucleus and one of the generative nuclei are undergoing division. P.Tcs.Sa.D.

Fig. 49. A trophozoite with a vegetative nucleus and seven generative nuclei, one of which is further dividing. C.S.F.D.

Fig. 50. A trophozoite with a vegetative nucleus and eight generative nuclei. C.S.Sa.H.
PLATE XVI.

Fig. 51. A fresh trophozoite with one vegetative nucleus and eight generative nuclei.  

Fig. 52. A trophozoite at a later stage.  

Fig. 53. A trophozoite showing a vegetative nucleus and twelve generative nuclei which are arranged in two groups. In each group, two capsulogenous cells, two sporoplasts and two valve cells are seen.  

Fig. 54. A more advanced stage.  

Fig. 55. A fresh trophozoite at an almost similar stage of the development to the last.  

Fig. 56. A more developed form.  

PLATE XVII.

Fig. 57. A trophozoite greatly spread out in the smear.  

Fig. 58. Another trophozoite.  

Fig. 59. A trophozoite with two more advanced spores.  

Fig. 60. A trophozoite with two spores in which the formation of polar filaments is clearly observable.  

Figs. 61-67. Young spores at various stages of development, the rest of the body of the trophozoites are not shown.  

PLATE XVIII.

Figs. 68-70. More advanced stages in the spore formation.  

Fig. 71. A section through a trophozoite attached to an epithelial cell of the host tubule. This section showed only one of the spores and the vegetative nucleus.  

Figs. 72, 73. Young spores, one showing the formation of polar filaments.  

Fig. 74. A young spore.  

Fig. 75. A spore showing foramina for the polar filaments.  

Fig. 76. A spore.  

Figs. 77, 78. Two different end views of a young spore.  

Figs. 79, 80. Tangential sections through the anterior region of spores, showing the foramina of polar filaments in front view.  

Fig. 81. A trophozoite with two mature spores of normal size.  

Fig. 82. A trophozoite with two deeply stained spores of normal size.  

PLATE XIX.

Fig. 83. A trophozoite with two spores of much smaller size.  

Fig. 84. A trophozoite with a normal mature spore and an abnormal spore with three polar capsules under formation.  

Fig. 85. A trophozoite with a mature spore of somewhat smaller size and a generative nucleus whose division has so far not taken place.  

Fig. 86. A monosporous trophozoite.  

Fig. 87. A fresh disporous trophozoite.  

Fig. 88. A fresh disporous trophozoite.  

Fig. 89. A fresh disporous trophozoite showing pseudopodia.  

Fig. 90. Optical section of a normal spore in the fresh state.  

Figs. 91-93. Two surface views (Figs. 91, 93) and an optical section (Fig. 92) of an empty spore membrane in the fresh state.  

Figs. 94, 95. Optical section and the upper surface view of a spore of somewhat larger dimensions.  

PLATE XX.

Figs. 96, 97. Fresh spores.  

Fig. 98. A fresh spore of smaller dimensions.  

Figs. 99, 100. Optical section and a surface view of a spore seen from one side.
Fig. 101. An abnormal spore with three polar capsules and shell-valves in the fresh state.  

Figs. 102, 103. Spores with extruded polar filaments under the influence of a solution of sodium glycocholate.  

Fig. 104. A red blood corpuscle in the fresh state.  

Figs. 105–108. Leucocytes and “spindle cells” in the fresh state.  

Figs. 109, 110. Mononucleate leucocytes.  

Fig. 111. A trinucleate leucocyte.
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SOME NOTES ON THE DIFFERENTIATION OF CLOSELY-ALLIED SCHISTOSOMES.

BY F. G. CAWSTON, M.D.,

First Streatfeild Research Scholar, Durban, Natal.

When fresh-water snails are examined microscopically, specimens are occasionally encountered that are infested with the cercariae of more than one species. Sometimes these cercariae are easily differentiated. A snail may harbour numerous eye-spotted amphistomes and a few distomes without eye-spots, or furcocercous forms may be associated with cercariae possessing undivided tails. In some collections of semi-stagnant water, however, the same individual snail may be found infested with at least two distinct schistosomes. In 1916, I found Physopsis africana (in an overflow pool along the course of the Umsindusi river at Pietermaritzburg) heavily infested with two distinct schistosomes, and it was not uncommon, as Dr E. E. Warren also observed, for the two forms to develop in the same host. One of these cercariae I named Cercaria secobii, the other was probably the cercaria of Schistosoma haematobium. Soparkar (1921 a and b) has moreover noted a double infection of Planorbis exustus near Bombay.

The two trematode species which cause Bilharziasis in Africa were long considered to be identical, but careful work, conducted during recent years, has demonstrated essential differences in the cercarial stages of the worms. At one time it was assumed that the worms could be determined in accordance with the species of snail in which the cercariae were found. Thus Leiper (1919) stated that cercariae “developing in the Bullinus molluscs always produce bilharzia worms which give rise solely to terminal-spined eggs, while those which have developed in Planorbis boissyi always become worms which produce solely lateral-spined eggs.”

But Physopsis africana has been shown to be the common intermediary host for both S. haematobium and S. bovis and less commonly for S. mansoni, the cercariae of these species resembling each other even more closely than do those of S. haematobium and C. secobii, the latter being a longer and narrower form with particularly long prongs to its divided tail (Cawston, 1917).

Though I have never succeeded in following the development of schistosomes in any other species of fresh-water snail than Physopsis africana, the presence of schistosomes in various other species which I have found infested in Natal streams and the development of S. mansoni and S. haematobium in other forms recorded by Porter (1920) shows that there are conditions under
Differentiation of Schistosomes

which these schistosomes may develop in other snails than those that serve as their common intermediary host. (See also Cort, 1918.)

In a specimen of *Physopsis africana* which I sent him from Natal, Faust (ix. 1920) has reported the presence of the cercariae of *S. haematobium* and *S. mansoni*, as well as *Cercaria octadentata* which he (ix. 1921) regards as a developmental stage of *S. bovis*. Since *S. bovis* in Natal has only been recorded from experimental animals and possibly some Natal boys and, as *S. mansoni* has been seen in only one patient in Natal and in experimental animals, it is difficult to understand how one individual snail from the Durban suburbs can have been exposed to infestation by the miracidia of all three schistosomes. However, the observation shows that the development of miracidia in *Physopsis africana* is of no value as a means of differentiating these three schistosomes.

The association of such closely allied species in the same individual host is of great interest and emphasises the need of identifying the various schistosomes in their free-swimming stage (Faust vi. 1920). The methods of locomotion of the various cercariae and the relative length of their prongs are of more differential value than their various total lengths. A determination of the number of pairs of mucin glands is one of the most reliable means of determining the species to which a cercaria belongs; but the chief means of differentiating cercariae is by a comparison of the adult forms and, where there is mixed infestation and the cercariae are not readily distinguishable, the task becomes more complex. Soparkar states that the "structural correlation between a known cercaria and one under investigation may at best be suggestive of their possible identity, but the final proof must rest with the experimental rearing from them of identical adults.”

I have treated guinea-pigs with cercariae from a snail which was heavily infested with eye-spotted cercariae resembling *C. frondosa* but, on post-mortem dissection, have found schistosomes which could not possibly have developed from eye-spotted forms. If the cercariae responsible for the infection were so few as to be overlooked amongst the commoner species of parasite, it will be readily understood how difficult it is to determine those cercariae which represent the larval stage of the rarer schistosomes. It is certainly surprising to obtain successful experimental infection by using schistosomes that are so few as to be overlooked, when other animals which are injected and fed on very numerous schistosomes on several occasions show no sign of infection at the end of four months.

With a large number of snails bred in captivity, and exposed to infection with the various miracidia, it should be possible to correlate the various cercariae with the adult worms used in the experiments, but the rarer schistosomes are hard to obtain and the miracidia of fasciolae take a long time to hatch out. However, from clean bred *Physopsis africana* to which numerous ova of *S. haematobium* (readily obtained in Natal) are added we can obtain typical cercariae of *S. haematobium* at will. We have yet to determine the life-history of many cercariae commonly encountered in fresh-water snails from
various parts of South Africa. A comparison of the various schistosomes found in South African snails and of closely allied forms occurring elsewhere, of which particulars are appended, may be of interest to those who are engaged in the study of such parasites in South Africa.

<table>
<thead>
<tr>
<th>Schistosome</th>
<th>Approximate length of cercaria in mm.</th>
<th>Pairs of mucin glands in cercaria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. haematobium</em> (C. criapa)</td>
<td>0.52</td>
<td>3 acidophilic with large nuclei</td>
</tr>
<tr>
<td><em>S. mansoni</em> (C. spinosa?)</td>
<td>0.39</td>
<td>2 acidophilic with large nuclei</td>
</tr>
<tr>
<td><em>S. japonicum</em></td>
<td>0.39</td>
<td>4 basophilic with small nuclei</td>
</tr>
<tr>
<td><em>S. bovis</em> (C. octadeca?)</td>
<td>0.43</td>
<td>5 acidophilic with large nuclei</td>
</tr>
<tr>
<td><em>S. ?</em> (C. indicae)</td>
<td>0.39</td>
<td>5 acidophilic</td>
</tr>
<tr>
<td><em>S. bovis</em> (C. octadeca)</td>
<td>0.43</td>
<td>2 acidophilic</td>
</tr>
<tr>
<td><em>S. ?</em> (C. oculata)</td>
<td>0.35</td>
<td>2 basophilic ? pharynx</td>
</tr>
<tr>
<td><em>S. ?</em> (C. secobii)</td>
<td>0.55</td>
<td>3 acidophilic with small nuclei</td>
</tr>
<tr>
<td><em>S. ?</em> (C. gladii)</td>
<td>0.92</td>
<td>4 all neutrophilic</td>
</tr>
</tbody>
</table>

Testes in adult | Ova in utero
--- | ---
*S. haematobium* (C. criapa) | About 4 | Numerous spine-pointed |
*S. mansoni* (C. spinosa?) | About 8 | Usually one lateral-spined |
*S. japonicum* | About 7 | Numerous ova, practically aspinose |
*S. bovis* (C. octadeca) | — | Numerous spine-pointed |

REFERENCES.


NOTES ON LARVAL FLUKES FROM CHINA.

By ERNEST CARROLL FAUST, Ph.D.,
Peking Union Medical College, Peking, China.

(With Plates XXI and XXII.)

In connection with a survey carried on during the past three years to determine the helminth parasites of China and their incidence in various parts of the country, I have had occasion to examine several thousand molluscs, particularly gasteropods, with a view to ascertaining the degree of their infection with larval flukes. Examinations thus far have been made in the areas around Peking, Yü Tai Hō (Shansi Province), Wuchang (Hupeh Province), Changsha (Hunan Province) and Kiukiang (Kiangsi Province). In Peking examinations have been fairly continuous during the entire period; in the other centres only summer examinations were possible.

The host species represented in the investigations, together with the localities where they were found, the numbers examined, and the parasites obtained, are presented in Table I. I am greatly indebted to Mr Bryant Walker, of Detroit, Michigan, and Mr Frank C. Baker, of the Natural History Museum, Urbana, Illinois, for identification of the hosts.

While the Peking molluscs show an average incidence of infection which compares favourably with that of the more humid areas in China, analysis of the data reveals a distinct seasonal variability. During the winter the only parasites present are encysted agamostomes and these are relatively few. However, from the latter part of March to the 1st June, there is a rapid development of larval flukes. This period represents the migratory season of birds, so that it is more than likely that the infection is an extra-territorial one superimposed on the area. This belief is borne out by considerable confirmatory data. Birds caught during their migration through North China especially in the spring, are heavily parasitised by flukes, while very few animals indigenous to the Peking area have an appreciable infection with this group of worms. Moreover, the infection-incidence of snails of the area is lowered during the summer but reaches a second peak in the autumn following the return of birds to the south.

On the other hand, the records from the Yangtze valley indicate just the reverse. In most cases the parasitisation of the mollusc is not overwhelmingly

1 Contribution from the Parasitology Laboratory, Department of Pathology, Peking Union Medical College.
high, probably because climatic conditions as well as the large areas covered by lakes and the large amount of irrigated land tend to foster the development of an enormous number of snails. But since fluke infection in all groups of vertebrates is unusually high in the Central Yangtze Valley, it is very probable that, of the larval flukes found in the samplings secured in this area, there are more that represent indigenous species than do those collected from North China. The absence in this collection of larvae of a large share of the well-known trematodes of man and domestic animals of Central China may be explained on the basis of chance distribution of many species of parasites in a tremendous number of host individuals. Random sampling explains why repeated examinations from various habitats in three centres (Wuchang, Changsha, Kiukiang) have not revealed more larvae of mammalian species; while the disproportionally large number of mollusces compared with definitive hosts in the area tends to reduce the percentage of infection. Furthermore, one needs to remember that certain species, such as *Clonorchis*, require a second intermediate host, which requirement necessarily reduces the number of individuals of the species that can "carry on." In addition to these data there is the fact that certain infections of man, such as *schistosomiasis japonica*, although present in various centres of the Central Yangtze Valley, are not ubiquitous, but definitely localised. It is quite necessary, then, to consider both groups of limiting factors in comparing the infection-incidence of larval trematodes in two areas as dissimilar as North and Central China admittedly are.

Certain species have been observed in both North and Central China. These include *Aspidogaster conchicola*, observed in two unrelated species of mollusc, as well as in *Amyda sinensis* and *Leuciscus aethiops; Cercaria pekinensis*; and *Cercariaeum mutabile*.

In all, 26 species of flukes have been observed from mollusc hosts, which are included in the following groups:

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspidobothridae</td>
<td>1</td>
</tr>
<tr>
<td>Holostomata</td>
<td>1</td>
</tr>
<tr>
<td>Monostomata</td>
<td>2</td>
</tr>
<tr>
<td>Amphistomata...</td>
<td>2</td>
</tr>
<tr>
<td>Distomata, divided as follows:</td>
<td></td>
</tr>
<tr>
<td>echinostome larvae</td>
<td>4</td>
</tr>
<tr>
<td>xiphidio-cercariae</td>
<td>8</td>
</tr>
<tr>
<td>cystophorous cercariae</td>
<td>1</td>
</tr>
<tr>
<td>cystocercous cercariae</td>
<td>1</td>
</tr>
<tr>
<td>leptocercous cercariae</td>
<td>3</td>
</tr>
<tr>
<td>microcercous cercariae</td>
<td>1</td>
</tr>
<tr>
<td>furcocercous cercariae</td>
<td>1</td>
</tr>
<tr>
<td>Cercariaeum (<em>C. mutabile</em>)</td>
<td>1</td>
</tr>
</tbody>
</table>
Larval Flukes from China

Aspidogaster conchicola has been recorded from both Europe and America, so that this record will place it in the group of widely distributed species. Cercariaeum mutabile has been described from the Occident (Cort, 1919), although from an entirely different host. Cercaria pekinensis and Tetracotyle orientalis (larva of Cyathocotyle orientalis) have recently been described from North China (Faust, 1921 and 1922). Of the remaining 21 forms, all are probably new species. Fourteen of this number, which I have had an opportunity to study, are described in this paper.

Cercaria plana nov. spec.

(Plate XXI, Fig. 1.)

This monostome larva, for which the name Cercaria plana is proposed, was found in two collections of Viviparus quadratus (Bens.) in the vicinity of Peking in 1921. The former material was secured at the canal gate near the East City Wall, while the latter was obtained in the North Lake of the Imperial City. The infection in each case was about 1 per cent.

The cercaria is a flat delicate larva, easily bursting under pressure of a cover glass. It measures 0.25 × 0.12 mm., and has a tail slightly longer than the body. Both the body of the cercaria and the tail are covered with minute spines. The movement of the mature animal is sluggish. The dorsal surface of the body is irregularly mottled with brown, particularly in the region of the eyes. The worm is trioculate, but the cyclopean eye is much slower in development than the lateral pair. The oral sucker is small (25 μ in transverse diameter). Behind it there is a distinct pharynx (Fig. 1, ph). Posterior to the pharynx there is a typical tuning-fork-shaped gut, the caeca of which end some distance anterior to the excretory bladder. The bladder is a subspherical organ, with a thin wall and a small pore. Proceeding from it anteriad are a pair of collecting tubules which appear to unite just posterior to the median eye. They are completely filled with excretory granules. On account of the opacity produced by cyst granules throughout the body, the finer details of the excretory system were obscured. The genital anlagen are similar to those of Cercaria spatula (Faust, 1919: 341, Fig. 1). Lateral to the excretory bladder are the lateral locomotor organs, which are simple invaginated pockets with a median attenuate lip capable of acting as a holdfast. The cystogenous cells are small and numerous. They are densely packed with minute rhabditiform granules. Encystment is slow but regular, with the laying down of a mucoid wall around the larva before the tail is dropped.

Cercaria plana develops in a large redia with conspicuous feet, a large spherical pharynx and an extensive gut filled with deep orange food particles.
Cercaria nervosa nov. spec.
(Plate XXI, Fig. 2.)

This amphistome larva, for which the name Cercaria nervosa is proposed, was found producing a heavy infection in Planorbus mollendorfii Cless., secured from a small stream in a temple ground in the Western Hills, some 35 miles from Peking.

The larva is pyriform, with a long narrow tail which has a jerky, nervous movement. The body measures 0.15 x 0.13 mm., while the tail averages about 0.3 mm. in length by 25μ in width. The entire body is covered with minute spines and, in addition, the head is beset with three rows of more conspicuous spines. A pair of pigmented eye-spots are found just posterior to the oral sucker.

The oral sucker has a small opening but a heavy muscular bulbus. At its inner limits definite pharyngeal pouches (pp) are seen. The greatest diameter of the organ is 33μ. From the mid-region of the oral sucker there leads inward a narrow prepharynx, lined with a mucoid thickening in the middle third and surrounded by a minute muscular pharynx in the distal third. The caeca arise directly from the pharynx and extend as a broad arch in a latero-posterior direction.

The posterior sucker measures 40 x 80μ, the transverse diameter being the greater. The excretory system consists of a small muscular bladder (b), with a posterior pore; collecting tubules in the body which arise from the anterior mid-line of the bladder, and by characteristic coiling proceed anteriorly and then return to the posterior part of the worm; and a caudal element extending to the distal region of the tail and there forking abruptly (see Fig.). Granules are found only in the middle part of the collecting tubule of the body. On account of the abundant supply of cystogenous granules throughout the entire body, details of flame-cell structure have not been observed. Three or four germ cell masses have been seen in the mid-region of the body.

The parthenita is a simple sacculate redia without feet, but having a definite pharynx and a small rhabdocoeel gut.

Cercaria syringicauda nov. spec.
(Plate XXI, Fig. 3.)

The cystophorous cercaria, for which I propose the name Cercaria syringicauda, was found producing a very heavy infection in two collections of Melania ebenina Brot., taken from the backwaters of the Yantze valley at Kinkiang. At the period when the collections were made this host species was the only common mollusc in the area.

C. syringicauda has an oval body measuring sensu stricto 0.13 mm. long by 0.09 mm. wide. The main caudal appendage has a pyriform bulbous region immediately behind the body and an extremely long tube continuing distad. The bulbus has measurements about one and one-half times those of the body,
while the caudal tube is eight to ten times the body length. At the junction of bulbus and caudal tube there is a noticeable enlargement. The lateral excretory tubule (excretory projection of Cort) has about the same bore as the caudal tubule and is about twice as long as the body. This lateral tubule connects proximally with the excretory bladder on the one hand and with the bulbus on the other. It is evident both from observation of the living worm and from examination of the preserved specimen that the main caudal tube as well as the lateral tubule serve as accessory excretory canals, since the main tube is hollow throughout its entire length and has been seen to extrude small excretory granules. The bulbus is filled with two kinds of secretory cells, consisting of a layer of large cells with coarse flocculent contents of a basophil nature lining the central lumen, and a second series of fine granular eosinophil cells loosely filling in the spaces between the integument and the first series. The function of these cells is not known. Neither in fully developed forms nor in younger stages has the body been found invaginated within the bulbus as described for C. yoshidae (Cort, 1920: 12) and other closely related forms.

While the body of the larva differentiates late, the internal structure is more fully organised than in other described species of the group. In addition to the oral sucker (43 μ in transection) and ventral sucker (37 μ in transection), the pharynx is well developed and the caeca provided with muscular walls. Furthermore, the anlagen of the genital organs have become separated into groups, with an anterior group \( (g_a_1) \) consisting of vitellaria and a median ovary and a posterior group \( (g_a_2) \) which cannot be determined with certainty but probably represents the fundamental male anlagen. I have not been able to make out many details of the excretory system within the body of the worm. The bladder is spherical with a posterior pore and collecting tubules which arise from its anterior margin near the mid-line.

The cercaria of this species develops from the germ-ball stage much more uniformly than does C. macrura Faust (1921 a, p. 16), for in this form the body and the appendages mature more nearly at the same time. In partially developed forms the caudal tube is only moderately long and performs only a slight, sluggish movement, while in the most mature specimens found it beats violently, propelling the worm at a considerable speed. It is apparent that C. macrura represents one line of development in which the tail organs mature long before the worm is expelled from the parthenita, while C. syringicauda represents a type with optimum synchrony of development. C. californiensis occupies an intermediate position. (Cort and Nichols, 1920.)

The cercaria develops in a simple redia which lacks "feet," but has a pharynx and long sacculate gut. Differentiation of parts in the embryonic cercaria takes place early. Growth from this stage into the mature cercaria is conspicuous because of the tremendous increase in size. No encystment of the body per se seems possible, since the body is not provided with cystogenous glands. However, there may be a stage later than those observed when the body is withdrawn into the bulbus and the glandular cells of that
organ then secrete a protective layer around the essential body, under which circumstances the larva could be transferred to the next host.

*Cercaria serpens* nov. spec.

(Plate XXI, Fig. 4.)

The larva, for which the name *Cercaria serpens* is proposed, was found in 3 per cent. of *Viviparus quadratus* (Bens.) examined at Wuchang during June and July, 1921.

The worm is a slowly moving larva, with a body measuring 0·1 mm. long by 0·06 mm. wide, and a broad tail nearly equal in length to the body. The body is entirely covered with minute spines and has a collar constriction with an interrupted series of 26 blunt spines. The tail has lamellate margins extending from the proximal region almost to the tip. In contracted specimens these have a fluted appearance.

The oral sucker is small, measuring 28μ in outer diameter and tapering inwardly like a caper-fig. The acetabulum is nearly isometrical but is sunken into a deep outer disc-like pocket (oo). The oral sucker leads into a long narrow prepharynx. The pharynx lies in the plane of the collar spines and measures 10μ in transsection. Leading inward from the pharynx the oesophagus is seen to consist of glandular epithelium with granular cytoplasm and clear-cut nuclei. This structure extends into the caeca which continue to the subdistal region of the body. The cephalic glands are situated just behind the acetabulum. They are numerous, with granular cytoplasm and clear nuclei and are acidophilic in reaction. The conspicuous elements of the excretory system consist of a rhomboidal bladder with a well-marked pore (ep), collecting tubules arising on the anterior side of the bladder and enlarging in the preacetabular region to accommodate the excretory concrement there, and the tubule system in the tail. The collecting tubules of the body become constricted in the region of the oesophagus and are flexed backward just posterior to the pharynx, to continue backward as the secondary tubules. These secondary tubules are provided with ciliated walls in the vicinity of the acetabulum. At the plane of the bladder the tubules are flexed forward, to continue as the tertiary tubules. On account of the closely massed cystogenous glands throughout the entire body of the larva the details of flame-cell structure could not be made out. Proceeding from the bladder into the tail one observes a short median shank which forks almost immediately to proceed to the lateral margins of the tail, there to open through large pores (see Fig. 4).

The redia possesses a pair of small "feet" which are situated near the posterior end of the body, a collar, a birth-pore, a conspicuous pharynx and an elongate gut with yellowish-brown opalescent content. Although none of the specimens of cercariae observed demonstrated the phenomenon of encystment, this method of transfer evidently obtains, in view of the large number of cystogenous glands in the body of the cercariae.
This species, for which the name *Cercaria cristacantha* is proposed, was found in 0.4 per cent. of *Viviparus quadratus* (Bens.), examined in Peking in the spring of 1921. It has an elongate body, 0.68 mm. in length by 0.25 mm. in width, and a tail somewhat longer, broad and stubby. The entire body is studded with a large number of minute spines and is beset with 24 stylet spines in an interrupted series around the collar. The tail is aspinose.

The oral sucker measures about 50 μ in diameter. The ventral sucker, which lies in the middle of the body, may reach a diameter of 100 μ, although it more usually averages about 80 μ in transection. Within the oral sucker is a short prepharynx which is soon replaced by the pharynx, an oval sphincter about 30 μ in transverse diameter. Behind the pharynx is a long oesophagus which leads backward as far as the pre-acetabular region, where it forks in a characteristic manner, with the furci extending to the subdistal region of the body. The cephalic glands are many and heavily massed. They lie in the middle half of the body, mesad to the excretory tubules. They contain a chromophobic mucoid substance and have small basophilic nuclei. They empty through capillary ducts (*cgd*) lateral to the oral sucker. The genital anlagen consist of two clumps of cells lying mesad, one over the anterior region of the acetabulum, the other anterior to the bladder, with a connecting chain of cells. This system is, indeed, very undifferentiated in the cercaria. The body is provided with cystogenous glands.

The redia is a typical parthenita of the echinostome group, with birth-pore and collar prominence, pharynx and gut. The "feet" are present but are not conspicuous. The region of the integument surrounding the pharynx is studded with coarse sharp spines. Encystment of the cercaria is readily effected by decaudation and the elaborate secretion of cystogenous material to form the heavy cyst wall. The cyst is found throughout the winter and early spring months, long after motile cercaria have disappeared. It is an adaptation, no doubt, for prolonging the period of transmission to the next host.

**The Excretory System of *C. cristacantha*.**

The main features of the excretory system of this species conform to type. There is an oval bladder, with a posterior pore and a pair of large collecting tubules running cephalad. Just anterior to the acetabulum each tubule enlarges to permit of the collection of excretory granules in this region (*eg*, Fig. 5). The tubule bends and is directed posteriad as the secondary tubule. As it again resumes its direction anteriad it gives off the capillaries of the system. The tubule system in the tail suggests an inverted Y, with the median shank and laterals of approximately equal length. It is the ultimate branching of the capillary system that deserves special attention.
A careful study of the flame-cells and connecting capillaries of *C. cristacantha* shows that there are 12 sets of two on each side of the body, making 24 for each side of the body or a total of 48 flame-cells for the larva. The picture of a dichotomous arrangement of flame-cells in the body of an echinostome is altogether unique, all previous records having indicated a trichotomous grouping. On this assumption (Faust, 1919: 334) I have indicated two groups, one consisting of a single cluster of three (α group) and a second containing the α cluster and an additional cluster or series designated as β. Thus, in *C. chisolenata* only the α group is found, while in *C. complexa* the α group (3) and the β group (12) make up a total of 15 flame-cells.

In *C. cristacantha*, however, although an analysis of very young larvae indicates an original α + β condition, subsequent forking of each series behaves in such a way that (1) a trifurcation of each group occurs, (2) followed by an additional bifurcation. Thus the original α + β groups obtain here as in heretofore described species and, primitively also, the 3-flame-cell cluster of each group, but, in addition, a later dichotomy has been superimposed on the original and fundamental pattern. Thus the system may be expressed as

\[ [(2 + 2) + (2 + 2) + (2 + 2)] + [(2 + 2) + (2 + 2) + (2 + 2)], \]

for which the formula \(\alpha^n + \beta^n\) holds. This, on expansion, becomes

\[ \alpha^1 + \alpha^{11} + \alpha^{111} + \alpha^{1IV} + \alpha^{1V} + \alpha^{1VI} + \beta^1 + \beta^{11} + \beta^{111} + \beta^{1IV} + \beta^{1V} + \beta^{1VI}. \]

Moreover, this same pattern has been impressed on the redia (α, β, Fig. 6), for here, too, there is a distinct anterior (α) group and a posterior (β) group for each side of the larva, the two joining to empty through a common excretory pore (ep). Furthermore, here, too, there is the identical pattern which was found to obtain for the cercaria, namely, for each group pairs of capillaries and flame-cells.

The circumstance of this \(2 \times 3 \times 2\) pattern for each α and β group is not, therefore, a variable phenomenon, but rather a sub-group characteristic, the analysis of which adds materially to our conception of the lines of divergence in the group.

**Cercaria leptoderma** nov. spec.

*(Plate XXI, Figs. 7-11.)*

This larva, for which the name *Cercaria leptoderma* is proposed, was found once out of a total of more than 2000 specimens of *Viviparus quadratus* (Bens.) examined from the Peking area. The parasites were taken from the liver gland of the host.

The mature larva is extremely active and of sufficient size to be easily seen without the aid of the microscope. The body measures 0·35 mm. in length by 0·15 mm. in width, while the long, narrow median shank of the tail has a length of about 0·6 mm. and the furci measure about 0·15 mm. The furci are flat, sharp blades. The entire worm is aspinose except for a cap of sharp hooks set on the anterior end of the cercaria.
The oral sucker is a powerful muscular organ, some 60μ in diameter. Within it there is a small anterior chamber, behind which is a pair of oral pockets (Fig. 11), and behind this region a straight narrow lumen which leads directly into the small muscular pharynx. This organ is followed in turn by a narrow oesophagus, which divides soon to form a pair of pouch-shaped caeca. These latter consist of a relatively small number of large glandular epithelial cells, with small condensed nuclei and poorly defined division walls, so that one is reminded of a syncytium. No ventral sucker has been observed. The cephalic glands are confined to the anterior third of the body, outside the caeca. They consist of a single granular acidophilous gland (acg) and a group of six mucoid basophilous glands (beg) for each side of the body. These glands secrete very powerful digestive ferments which are poured out through hollow boring spines (cgd) just in front of the oral pockets.

While this species of furcocercous cercaria cannot be thought of as a mammalian parasite, its adaptability for penetration is shown experimentally. I placed a few of these larvae in a drop of water on the back of my hand for 15 minutes and found that they produced a noticeable irritation with the typical exanthem of the invasion stage of schistosomiasis. This condition lasted for a period of two hours, after which it disappeared. While penetration of the skin was not effected, the ability to irritate exposed human skin was demonstrated. The anlage of the genital organs (ga) is found in a single mass of cells just in front of the excretory bladder. The excretory system deserves special mention.

The Excretory System of C. leptoderma.

In this larva the bladder (b) lies at the posterior limit of the body on top of the proximal end of the tail. It is vesicular, with a pore on the dorsal side somewhat anterior in position. The main collecting tubules of the body arise from the anterior side of the bladder and run forward on the outside of the digestive caeca.

Midway in their course they give off the secondary collecting tubules (ost). After running forward to the anterior region of the caeca they bend mesad and fuse on the mid-line with the median collecting tubule (mel). This latter tubule is itself a fused anterior portion of the inner (anterior) collecting tubules of the system.

The secondary tubules consist of a group of three anterior and three posterior units for each side. Each of these runs inward to a cluster of three capillaries, each having a flame-cell at its inner end. There are, therefore, three anterior groups of three flame-cells and three posterior groups of three flame-cells for each side of the body, making 18 flame-cells on each side, or 36 for the entire body. The outermost (posteriormost) cluster (β111) is found in the tail and performs its function long after the tail is severed from the body. Since the pattern consists of an anterior and a posterior portion the formula is expressed as (3 + 3 + 3) + (3 + 3 + 3) or \( a^1 + a^{11} + a^{111} + \beta^1 + \beta^{11} + \beta^{111} \), and the basic formula is \( a^n + \beta^n \).
Posterior to the bladder is a small central portion of protoplasm, the Island of Cort (Fig. 11, IC), lateral to which a pair of tubules are found with connection to the bladder on the anterior side and common junction with the long median tubule of the tail on the posterior side. This caudal element forks with the furci and opens at the tip of each furcus. It is evidently an accessory tubule assisting in the conduction and expulsion rather than the original collection of excretory wastes.

Additional information relative to the basic structure of the flame-cells and capillaries is found in the successive stages of development of the system in the cercaria. In the germ-ball (Fig. 7) one sees a single flame-cell at the anterior end of the tubule for each side of the body.

As the germ-ball elongates (Fig. 8) the flame-cell and capillary are found to divide, providing the basic $\alpha + \beta$ grouping for the system. In the next stage (Fig. 9) the $\alpha$ and $\beta$ groups have each divided into three. Moreover, the posterior (outer) and anterior (inner) branches of the collecting system have divided but without complete separation, leaving a communicating trunk. In the next stage (Fig. 10) this common tubule has become minimised (ost of Fig. 11), the anterior portion of each of the anterior tubules has become fused with its mate and only the caudal portions of the collecting system remain immature. Finally, in the fully developed cercaria (Fig. 11) the complete picture is expressed.

With the analysis of the system, the question arises as to the relation of the system to the series previously worked out. A comparison (Faust, 1919: 334) shows that it has the same primitive pattern as the cercaria of Schistosoma japonicum (cp. Fig. 8 with Fig. 2 a of Cort, 1918), but with this difference obtaining, that the pattern in the cercaria of $S. \text{japonicum}$ has remained primitive with only a total of 22 flame-cells to represent the formula $\alpha + \beta$, whereas, in the case of $C. \text{leptoderma}$, remarkable modifications have been made and a trichotomy has been imposed twice on the system (cp. with $C. \text{pekinensis}$, a cystocercous form, Faust 1921: 210–211). Thus in this type the complete formula is $(\alpha)^2 + (\beta)^2$, where the factor “3” is indicated for this particular species. The system in this species is an excellent example of divergent evolution.

*Cercaria stylo Buccalis* nov. spec.

(Plate XXII, Fig. 12.)

The xiphidiocercaria, for which the name *Cercaria stylo Buccalis* is proposed, was obtained from 10 out of 13 specimens of *Limnaca plicatula* Bens., obtained from the grounds of The Live Oak Temple in the Western Hills, near Peking.

The cercaria has a body ovate in form, measuring about 0.27 mm. in length by 0.15 mm. in width, and a tail slightly longer than the body. The body is covered with short acicular spines, which are replaced at regular intervals by longer spines of the same kind. The tail is aspinose. The body surrounds the tail at the junction of body and tail, and is provided with a pair of special
Larval Flukes from China

suctorial pockets (lsp). In these pockets there are sharp spinose processes, which assist in locomotion of the body after decaudation.

The oral sucker lies ventrad and measures 64 μ in transverse diameter. The ventral sucker is situated slightly behind the middle of the body and has a transverse measurement of about 72 μ. Placed in the dorsal wall of the oral sucker is a characteristic stylet. The oral sucker leads directly into the spherical pharynx, while the short oesophagus is also reinforced with a muscular sphincter (see Fig.). The caeca are simple pockets which extend to the region of the bladder. Lateral to the pharynx are paired groups of cephalic glands (acg), four in number, with a granular cytoplasm and acidophilic reaction. They empty through small ducts (cgd) at the sides of the stylet.

The genital anlagen have not been observed.

The excretory system consists of a vesicular bladder with enlarged anterior cornua, from which the collecting tubules arise, and a long channel through the middle of the tail, which connects proximally with the bladder. The excretory pore (ep) is the only opening observed for the system. After emerging from the lateral cornu each primary collecting tubule soon divides into five secondary tubules, each of which ends in three capillaries with their respective flame-cells. There are, therefore, 15 flame-cells on each side of the body, arising from five tubules of equal rank. This may be expressed as $3 + 3 + 3 + 3 + 3$, and requires a formula of $a + b + c + d + e$, indicating that the larva belongs to a different group of stylet larvae than any previously reported where the excretory system has been thoroughly analysed (Faust, 1919: 334).

No cystogenous glands have been observed, but the caudal pocket setae (lsp) indicate that decaudation takes place. With it there is probably formed a thin cyst-wall around the larva.

The sporocyst in which *C. stylobuccalis* develops, is a simple sacculate structure completely lacking differentiation.

Cercaria styloidea nov. spec.

(Plate XXII, Fig. 13.)

The larva for which the name *Cercaria styloidea* is proposed, was secured from one specimen of *Viviparus lecythoides* (Bens.), taken from a lily pond at Changsha during June, 1921, and from 0.4 per cent. of *V. quadratus* (Bens.) from Changsha during the same period.

The body of this cercaria is oval and measures about 0.15 mm. in length by 0.1 mm. in width, while the tail is somewhat longer. Both body and tail are aspinose.

The oral sucker is a large spherical mass directed ventrad and has a transverse diameter of 45 μ. The ventral sucker is located far posteriad, and has only one-half the diameter of the oral sucker. The stylet is set in the anterior roof of the oral sucker. It has the characteristic shape but has distinctive reinforcements in the middle of the anterior portion and sides of the posterior
part. The oral sucker leads directly into the spherical pharynx, which in turn opens into the oesophagus. This organ runs a tortuous course to the anterior margin of the acetabulum, where it forks to form two short solid non-functional caeca. With the exception of the bladder the entire series of post-acetabular structures is undeveloped. The cephalic glands consist of two mucoid basophilic and one granular acidophilic cell for each side of the body. These open through separate ducts lateral to the stylet (cgd).

The excretory system is composed of a transversely compressed bladder, with the collecting tubules arising from the antero-lateral angles and an undifferentiated drainage tubule in the tail. The excretory pore (ep) is the only opening of the system which I have observed. The primary collecting tubule branches once, giving rise to an anterior and a posterior secondary tubule. Each of these drains three capillaries with their respective flame-cells. The system is, therefore, exceedingly simple, consisting of the two groups of three cells each, expressed as $3 + 3$ and referable to the formula $\alpha + \beta$. This is extremely suggestive of the flame-cell grouping for the adult brachycoeloid, *Acanthatrium nycteridis* (Faust, 1919b: 210-211) which is $3 + 3 + 3 + 3$; and, moreover, is known to have the formula $\alpha^4 + \alpha^{11} + \beta^1 + \beta^{11}$, with the basic formula $\alpha + \beta$. It is highly probable, therefore, that *C. styloidea* is a larva of the subfamily Brachycoeliiidae, in which the same flame-cell components obtain and where the complete formula is expressed as $2\alpha + 2\beta$.

Finally, this belief is reinforced by an analysis of the digestive system, which has short caeca, and by the late development of the genital organs. These are substantial data for supporting the thesis that relationships between larval and adult trematodes may be predicted with certainty by a careful analysis of the excretory system.

*Cercaria diophthalmica* nov. spec.

(Plate XXII, Fig. 14.)

This cercaria, for which the name *Cercaria diophthalmica* is proposed, was found once in a 1·2 per cent. infection of *Viviparus quadratus* (Bens.) and in a 1·8 per cent. infection of *V. lecythoides* (Bens.) at Changsha in June, 1921. The larva is a styleted worm and has a body covered with long, delicate spines and an aspinose tail. The body measurement averages 0·135 mm. in length by 0·055 mm. in width, while the tail length equals 0·1 mm. The anterior end of the cercaria is provided with a large oral sucker, 35 $\mu$ in diameter, the inner dorsal surface of which has an acute stylet, which consists of base, reinforced stem and tip. The small ventral sucker is situated in the posterior third of the body and measures only 18 $\mu$ in diameter. The prepharyngeal region within the oral pocket is lined with small head glands (pg), which are of a mucoid nature and open anteriad through conspicuous ducts (pgd). Immediately behind the prepharynx is the pharynx, a small spherical sphincter 10 $\mu$ in section. Behind the pharynx is the oesophagus, a long narrow structure which divides to form paired caeca just anterior to the ventral sucker.
There are three pairs of cephalic glands, lying on each side of the ventral sucker. A single, finely granular, basophilic pair lie in the plane of the sucker, while the two coarsely granular acidophilic pairs lie slightly anterior. They discharge their products through long ducts emptying at the sides of the oral opening.

The excretory system consists of a compressed bladder with median dorsal opening, conspicuous lateral collecting tubes which divide in the plane of the ventral sucker to form anterior and posterior branches, and a median caudal collecting tube. Each of these two elements is traceable to two pairs of flame-cells. There are, then, eight flame-cells for each side of the body, arranged as 

\[(2 + 2) + (2 + 2)\]

represented by the formula \(\alpha^1 + \alpha^{11} + \beta^1 + \beta^{11}\), with the basic formula \(\alpha + \beta\). It happens that this expanded formula \((2 + 2) + (2 + 2)\) is the one originally found to obtain for Microphallus opacus (Wright, 1912), and while it cannot be said with certainty that \(C.\) diophthalmica is the larva of the genus Microphallus, it belongs, without doubt, to the same sub-family.

\(C.\) diophthalmica is characterised further by the presence of a pair of pigmentless eye-spots (le), located on the dorsal side in the prepharyngeal plane. There are also pocket spines on the inner face of the caudal pocket (lsp).

The cercaria develops in an elongate sporocyst which is without conspicuous marks of identification.

**Cercaria acrodonta** nov. spec.

(Plate XXII, Fig. 15.)

This larva, for which the name *Cercaria acrodonta* is proposed, was secured from *Viviparus polyzonatus* Frld. and *V. quadratus* (Bens.) at Wuchang during June, 1921. It is a minute fluke entirely covered with delicate spines. It has a sluggish movement, with a lash-like motion to the long heavy tail. The body measures 0·11 mm. in length by 0·07 mm. in width.

At the anterior end of the body is a large oral sucker 44 \(\mu\) in transection. The anterior (dorsal) portion of this organ appears to have a muscular adjustment separate from the larger posterior element, and includes the stylet 12 \(\mu\) long, which is divided into a single median element and paired elytra. The ventral sucker lies in the posterior third of the larva. It has a diameter about one-third that of the oral sucker.

The buccal cavity leads directly into the pharynx, which surrounds the anterior part of an extremely short oesophagus. The caeca are puffy pouches lying in the middle third of the body. The cephalic glands are unique in number and arrangement. They consist of seven pairs in tandem, with a middle basophilic gland, anterior and posterior to which are a pair of acidophilic glands. The anterior two pairs and the posterior two pairs are basophilic. Thus the complete series consists of five pairs of basophilic glands and two pairs of
acidophilic glands. The former have mucoid content and the latter are granular.

Only the reservoir elements of the excretory system have been studied. The median bladder is small, while the cornua are conspicuously large. The excretory pore is surrounded by a strong sphincter.

The larva develops in a simple sporocyst.

_Cercaria circumstricta_ nov. spec.

(Plate XXII, Fig. 16.)

The minute larva, for which the name _Cercaria circumstricta_ is proposed, was found in 2 per cent. of _Viviparus quadratus_ (Bens.) and in 2 per cent. of _V. polyzonatus_ Frild., examined in Wuchang in June, 1921.

The body of the cercaria is broadly oval from the anterior aspect and truncate posteriorly, and measures 0.088 mm. in length by 0.074 mm. in broadest cross-section, while the tail is an elongate cone. The animal is propelled by the sluggish lashing of the caudal organ. The body is covered with delicate spines. There is a constriction of the body just behind the oral sucker. The latter organ is 26 μ in diameter and is surrounded anteriad by a crown of 20 sharp reversed hooks. No hooks or spines have been found in the region of the nuchal constriction. There is a short prepharynx anterior to the pharynx and an oesophagus mesad to the pharynx. The caeca arise from the oesophagus and extend to the subcaudal region of the body. The ventral sucker lies somewhat behind the mid-region of the body and is only slightly smaller than the oral sucker.

The excretory tract centres in a flattened bladder with postero-dorsal pore and with collecting tubes emptying into it from the antero-lateral aspect. These tubes are distended and contain concretions which are highly refractive. On reaching the region of the pharynx the tube becomes constricted and flexes backward, giving off in its course three secondary tubes. Each of these tubes leads into a series of three capillaries, each with a flame-cell at its head. The flame-cells are relatively large and have a few thick cilia. Thus the number of flame-cells for each side of the larva is 9 and the probable factoring is $3 + (3 + 3)$, with the formula $a + b^2$ obtaining. Leading from the bladder into the tail is a conspicuous duct which forks in the proximal fourth and opens laterad about a fourth distance from the posterior end of the tail.

A portion of the parenchyma is filled with cystogenous glands (eg) which contain minute elongate granules. These lie in two series on each side of the body. The intervening spaces are occupied with many cephalic glands, which empty through ducts around the oral sucker. It seems highly probable that the larva encysts by use of the former variety of secretory apparatus, while the latter serve in the digestion of host tissue after ingestion of the cyst.

The generative anlagen were entirely obscured by the secretory glands.
C. circumstricta develops in a redia with small pharynx and a long orange-coloured gut. The larva has many points in common with the echinostome cercariae, but since it lacks the incomplete collar of spines in the neck region and has a complete ring in the anterior oral aspect, there is hesitancy in placing it in the group. It may represent a transition to some group of leptocercous larvae.

**Cercaria photifera** nov. spec.
(Plate XXII, Fig. 17.)

This larva, for which the name *Cercaria photifera* is suggested, is a leptocercous form with pear-shaped body and a tail with fluted margins. It was found as a 2 per cent. infection in *Viviparus polyzonatus* Frld. at Wuchang in June, 1921. The body is completely covered with delicate spines. The anterior end is snout-like. On the dorsal face close to the pharynx are a pair of pigmented eye-spots (*le*) with the pigment cup posterior. The body measures 0.186 mm. in length by 0.114 mm. in width, while the tail is nearly a third longer. The oral sucker measures 30 μ and is directed anteriad. The ventral sucker which is 22 μ in diameter lies mesiad. The pharynx is uniquely large, measuring 30 μ in cross-section. The bladder consists of a poorly defined mid-region and large muscular cornua. Details of other parts of the excretory system have not been observed.

Practically the entire body is filled with about 36 cephalic glands (*beg*) with fine granular contents and basophilic reaction. These empty through a corona of ducts (*cgd*) surrounding the sucker. They are tipped with sharp hollow spines. It is evident that these glands which occupy so large a mass of the larva, play an important part in the migration to or within the next host. They obscure the whole central region of the worm, making it impossible to make out the underlying or intermediate organs and tissues. The difficulty is further accentuated by the presence of a brown pigmentation over the surface (omitted in the figure).

*C. photifera* develops in an irregularly branched sporocyst without any particular differentiating characters.

**Cercaria chromophila** nov. spec.
(Plate XXII, Fig. 18.)

The larva, for which the name *Cercaria chromophila* is proposed, was found in a 0.6 per cent. infection of *Melania ebenina* Brot. collected at Kiukiang in August, 1921. The fluke has a long body with cylindrical contour and a heavy tail which is provided with dorsal and ventral integumentary folds giving a graceful sluggish movement to this organ. The body throughout is aspinose. On the dorsal side there is a pair of pigmented eye-spots somewhat posterior to the pharynx. The entire body is covered more or less with a mottling of brown pigment which is especially marked in the region around the eye-spots. At the distal end of the worm just lateral to the portion of the tail included
within the body is a pair of lateral suctorial pockets (Isp). The body is 0.16 mm. long by 0.065 mm. in diameter, while the tail is 0.3 mm. long.

The oral sucker constitutes an anterior protrusion of the body, lying almost entirely anterior to the body proper. It has a transverse diameter of 30μ and opens through an extremely small anterior pore. Behind it is a short pre-pharynx followed by a small muscular pharynx. Posterior to the pharynx lies the long narrow oesophagus which disappears within the group of cephalic glands and cannot be traced to its distal termini. The cephalic glands consist of huge masses of mucoid cells filling the middle third of the body. The ducts parallel the digestive tract and open laterad to the oral suctorial pore. The excretory system consists of a singularly small central bladder with dorsal pore, and three main canals, two running antero-laterad in the body and one through the middle of the tail, terminating in a pore at its distal end. This caudal canal seems, therefore, to be a drainage rather than collecting tube. The flame-cells and capillaries of the system have not been made out. The genital anlagen consist of a group of undeveloped cells lying medially behind the cephalic glands. The ventral sucker is vestigial, containing only glandular elements.

*C. chromophila* develops in a simple sacculate redia, which lacks feet, birth-pore or any other differentiating features except a well-developed pharynx and small rhabdocoel gut. Small cystogenous granules are found throughout the body. While cyst formation has not been observed it seems highly probable that this process occurs and that the distomule is transferred passively to the next host.

*Cercaria abbrevicauda* nov. spec.

(Plate XXII, Fig. 19.)

This microcotylous larva, for which the name *Cercaria abbrevicauda* is suggested, is ovate in outline with a small knob of a tail consisting of undifferentiated parenchyma. The body measures 0.1 mm. in length by 0.06 mm. in width and is covered with minute spines. The larva was found in a 0.6 per cent. infection of *Melania ebenina* Brot. collected at Kiukiang in August, 1921.

The oral sucker is the largest organ in the body. It measures 40μ in transverse diameter and is slightly longer than broad. The ventral sucker is less than half this diameter. The dorsal lip of the oral sucker is much thicker and more pronounced than the lower lip. There is a mucoid stylet inserted in the dorsal lip, which can be readily protruded and retracted. Within the oral atrium is a small pharynx which has lost its muscular function but serves as the focal centre of mucoid pharyngeal glands (pg) which envelop the area. The remaining parts of the digestive tract, consisting of oesophagus and caeca have not been found after careful search. Cephalic glands are of two types. There are paired groups of probably ten mucoid basophilic glands strung in tandem arrangement from pharynx to excretory bladder. In the pre-acetabular region there are also four pairs of granular acidophilic glands. All of these
glands have ducts opening at the sides of the stylet on the dorsal lip of the oral sucker. The only portion of the excretory system observed is the bladder which is oblong-ovate in contour and has heavily thickened walls and a distal pore. The genital cells are relatively well differentiated, with posterior, median and anterior elements.

The redia in which the cercaria develops is a simple organism with simple contour and a digestive tract consisting of muscular pharynx and vestigial gut. The cercariae mature in large numbers in the rediae.

A comparison of this cercaria with that which Kobayashi (1921) has described as the larva of Paragonimus westermanni, shows certain striking resemblances. While C. abbrevicauda is described as smaller, the relative proportion of suctorial organs, type of stylet, excretory bladder, types of cephalic glands, spinose integument, tail, and even the redia, are all evident signs of relationship. In both Kobayashi's material and my own the differentiation of the two types of cephalic glands was readily effected although the exact number of each was not easily made out. It is important to note, also, that the larva is found in a species of the mollusc genus Melania. In view of the known records of paragonimiasis in the mountains of Kiangsi and Hunan Provinces in Central China and the similarity of my material with Kobayashi's, it is not unlikely that this represents the larval stage of the fluke, described for the first time from China.

**Discussion.**

The species of cercariae described in this paper include the more common groups which one usually obtains from representative samplings in any area surveyed, such as trioculate monostome, oculate amphistome, echinostome and stylet cercariae. As the study of these larvae progresses subdivision of these larger groups on the basis of fundamental characters becomes more and more possible. Even though the echinostome group is a natural one with relatively clean cut characteristics, members of the group have differentiating features which can be determined by a careful study of the larva. The classification on the basis of flame-cell pattern clearly proves the applicability of such a method. On the other hand, the "stylet cercariae" are an artificial group, only a few species of which have known correlations with adults. The simpler xiphidio-cercariae belong to at least three groups, Allocruciidiidae, Plagiocruziidae and Brachycoeliidae, while the leptocercous and microcercous forms with a buccal stylet represent entirely different groups. It is thus seen that the stylet is an organ having a wide adaptation, an organ probably originally of definite use in penetrating host tissue. In some species it may still serve such a purpose, but in others a new method of entry into the host has been effected, namely, passive admittance of the encysted agamostome with food. In this latter type the stylet never comes to function but is included in the cyst and soon degenerates. It is noteworthy that in all instances where stylets have been recorded for cercariae paired cephalic glands, emptying
through hollow spine-capped ducts at the sides of the stylet, have also been observed. This is definite proof of the combined action of abrasion and chemical erosion utilised primitively by the animal to invade the host.

Yet the cephalic gland is not a unique accompaniment of the stylet, for it occurs in the furcocercariae and non-styleted leptocercariae and in the echinostome larvae. The study of known species of cercariae has been too fragmentary to formulate any broad statement regarding the specificity of the cephalic glands in various groups. As far as is now known, their number is specific for mammalian schistosome larvae. In the two brachycoeliid larvae, \textit{C. styloidea} (Fig. 12) and \textit{C. diophthalmica} (Fig. 13), belonging to different subfamilies, there are paired clusters of three such cells, differing in intimate structure and chemical secretions, but fundamentally the same. It is impossible to conceive how these three cephalic glands can be even generically diagnostic in this group.

Ontogenetically the organs of the larva are important in as far as they develop into organs functioning in the adult worm; phylogenetically they are of significance in as far as they relate the organism to other similar organisms in the past; systematically they are of value only in as far as they show by inspection the relation of larva and adult. For the last-named purpose differentiation within the groups is highly desirable. Thus far, the only two systems which have furnished evidence of such unique properties are the excretory system and the cephalic glands. All other systems investigated have proved too immature, too generally distributed or too ephemeral in their nature to be of fundamental importance in relating larvae to adults.

It seems highly probable that careful study of cercariae will make it possible in the near future to relate entire series to adult groups and, what is more important, establish natural groups where artificial groups now exist.

\textbf{Summary.}

1. Collections of flukes made in several centres in China include 25 species of cercariae.
2. Fourteen of these species are described as new.
3. Details of the excretory system are presented for six of the species described. In two of these instances (echinostome larvae and furcocercariae) differentiation within the natural group is demonstrated, while in two other larvae, \textit{C. styloidea} and \textit{C. diophthalmica}, proof of relationship and correlation with adult groups is established.
4. \textit{C. abbrevicauda} shows striking resemblances to the larva described as the cercaria of \textit{Paragonimus westermani} and may possibly be regarded as identical with the larval stage of that worm. Moreover, the host genus is the same.
5. The stylet organ is shown to be non-specific and the group of the "xiphidio-cercariae" to be a composite of several natural families.
6. No organs of the cercaria save the cephalic glands and flame-cells have thus far proved satisfactory for differential diagnosis of the larva.
7. Further study along these lines will make it possible to demonstrate natural groups where only artificial groups now exist.

**EXPLANATION OF PLATES XXI—XXII.**

Note. The line at the side of each figure has a value of 0.01 mm.

**PLATE XXI.**

Fig. 1. Cercaria plana, ventral view. ×145.
Fig. 2. Cercaria nervosa, ventral view. ×190.
Fig. 3. Cercaria syringicauda, dorsal view, showing body, syringe bulb and terminal excretory tube. ×236.
Fig. 4. Cercaria serpens, ventral view. ×440.
Figs. 5, 6. Cercaria cristacantha. Fig. 5, ventral view of the cercaria. ×90. Fig. 6, lateral view of the redia. ×34.
Figs. 7—11. Cercaria leptodera. Figs. 7—10, developing stages of the larva showing differentiation of the excretory system. Fig. 11, detail of the mature cercaria. ×200.

**PLATE XXII.**

Fig. 12. Cercaria stylobuccalis, dorsal view. ×170.
Fig. 13. Cercaria styloidea, dorsal view. ×256.
Fig. 14. Cercaria diophthalmica, dorsal view. ×338.
Fig. 15. Cercaria acrodonta, dorsal view. ×398.
Fig. 16. Cercaria circumstricta, dorsal view. ×486.
Fig. 17. Cercaria photifera, dorsal view. ×245.
Fig. 18. Cercaria chromophila, ventral view. ×358.
Fig. 19. Cercaria abbrevicauda, ventral view. ×350.

**LEGEND FOR FIGURES.**

acg, acidophilic cephalic gland; b, excretory bladder; beg, basophilic cephalic gland; cb, caudal bulb of excretory apparatus; ce, caecal epithelium; cg, cystogenous glands; cgd, cephalic gland ducts; cp, caudal excretory tube; eg, excretory granules; ep, excretory pore; ga, ga₁, ga₂, genital anlagen; IC, Island of Cort; ict, inner collecting tubule; le, lateral eye; lp, lateral excretory tube; lsp, lateral suctorial pocket; mel, median collecting tubule; me, median eye; os, outer acetabular disc; os, oral sucker; ost, origin, secondary collecting tubule; pg, pharyngeal gland; ph, pharynx; ppgd, pharyngeal gland duct; pp, pharyngeal pocket; s, stylet.

**REFERENCES**


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TABLE I.

Infection-Incidence of Flukes in Molluscan Hosts from Five Centres in China,
1920—1922.

<table>
<thead>
<tr>
<th>Hosts (1—12) and Parasites</th>
<th>Peking</th>
<th>Yü Tai Hô (Shansi)</th>
<th>Wuchang (Hupêh)</th>
<th>Changsha (Hunan)</th>
<th>Kukiang (Kiangsi)</th>
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<tr>
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CONSIDERATIONS ON THE LIFE-HISTORY OF TAPEWORMS OF THE GENUS *MONIEZIA*.

By F. W. FLATTELY, M.Sc.,

*Lecturer in Zoology, University of Durham.*

The following account of observations and experiments in connection with the life-history of *Moniezia* tapeworms, though embodying only negative conclusions, is put forward in the hope that it may mean a certain amount of ground cleared for the benefit of other workers.

As is well known, the *Moniezia* tapeworms are parasitic in various species of herbivorous animals, mostly Ungulates, but are particularly common in the domestic sheep, from which they can be obtained in large numbers in the public abattoirs of any big city. Previous to 1915 a certain number of experiments had been carried out in connection with this problem but all with negative results. A few zoologists had also put forward suggestions, but these had been either insufficiently tested or had been more or less definitively shown to be incorrect.

The genus *Moniezia* was established by Blanchard in 1891 to include 13 species of unarmed tapeworms occurring in *Bos*, *Ovis*, *Macropus*, *Lepus* and *Arctomys*, and revised by Stiles in 1893. Stiles's emended diagnosis is as follows:

*MONIEZIA* R. Bl., 1891. Char. emend. Head without hooks, segments generally broader than long and longer than thick, end segments showing a tendency to become longer and narrower. Two full sets of genital organs, with two uteri and two lateral pores in each segment. On the right side the vagina is ventral, cirrus dorsal; on the left side vagina dorsal, cirrus ventral. Dorsal canal lies dorso-median of ventral canal. Genital canals cross the longitudinal canals and nerves dorsally. Interproglottidal glands generally present. Calcareous bodies absent from parenchyma. Eggs with well-developed pyriform body.

Stiles recognises the following species as belonging to this genus: *M. planisima*, *M. Benedeni*, *M. Neumannii*, *M. expansa*, *M. oblongiceps*, *M. trigonophora*, *M. alba* and *M. denticulata*. The host species include: Domestic cattle (*Bos taurus*), Zebu (*Bos indicus*), Goat (*Capra hircus*), Spanish Ibex (*Capra pyrenaica*), Roe Deer (*Cervus capreolus*), Pampas Deer (*Cariaecus campestris*), Cariacus paludosus, Cariacus nambi, Brocket (*Cariaecus rufus*), Cariacus simplicicornis, *Cariacus* sp.?, Gazelle (*Gazella dorcas*), Musk-ox (*Ovibus moschatus*), Domestic Sheep (*Ovis aries*), Chamois or Gemse (*Antilope rupicapra*).
In addition to *Moniezia*, Stiles recognises two related genera of tapeworms as having representatives in cattle, sheep and allied animals, viz. the genus *Thysanosoma* (Diesing, 1834) and *Stilesia* (Railliet, 1893), neither of which has so far come under my notice in Britain.

It is remarkable that notwithstanding the wide distribution of the various species of *Moniezia* and the commonness of their occurrence, the life-history has not in any single instance been worked out. The same is true of the other two genera of tapeworms found in herbivorous animals, viz. *Thysanosoma* and *Stilesia*. Doubtless our ignorance is to be attributed rather to lack of investigation than to anything inherently peculiar in the development of these forms, attention, naturally enough, having been focussed mainly upon the species directly affecting man or coming more closely under his notice. From an economic point of view alone, however, the life-history of *Moniezia* tapeworms deserves the closest attention, since the total loss to the country, owing to depreciation in the value of the lambs affected by these parasites, must be considerable.

Suggestions which have been put forward hitherto as to the life-history of *Moniezia* have been made from two distinct view-points. Either they require an intermediate stage in the body of some invertebrate animal or they assume that the adult tapeworm may develop directly from the egg without change of host. The first view is the one most generally held since it accords with our knowledge of the great majority of other tapeworm histories. The latter view has been supported principally by Mégnin and Curtice and, in spite of its apparent improbability, should not be hastily rejected. It has been opposed by Moniez (1879) and Railliet (1880) among others, the former, however, not altogether excluding the possibility of a direct development in the one host. Experience shows that it is rash to dogmatise about tapeworm life-histories. Grassi, for instance, in 1889, was able to infect mice with *Taenia murina* by feeding them on the eggs, the cysticercoid stage appearing in 3–4 days in the intestinal villi and the adult *Taenia* in 15–30 days. Recently a case came under my own notice of a soldier who had developed literally scores of cysticercoids of *Taenia solium* as a result of having accidentally ingested the eggs of this form (see Hughes, 1921). On the other hand, attempts made by Curtice, Stiles and recently by the writer to produce direct infection have all been fruitless. The thought suggests itself, however, that before finally rejecting the direct infection theory, as supported by Mégnin and Curtice, it would perhaps be advisable to determine whether some form of maturation is not necessary before the eggs will develop. In natural conditions the proglottids would hardly ever be ingested immediately they are dropped. They would probably lie about in the faeces or on the grass for some time.

Another consideration presents itself with regard to this question of direct infection, viz. the multiplicity of closely related species affecting the same host. Are we to regard this as evidence against the direct infection theory? One feels tempted to do so and to associate each particular species of *Moniezia* with
Life-history of Moniezia

A corresponding species of intermediate host: for the sake of argument, let us say, with particular species of dung beetle (Aphodius), remembering in this connection that the invertebrate host is, probably, historically the earlier and was at one time, possibly, the only host. Nevertheless, we have to bear in mind the fact that several distinct species of Strongylus, for example, which all develop directly, often occur in the same sheep and occupy precisely similar positions in the gut. We can possibly explain this fact by assuming various species of Strongylus, or of Moniezia, to have become specialised originally in connection with distinct species of wild Ungulates and to have severally adapted themselves to the sheep later on, when it came under domestication. Whether or not, then, the fact that several distinct species of Moniezia occur in the sheep affords a clue to the nature of the life-history must be left to the judgment of the individual worker.

Some experiments performed by Curtice as long ago as 1888 merit attention, but they were unfortunately too loosely controlled and indecisive to furnish reliable conclusions.

Neglecting complicating factors, we may say that Curtice was able to obtain infection among some of the lambs of a small flock under conditions such that “none of the invertebrates which are usually suggested as being the intermediary bearer were present.” The most important of these conditions were as follows: accommodation, a small stable with adjoining, very dry, hill-side pasture; food, clover and grain from the market; drinking water, from an adjacent well. Curtice, however, makes no mention of ectoparasites, some species of which, in the conditions of his experiment, might well have been responsible for conveying the infection, nor of coprophagous forms; in short, there is no real ground for his claim to have disposed definitively of the idea of an intermediate host.

In 1893 Stiles reported only negative results from a series of experiments in connection with the life-history of Moniezia. These included attempts to produce direct infection by feeding eggs to sheep, and experiments to produce an intermediate stage in Melophagus ovinus, numerous coprophagous insects and earth-worms. He gave as his conclusion that some insect, worm or snail would eventually be found to contain the larval stage, and announced his intention of continuing the experiments on a larger scale. Apparently, no account of these later experiments was published.

With regard to Melophagus ovinus, this ectoparasite had already been suspected as a carrier, and examined, by McMurrich (1884), in connection with a bad outbreak of tapeworm disease (M. expansa) in Manitoba. He found nothing incriminating.

In 1915, Miss Lebour published an account of experiments she had conducted in feeding proglottids of Moniezia to the slugs Agriolimax agrestes and Arion circumspectus. Her results were negative, the eggs, although readily ingested by the snails, failing to develop, and being recovered unchanged in the faeces. These were the last experiments previous to those of the writer.
Among much that is doubtful in regard to the life-history of species of *Moniezia* one fact seems quite clear and provides a valuable hint from which the investigation of the problem may perhaps most profitably be tackled. It is the tender age at which adult, fully grown worms are found in the lamb’s intestine. In America, according to Curtice (1890), individuals of *Taenia (Moniezia) expansa* are found measuring 2–5 yards long in lambs from 2–4 months old. This is one of the first points to which I directed my attention and I found that on a farm close to Aberystwyth proglottids began, in 1914, to appear in the faeces of lambs on April 10th, approximately six weeks after the lambs had been dropped. (I have since observed proglottids in the faeces of a lamb not less than four, and not more than six, weeks old.) On April 28th I observed a string of proglottids some 12 inches long in the faeces of a lamb. These showed the ovaries and yolk glands fully developed, but the uterus had not begun to spread. It is evident that for the worm to attain this condition in so short a period infection must take place either at, or very shortly after, birth. If we accept provisionally the idea of an intermediate host, then it is evident that the creature—slug, earth-worm, beetle, tick, louse, mite or what not—must be present on the pasture at lambing time, and, moreover, must be present in considerable numbers, for it is not an isolated lamb that is infected but practically all the lambs of a flock. Further, most lambs harbour not one worm but a number. In Britain, however, the variety of invertebrate life to be observed upon a pasture at lambing time, is, generally speaking, so small that if an intermediate host really does occur, the task of finding it should not be one of extraordinary difficulty.

A first visit to the above-mentioned farm near Aberystwyth was employed in examining, as an obvious preliminary step, the udders and wool of a number of ewes for parasites, but none was present. The udder region was scraped with a knife and the resulting epidermal scales, dirt, etc., were removed to the laboratory for microscopical examination. The object of this was to establish the possibility of tapeworm eggs or larval forms being transferred from the pasture to the belly of the ewe, and thence being ingested by the lamb when sucking. For, assuming that infection should occur directly from the egg, the lamb would hardly pick up the latter immediately from the pasture since in the first few weeks of its life it does little more than nose the grass in a very superficial manner.

The result of this microscopical examination was also negative. Next, grass was collected from the pasture at random, washed thoroughly, and the washings examined for possible larval forms. The result was negative. The same water was allowed to stand and was re-examined from time to time, but still without result.

On March 12th a number of specimens of the slugs *Agriolimax agrestis* and *Arion hortensis* were collected from the pasture and dissected on the chance of their furnishing an intermediate stage, particular attention being paid to the pulmonary cavity, the wall of which was cleared and examined micro-
Life-history of Moniezia

scopically. Result, negative. Shortly before this, as already noted, Dr Lebour
had conducted experiments on feeding proglottids to various slugs and snails
but had not been able to get the eggs to develop. My own conviction is that
in spite of their appetite for tapeworm proglottids (see Lebour, 1915 and Grassi
and Rovelli, 1892), they are very unlikely hosts for the parasite since in the last
resort the lamb would require to swallow them, and this, for the reason we
have seen above, it would be very unlikely to do. On the other hand, of course,
it is always possible that, as noted by Miss Lebour, the eggs may undergo
some alteration during their passage through the slug’s intestine which, though
not apparent, may influence their development in other ways. Slugs are not,
however, by any means the only animals capable of rendering such a service
to the tapeworm. For instance, my colleague Mr C. L. Walton and myself
once derived considerable amusement from watching hens busily engaged in
swallowing tapeworms lying on the floor of the Aberystwyth slaughter-house.
In thinking over the various invertebrate animals capable of acting as an
intermediate host, one cannot avoid being struck by the fact that while, as
was already remarked, the variety of invertebrate life visible on a pasture
at lambing time is very small yet one class of animals at least is exceedingly
well represented, even at that early period in the year. These are the many
coprophagous forms which live in sheep and cattle dung, especially Aphodius
spp., small Staphylinids (Aleocharis) and mites. I have repeatedly seen species
of Aphodius feeding in sheep dung which contained tapeworm proglottids and
it seems inevitable that they should ingest tapeworm ova. It was not till
some years later, however, that I was able to explore this possibility further
and my conclusions concerning it are given later in this paper.

The remainder of a short research period in Aberystwyth was devoted to
testing the possibility of the lamb becoming infected directly from the mother
ewe, while sucking. In view of the early age at which lambs are infected it
seemed not unreasonable to make the following hypothesis. Sheep when
feeding ingest the ova which lie about the pasture. These ova then develop
into hexacanth embryos which, in the case of the ewes, make their way, or
eventually get carried, to the region of the udder. There they may develop
into larval forms and remain till the ewe drops her lambs when they
are passed to the lambs while sucking. The feeding process is a very energetic
one on the part of the lamb and might well provide the stimulus necessary
to liberate the larval Moniezia from the tissues in which it is lying into an
adjacent milk duct and thence into the teats. There is nothing to prevent such
a course of events provided the larva is of minute size, which it may quite
well be. We may note, for instance, that Curtice and, later, Hassall, found
numbers of very young forms of an unarmed tapeworm—probably Cittotaenia
variabilis—in rabbits, which were less than 1 mm. in length, one being as small
as 0.304 mm.

Obviously, the experimental work required to test such an hypothesis
ab initio would need to be of a very careful kind. A short cut would be effected
if the presence of the larval forms could be demonstrated microscopically in
the udder of a ewe selected from what was known to be a regularly infected
pasture. An opportunity to do this was afforded by the death of a six year
old ewe at the farm under investigation. The udder was accordingly removed
and a careful microscopical examination of it was made. Nothing was found
in this way, which was perhaps only to be expected. Next, small snips of udder
tissue were taken at various points, embedded and sectioned. For a time hopes
were raised by the discovery in these sections of stages in the life-cycle of
some organism or other encysted in the udder tissue. These cysts seemed more
frequent in the portions of tissue taken high up the udder, in the neighbourhood
of the larger branches of the lacteals and blood-vessels. They were thought
to have some resemblance to hexacanth embryos. Examination under the
highest powers of the microscope, however, failed to reveal any spines, which
would definitely determine their character. What under the lower powers
seemed to resemble spines turned out to be tears or folds in an internal mem¬
brane. I now hold the view that these are stages in the life-history of some
Protozoan parasite.

In pursuance of the same idea a quantity of milk from the ewes on this
farm was examined microscopically, but failed to give anything of interest.
This is obviously a very slow process and various methods of speeding it up
were tried: evaporating, centrifuging, etc., but always without result. It was
of considerable interest to notice that the small intestine of the old ewe already
mentioned contained several tapeworms all of which were in a very ill-nourished
condition, quite unlike those occurring in young lambs. How long the ewe
had harboured these worms it is, of course, impossible to say. It is well known
that infection with tapeworm only reaches serious proportions in young lambs:
shepherds will say that they hardly ever notice proglottids in the faeces of
older sheep and, similarly, butchers hardly remark the presence of worms in
older sheep slaughtered at the abattoirs. Had these emaciated-looking worms
hung on from the early period of the ewe’s life or were they acquired sub¬
sequently? From the point of view of the life-history it is of capital importance
to establish whether infection is contracted only in the period before the lamb
is weaned or continuously all through life. If the latter is the case then it
would effectually dispose of the idea that the lamb acquires infection through
its mother, and the fact that the worms are so much less often observed in older
sheep would simply mean that the conditions for the development of the tape¬
worm are then less favourable, owing to the substitution of an herbivorous
diet for one of milk. Speaking of the occurrence of *M. expansa* in the United
States, Cooper Curtice (1890) says that

the worm, though present throughout the year, is more abundant in the locality of Wash¬
ington during May and June than at any other season....In Colorado an outbreak was heard
of in a flock of Merinos which occurred annually about July and August, after which the
lambs would improve. The disease is more prevalent in the summer season, and causes the
greatest damage in lambs less than six months old. If the young animals can be carried beyond
this age they seem to be either better able to withstand the ravages of the parasite, or
Life-history of *Moniezia*
to have reached a season unfavourable for its development....The broad tapeworms do not last long in their adult state but after maturing all their segments are shed at once. From the time that the segments are shed the afflicted lambs will begin to receive and rapidly lay on fat.

The above remarks of Curtice's on the occurrence of *Moniezia* in the States describe exactly the conditions prevailing with regard to the disease in this country, and the recrudescence of the trouble yearly in early summer goes some way towards supporting the view that the lambs are infected only at, or soon after, birth, and do not become re-infected later. It may also mean, of course, simply that the conditions for the development of the parasite in the older sheep are less favourable. The writer does not altogether agree with Curtice's statement that the worms are at once shed after maturing all their segments, but inclines to the view that in many cases the scolex will hang on for a considerable period and make an attempt at maturing fresh segments.

On the whole, investigations at Aberystwyth left the writer without any particular conviction as to what lines future research should follow, but with the feeling that two hypotheses merited careful testing. They were (1) that infection may be conveyed from the mother to the lamb when sucking, the intermediate stage being assumed to be present in the ewe's udder; in other words that intermediate and adult stages occur in different parts of the same host, and (2) that infection may be conveyed through the intermediary of some coprophagous mite or beetle which is picked up by the lamb, directly, when nosing the grass or, indirectly, from the fleece of the mother-ewe when the lamb is sucking.

The following notes were made on the occurrence of tapeworms in lambs approximately three months old from the Aberystwyth slaughter-house, and serve to illustrate what has been remarked above regarding the early age at which lambs become infected, and also to indicate that the whole of the infection does not take place simultaneously but that re-infection occurs at intervals.

June 9th, 1915. "String" of lamb from slaughter-house. Five individual tapeworms were found. The specimens varied considerably in size and degree of maturity. In one case the proglottids reached a size of 11 mm. and mature ova were present in segments situated approximately 120 mm. behind the scolex, the number of segments in this length being roughly 530. The remainder showed no ova at 275 mm. behind the scolex, in which length there was approximately the same number (530) of segments, the latter being a little over twice as long as in the previous case.

Same date. String of lamb from slaughter-house. Two worms, one with fully mature proglottids, broad and extremely short, the other with appreciably longer proglottids and no mature ova.

June 11th. String of lamb supplied by local butcher. Seven individual worms. There is a considerable difference in appearance among the worms of
this batch. In some the "neck" is extremely long and thin, and it is only beyond a distance of several inches that the proglottids begin widening out. In others the neck is very short and the proglottids rapidly become very broad.

June 12th. In the string of a single lamb from the slaughter-house on this date, I found 75 individuals, the aggregate length of which was 150 feet. The worms showed the same differences in appearance as those in the preceding batch. There is no doubt about the extraordinary degree of infection in this case, as 75 was the number of scolices found. Curtice states that the number of individuals occurring in the same intestine may be from two or three to a hundred, but that it is unusual to find more than half a dozen adults together. He himself observed as many as 14 adult worms in a lamb four months old.

According to observations made at the time, the above worms belonged to one or other of two species, viz. *M. expansa* and *M. trigonophora*, the former being much the more numerous. If my identification of the latter species is correct then the two species occurred together in the same lamb. The identification was based on microscopic characters and not on externals which, as Stiles has pointed out, vary tremendously according to the age of the worm and the state of contraction. Thus, *M. expansa* may show precisely the differences which have been above noted, viz. in some individuals the neck may be extremely long and thin, the proglottids widening out very gradually, while in others the neck is very short and the proglottids become quite broad only a short distance behind the scolex. The characters which led me to identify some of the individuals as *M. trigonophora* were mainly (1) the presence of interproglottidal glands grouped around blind sacs, and (2) the arrangement of the testicles in two triangles, one at each side of the segment. I am anxious, however, not to attach too much importance to the occurrence of *M. trigonophora* in Wales, since I have not since found it elsewhere, and since also the triangular arrangement of the testicles is not absolutely decisive, *M. expansa* itself occasionally showing the same arrangement in certain segments.

In addition to the specimens above noted which were obtained by myself from the Aberystwyth abattoirs, I was later able to examine a large batch procured for me from the same source through the kindness of Mr T. A. Stephenson, M.Sc. These proved without exception to be specimens of *M. expansa*. A few notes on these are appended. In Nov. 1919, I further received from Mr C. L. Walton, M.Sc., a strobila from a lamb in all probability reared in Carnarvonshire and grazed in Anglesea. From the shape of the proglottids (longer and narrower than those of *M. expansa*), position of the genital pores, absence of interproglottidal glands and, finally, the character of the scolex, I have no hesitation in putting this specimen down as *M. alba*.
OBSERVATIONS ON THE OCCURRENCE OF MONIEZIA IN THE ROMAN CAMPAGNA.

When in Rome I was able to make a few observations on the occurrence of tapeworm in this area which may prove of interest. At the outset I was inclined to think that the disease would be of relatively rare occurrence on the wide and rough pastures in the neighbourhood of Rome. The contrary proved to be the case.

So far as my inquiries went there was relatively little infection on the mountain pastures and sheep walks in Wales, and the explanation of this I took to be that the rough nature of the pasture and the wide area over which the sheep roam would mean that the eggs were less likely to be encountered by a fresh host. The same argument might reasonably be considered applicable to the Roman Campagna. The following fact, however, destroys the analogy. The flocks in the Campagna are large (two or three hundred head or more) and though they pasture over large areas under the care of a shepherd, they are regularly rounded-up at night and closely folded until well on in the following morning. In order to discover whether infection exists it is only necessary to visit the spots where the animals have spent the night and where quantities of faeces have collected. Except in one or two cases, lambs of two, three and four months old were always found to be infected. There is thus ample scope for infection, either directly, or by means of an intermediate host. Even if infection does not occur at the time (owing, say, to the period of the flock's stay being too short to allow of infection by means of an intermediate host), nevertheless these areas, being literally saturated with eggs, may well serve as centres of infection which the sheep will be almost bound sooner or later to cross again.

The same ideas regarding the conditions of infection prevail as in England, viz. there is a marked tendency to associate it with wet pastures. I have always tried to resist an undue bias in this direction since the judgment seems to be based on the general tendency to connect all ailments, human and otherwise, with damp situations and is not the result of observation. There was no difficulty in obtaining evidence of infection from wet localities, but I had great trouble in persuading the laboratory attendant who was helping me to accompany me to a portion of the Campagna which could be regarded as really dry. On our first visit to such a district we found a large flock of lambs about four months old to be infected. Unfortunately, it is extremely hazardous to draw any conclusions from this fact as the flocks in the Campagna are continually being moved about, so that the spot at which one finds evidence of infection at any particular moment may be at a considerable distance from the one where infection was contracted. Lambing in the Campagna takes place as soon as the sheep return from the hills, i.e. September, and continues until January and to a certain extent until February. It was, unfortunately, quite impossible to get the history of any one flock and to keep in touch with it,
as was imperative if any definite conclusion was to be drawn as to the conditions favouring infection.

Lambs are slaughtered for the market at a very tender age around Rome—at two to three weeks old, in fact. Advantage of this was taken to endeavour to fix how soon after birth it was possible to get direct evidence of infection. A most careful examination was made with a lens of a number of intestines of lambs a fortnight old but failed to reveal any scolices. I was therefore forced to conclude that infection had not yet occurred. Curtice took some very small individuals about 2 mm. long (which he figures) from a lamb, but does not state the latter’s age. The point is not perhaps of fundamental importance for, as we have already seen, we are bound to infer that infection takes place at a very early stage.

A point worth mentioning is that I learned from the Roman abattoirs that whereas lambs from the Campagna Romana and Sardinia are regularly infected with tapeworm, those from the Marches of Ancona, from Romagna (Ravenna), Perugia and Umbria, where the sheep are stable reared, are free from infection. How far this statement can be relied on it is difficult to say. Dr Zürn, on the other hand (1882), states that the disease also occurs among sheep which have been fed entirely in the stalls, but more especially among the younger and youngest of a flock which are put to graze.

OBSERVATIONS ON MONIEZIA IN THE ABERDEEN AREA.

There are very few flocks in the country immediately round Aberdeen and none of which any history of infection could be procured. Through the kind offices of Mr W. Brown, M.R.C.V.S., Lecturer in Veterinary Science at the University, I was able to get in touch with a farm in Forfarshire where a certain amount of infection was known to occur with fair regularity. I visited the farm in March, 1919, when lambing was well begun and examined the fleece of a number of ewes for ectoparasites, paying particular attention to the belly region, but found no signs of any parasites: keds, lice, etc. whatever. A closer scrutiny of a quantity of wool which was removed to the laboratory also proved fruitless. The farmer himself was confident that none would be found, his being pedigree sheep living under ideal conditions. It is a distinct disadvantage in connection with this research that while one is compelled to look for the agent of infection in February—March, it is not till three months later that one gets to know whether infection has actually been heavy, light, or absent. This is one reason why it is essential to conduct one’s researches on a farm where infection is not sporadic but is known to occur regularly year after year. This notwithstanding, one may go so far as to say that here was a farm where ectoparasites would probably be at a minimum and which nevertheless had a fairly regular history of infection. The fact seems significant.

There seems to be only one plausible reason why infection occurred fairly regularly on this particular farm, viz. the fact that lambing was always carried out in the same meadow, which was therefore likely to be heavily infected with
Life-history of *Moniezia*
epps passed by the ewes. A number of invertebrates were collected from this meadow: *Aphodius punctato-sulcatus, A. ater, Aleocharis*, dung-flies (*Scatophaga stercoraria*), earth-worms (*Lumbricus terrestris*), and carefully dissected in the laboratory (the earth-worms were sectioned), but without result.

During the spring of 1919 and again in the spring of 1920 it was determined to test the possibility of dung beetles acting as carriers more thoroughly. From the farm above discussed, and from another in the same neighbourhood which also had a history of infection, over a hundred individuals of *Aphodius* (various species, mostly *A. punctato-sulcatus*, but also *A. prodromus, A. ater* and *A. rufipes*), about three-score Staphylinids (*Aleocharis lanuginosa*), a score of dung-flies (*S. stercoraria*) were collected and dissected, all without result.

The following experiment was also performed:

A number of dung beetles, mainly *A. punctato-sulcatus*, were kept among sheep faeces liberally sprinkled with tapeworm ova, for a period of several weeks. The faeces were spread upon turf in the open and covered by a rectangular frame $18" \times 18" \times 5"$ with a top of narrow-meshed wire gauze, the sides of the frame being sunk a couple of inches into the grass. Under natural conditions, the beetles do not occur either in perfectly fresh or in comparatively stale dung since they require time in order to locate and enter the dung, and vacate it when it begins to dry up. Consequently it was necessary to remove the stale dung periodically and to substitute fresh dung which was also, of course, sprinkled with eggs. At the end of three weeks most of the beetles still present were removed and dissected under the microscope. Occasional eggs were detected in the gut of some of the beetles, but nothing in the nature of an intermediate stage was found. A few further beetles were collected from the faeces subsequently but they too gave negative results. Altogether some 20 beetles were dealt with in this way. This was only a small fraction of the beetles originally present in the faeces, a number having died or escaped.

Another set of observations in Aberdeen was directed towards ascertaining the effect of long exposure to weather upon the eggs of *Moniezia*. Short series of proglottids were scattered upon a medium of (a) pure, washed sand, and (b) garden soil contained in seedling boxes, and exposed to the weather from early October till shortly before Christmas. The boxes were covered with perforated zinc through which the rain had free access; they had the usual arrangements for drainage but were lined at sides and bottom with a double layer of the finest bolting cloth to prevent the eggs from being washed away. A further lot of isolated proglottids were placed in a jar full of tap-water (c) covered with fine bolting cloth which was left in the open under the same conditions as the boxes.

Notwithstanding that the season was a fairly wet one the proglottids in both boxes (a) and (b), when examined after nearly three months were distinctly desiccated. They had not so much decomposed as shrunk to a thin film. The effects were more marked in the case of those which were laying on sand. The eggs recovered from these proglottids by teasing in water were in the case
of (a) burst practically without exception. In the case of (b) the eggs remaining apparently intact were slightly more numerous. Very strikingly different was the case of the eggs kept in water: these seemed to have increased slightly in size, were perfectly regular in shape, their envelope intact and, in short, appeared all ready for development except that there was no movement to be detected within the egg. Judging by appearances, therefore, the viability of the egg seems to be distinctly favoured by the presence of water. This conclusion is not new of course (see Ministry of Agriculture Leaflet, No. 119), but it is interesting to have obtained experimental evidence in a matter which is of considerable practical importance. It does not necessarily follow from this that the presence of water is essential to the life-history (although it may be so); if the intermediate host should be a member of the dung fauna then the eggs will either be ingested within a comparatively short period (say a week), or will have lost their opportunity definitively, in which case their subsequent viability is not of much moment.

A considerable number of worms were collected from the Aberdeen abattoirs at various times and proved to be *M. expansa* without exception.

**OBSERVATIONS ON MONIEZIA IN THE NEWCASTLE-ON-TYNE AREA.**

Through the courtesy of Prof. Gilchrist and the members of the Northumberland Education Committee, I was able to carry out a feeding experiment to test the possibility of a sheep acting both as intermediate and as final host to the worm. I thought it possible that by feeding eggs to a ewe, larval stages (hexacanths, cysticercoids or what not) might be recovered later from the tissues, notably the udder region. If this should prove to be the case then it would not be difficult to round off the life-cycle by supposing the embryo to remain dormant in the udder region of the ewe until it had dropped its lambs, when the embryos would be passed to the offspring when sucking. The arguments for and against such a theory were discussed earlier in this paper. The feeding experiment was actually performed upon a Sussex half-bred ewe-lamb only a week old. It would have been more logical to select an older sheep but this was not found practicable; moreover, by selecting a newly-born lamb the experiment would serve at the same time to demonstrate the possibility or not of direct infection. Again, it seems not unlikely that a lamb would pick up eggs from the pastures at an early date under natural conditions, though possibly not until after it had begun to graze in earnest.

A quantity of eggs were isolated from ripe proglottids and administered in 50 c.c. of water to the lamb on Thursday, April 14th, 1921, with the aid of a small syringe, being squirted over the tongue and sides of the mouth. Care was taken to use eggs from as many different individual tapeworms as possible and from proglottids which had all the appearance of being properly mature. The dose was repeated a fortnight later. After being dosed, the lamb was put back with the mother-ewe and allowed the same liberty as the rest of the flock. No control lamb was used and the fact that the experimental lamb was
allowed complete liberty also complicated the experiment. The reason for not adopting a control was simply that of expense. The chances of success were so doubtful that the sacrifice of more than one lamb seemed unwarranted. The present experiment was intended as a “sighting shot” merely; if it were to furnish a clue it could be repeated the following season under the strictest control.

On August 9th, that is to say, nearly four months after the administration of the first dose, the lamb was slaughtered and the udder region and also the alimentary canal were taken away for examination. Microscopic examination showed nothing out of the ordinary, the lamb was fat and had thriven well; there were no signs of adult tapeworms in the gut. A number of preparations were made of the udder and small intestine and were carefully examined, but no larval stage was found. The result of the experiment was therefore negative, both as regards the possibility of direct infection and in respect of an intermediate stage in the same host as the adult worm.

As already noted, it is of critical importance in connection with this supposed mode of infection through the intermediary of the ewe, to establish whether Moniezia infection may be contracted by the lambs after they have been weaned. If this is really proved to be the case, then the above experiment loses most of its raison d’être, unless there is a dual method of infection, which seems most unlikely. I am not satisfied, however, that infection with species of Moniezia does occur after the lamb has been weaned. Certainly, I have neither obtained myself nor read any conclusive evidence on this point.

All the worms collected from Newcastle sources belonged to the one species, M. expansa.

SUMMARY.

Lambs contract Moniezia infection either at or very soon after birth, since they have been observed to harbour adult worms at 2–3 months old and in one case, to pass proglottids at 4–6 weeks.

The intermediate host, if such exists, must be frequent on the pasture in early spring, otherwise lambs would not be found to harbour adult tapeworms so regularly or in such numbers when slaughtered in early summer. In the small intestine of a lamb from 3–4 months old slaughtered at Aberystwyth, there occurred 75 individuals.

The fact that lambs regularly harbour adult tapeworms before they are weaned suggests the possibility of their contracting the infection from the mother-ewes. No direct evidence in this direction has been obtained, however, and an attempt to produce a larval stage in the udder region of a ewe by feeding to it the eggs of a tapeworm proved abortive.

Hitherto, all attempts to produce the adult tapeworms directly by feeding the eggs to sheep have failed; there is, however, the remote possibility that the eggs require to undergo some kind of maturation process outside the body of the sheep before they will develop. The fact that several species of Moniezia occur in the domestic sheep would seem to require an intermediate stage, which would occur in a corresponding number of intermediate-host species.
The disease seems prevalent in flocks which are singularly free from ectoparasites.

The invertebrates which seem most likely to harbour an intermediate stage are coprophagous insects, etc. (beetles, flies, mites). Attempts to infect species of *Aphodius* have nevertheless proved fruitless.

Moisture favours the survival of the eggs of *Moniezia*: eggs kept in water for a period of several months seemed to remain perfectly viable. Nevertheless tapeworm is common among flocks on pastures about Rome which are characteristically dry.

A comprehensive series of experiments under conditions of the most complete control would almost certainly clear up the life-history; on economic grounds alone the problem is urgent.

The overwhelming majority of a quantity of worms collected from slaughterhouses in Aberystwyth, Aberdeen, Beauly (Inverness-shire) and Newcastle-on-Tyne proved to be of the species *M. expansa*. The only other species found were *M. trigonophora* and *M. alba*. The identification was based on anatomical characters and not on externals, which are useless.

The writer intends directing his attention to coprophagous mites as carriers, viz. *Gamasus coleoptratorum*, *G. fimetorum*, *Macrocheles glaber*.

**BIBLIOGRAPHY.**


OBSERVATIONS ON WILD RATS IN ENGLAND, WITH AN ACCOUNT OF THEIR ECTO- AND ENDOPARASITES.

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(With Plates XXIII and XXIV, 1 Text-figure and 2 Charts.)

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INTRODUCTION.

It is generally recognised that the measures at present in vogue for the reduction of the rat population are far from satisfactory. A great deal can no doubt be done in the way of lessening the numbers of these destructive and dangerous rodents by poison, bacterial viruses, trapping, the use of natural enemies and so forth, but the rat is so prolific that the losses inflicted by these means are soon made good. The Rodier system attacks the problem from a somewhat different aspect, inasmuch as it is primarily a method of interference with the reproductive life of the rat. Both sexes are trapped, the females destroyed and the males liberated. The relative number of males to females is thereby altered, with the result that the males wage warfare amongst each other, many being killed, while the female also frequently perishes in the struggle for possession.

It would seem that in certain places and under certain conditions the Rodier system works well but it appears more adapted for use in limited areas.
A suggestion has already been made to the effect that its value might be determined by experimental work on ship-board but so far nothing has been done in this direction. The method has certain drawbacks. It is not easy to handle wild rats; only a professional rat-catcher or someone with special experience can do so with impunity. Again it is by no means always easy, in the case of young specimens, to determine the sex.

In thinking matters over the idea occurred to one that if only some bacterial agent could be found which would either produce sterility in rats or induce abortion it might be possible to employ it to advantage and in such a way that the male rat might infect the female during the sexual act.

It was clear that in the first place it would probably be difficult to find any such organism which could be safely used and in the second that even if it were forthcoming and proved pathogenic only to the rats there was every likelihood that the rodent would soon develop an immunity against it. Still a research of this nature appeared to offer some possibilities, for it is easy to understand how useful a wholesale interference with the rat's family life, even for a limited period, might be if the rat population were found harbouring $B. pestis$ and there was a danger of an outbreak of human plague.

It appeared, therefore, worth while to set such an enquiry afoot, especially as, during its course, other matters of interest and importance could conveniently be studied.

Eventually a system of team work was evolved with a view to collecting information on the following points:

1. The possibility of obtaining, either from rats themselves or from some outside source, an organism which could be safely used on a large scale for producing sterility or inducing abortion in rats and which could be transmitted from the male to the female rat and possibly also conversely during coitus.

2. The occurrence of rat parasites, including ecto-parasites, helminths, intestinal protozoa and haematozoa.

3. The incidence of $Leptospira icterohaemorrhagiae$ and the mechanism of its transmission.

4. The presence of pathological conditions, apart from those associated with the genito-urinary system, in the rats examined.

5. Any other matters of general interest regarding the rat population as, for example, the relation of the pregnant state to season.

At the outset I wish to point out that personally I have been but little associated with this research work. Indeed, for a large part of the time during which it was being prosecuted I was abroad, but as I initiated it and have been more or less in touch with it throughout it has been considered advisable that I should place the results, such as they are, on record.

The main object of the enquiry was in the hands of Major H. C. Brown who was entrusted with the bacteriological work. Lt-Colonel G. E. F. Stammers performed most of the post-mortem examinations on rats, examined blood films, and kept a record of the findings.
Rat parasites were studied by Dr A. C. Stevenson and Mr Gerald Dudgeon. Dr Stevenson made the majority of the examinations for intestinal protozoa and determined the incidence of Leptospira infections. Mr Dudgeon carried out a study of the helminths and ecto-parasites. Dr Wenyon, with a view to determining if the rat harboured cultural flagellates other than trypanosomes, examined by culture in NNN medium the blood of a hundred rats, but found the only organism present was Trypanosoma lewisi.

Throughout, our Senior Laboratory Assistant, Mr W. J. Muggleton, rendered valuable aid, arranging for the supply of material and assisting in all branches of the enquiry.

Mr H. A. Baylis of the British Museum was good enough to aid Mr Dudgeon in the identification of worms and to advise on certain points connected with them.

In all 478 wild rats were examined, of which 34 were Rattus rattus and the remainder Rattus norvegicus. No conclusion can be drawn from these figures as to the relative proportion of the two species, as the dealers only sold black rats when these were specially wanted, the skins of black rats being of greater value than those of grey rats. It seems also that the black rat is rarely captured save on board ships or at the docks, and this although a higher price is paid the rat-catchers for black rats.

The rodents were for the most part obtained from dealers in London and were London rats, but specimens were also secured from such towns as Bedford, Brighton, Chester, Eastleigh (Hants), Horsham, Liverpool, Luton, Maidstone, Preston, Reading and Sandwich. Occasionally rats from country districts were secured and a few were captured from ships at the London Docks. We were much indebted to Mr G. L. Moore of Maidstone for the kind help he gave us in obtaining country rats.

The period of work covered more than a year. The investigation was started on August 16th, 1920, and, with a month's intermission in August, 1921, ended on December 14th, 1921.

Generally speaking the condition of the rodents was good. As a rule they were well nourished and, beyond showing recent and sometimes septic wounds and the scars of old lesions, rarely exhibited any external sign of injury or deformity.

Every rat was weighed and this gave some idea regarding maturity. So far as female rats went, the almost invariable presence of corpora lutea indicated that we were dealing with adults and it would seem that a minority of immature animals were examined. The weights of male Rattus rattus varied from 75 to 180 grammes and of females from 75 to 225 grammes, no pregnant black rats being found. The heaviest female black rat not showing corpora lutea weighed 118 grammes.

The weight of male Rattus norvegicus ranged from 100 to 510 grammes and of females from 85 to 470 grammes, excluding pregnant animals.

The heaviest female brown rat not showing corpora lutea turned the scale at 170 grammes.
Of 217 female brown rats examined 32 were found to be pregnant, litters varying from 1 to 12, the average in 27 cases where an estimate could be made being 7.

I. BACTERIOLOGICAL EXAMINATION OF RATS.

After opening the abdomen, organs from which bacteriological preparations were made and cultures were taken were seared on the surface and incised with sterile instruments under aseptic conditions. At first in the case of female rats cultures were taken in every instance, save when the animal was pregnant, from the upper portion of the vaginal tract or from the uterus and incubated both aerobically and anaerobically.

A number of different organisms, both cocci and bacilli, were isolated which could be grouped according to their sugar reactions. As nothing of any practical interest was found, either by these fermentation tests or by subjecting the organism isolated to serological tests (i.e. organism versus blood serum of host, organism versus serum of other rats or organism versus group high titre serum), this routine was discontinued and attention directed solely to the recovery of organisms from the genital tracts of female rats which exhibited definite pathological changes.

There were only four instances of this kind, three of which were fully examined. One was a case of pus in the Fallopian tubes in a non-pregnant rat, two were cases in which the uterus contained dead foetuses, the fourth an example of extra-uterine gestation.

The protocols of the three cases examined are as follows:

(i) Rat 207. Non-pregnant. Pus of a cheesy character present in the uterus and in both Fallopian tubes.

On aerobic culture from this pus two organisms were found, i.e.: (a) a gram-negative coccus and (b) a gram-positive streptococcus.

Cultures of both these organisms were inoculated together intraperitoneally into an apparently healthy mouse, which died in 24 hours. A blood culture from the heart of this mouse revealed the presence of another organism (c) while the original (a) and (b) were not recovered. Organism (c) was eventually proved to be *B. enteritidis* Gaertner. A culture of this organism before it had been definitely identified was introduced into the vagina of a tame rat on January 21st, 1921. The animal died on February 17th, 1921, and it is interesting to note that the bacillus was recovered not only from the heart-blood but also from the Fallopian tubes. Various other passages were carried out, both in the case of male (preputial infection) and female rats but as the organism was eventually found, as stated, to be a species pathogenic both to man and animals and hence unsafe to use for our purpose, further work with it was abandoned.

Organisms (a) and (b) were inoculated separately intraperitoneally into laboratory mice on February 16th, 1921. No ill effects were produced. On March 16th, 1921, three rats were given a vaginal injection of (a), three rats were given a vaginal injection of (b), three were given a vaginal injection of...
Observations on Wild Rats in England

(a) + (b). Two of the rats infected with (b) died on April 3rd from pneumonia. Nothing abnormal was found by the naked eye in their genital tracts which were not further examined. The remainder were kept and watched for two months to see if any sub-acute or chronic malady resulted. Nothing was observed. They were then placed with males.

All three of the rats injected with (a) had healthy litters, on June 5th, 12th and 14th respectively. The third injected with (b), which was placed with a male on two occasions, had a healthy litter on July 20th, 1921. Of those infected with (a) + (b) two had litters on June 5th and the third was obviously pregnant on June 21st.

Hence organisms (a) and (b) were ruled out of court.

(ii) Rat 330. Chloroformed May 10th, 1921, and two dead foetuses found in the uterus.

Aerobic cultures from the uterus resulted in the isolation of (d), a gram-negative coliform bacillus, and (e), a gram-positive coccus.

The vagina of a tame rat was smeared with material from the uterus of Rat 330 on May 11th, 1921, and this animal was placed with a male on May 14th. It had a healthy litter on June 11th, 1921.

Two other tame rats were injected per vaginam with a mixture of a 24 hours growth of (d) and (e) on May 12th. They were placed with males on May 14th and had healthy litters in due course.

Apparently, therefore, neither of these organisms was of the type required.

(iii) Rat 369. Chloroformed June 27th, 1921. Found to have an extra-uterine gestation from which cultures were taken. Two organisms were isolated (f), a coliform bacillus, and (g) a gram-positive streptococcus.

Material from cultures of both organisms was introduced into the vaginas of two rats on June 28th, in one case by injection, in the other by smearing. The smearing method was employed as it was thought it might produce slight surface abrasions such as possibly occur during coitus. Both were placed with males on June 30th and both had litters.

Six other white rats were injected per vaginam on July 11th, 1921, with a broth emulsion of (f) and (g) and subsequently placed with males. All were pregnant on July 29th. In no instance was any ill effect produced as the result of injection.

It therefore appeared clear that organisms (f) and (g) were not of service.

Having failed to obtain in wild rats under natural conditions any organism having the effect desired, attempts were made to infect the genito-urinary tract of tame rats with an organism which it was hoped might prove efficacious either in producing abortion or sterility.

The choice was limited, because care had to be taken that any organism employed should be of such a nature as to obviate any risk of producing serious effects in man or the domestic animals. The ideal would, of course, be one which, while capable of producing the desired effect in wild rats, was incapable of transmission to man or the domestic animals or, if transmitted
to them, was perfectly harmless, existing in them only as a saprophyte. It was not possible to find any such organism and in any case it was not feasible to employ wild rats for experimental purposes. Hence, as in the previous experiments, recourse was had to laboratory animals.

It was considered best to make a start with *Micrococcus catarrhalis* as this organism affects mucous membranes, is very unlikely to be transmitted from rats to man and, even if so transmitted, is so mildly pathogenic to human beings and in any case so common in their nasal passages that any fresh source of infection might, it was felt, for practical purposes be disregarded.

Accordingly a strain of *M. catarrhalis* (Gordon) was obtained from the National Collection of Type Cultures, Lister Institute, and cultures of it were used for the purpose of smearing the vaginas of tame rats.

In the first instance the vagina of a tame rat was smeared with a 24 hours culture of this organism. This rat was put with a male a few days later. She did not become pregnant but this may have been due to the experiment being performed at a time which was not the normal breeding season.

In the second case in which this organism was used a 24 hours culture was mixed with one of *Staphylococcus albus* which had been recently isolated from a case of gleet, and a heavy growth of these organisms was smeared on the walls of the vagina of one white rat which was afterwards put with a male. The former rat had a healthy litter 27 days after the inoculation. The mixed culture was used to see if symbiosis might play any part in pathogenicity.

My attention having been directed to the work of Teacher and Burton on infective abortion in guinea-pigs, I wrote to Dr Teacher and he very kindly supplied me with two strains of the diphtheroid bacillus which he and his co-worker had shown to be the cause of the second epizootic which they reported. The first was due to streptococci, of which pure cultures had not been obtained, so it was not possible to employ these organisms in our investigation.

Although it appeared advisable to test the diphtheroid bacillus above-mentioned the outlook regarding its possible value was not very promising. Teacher and Burton were unable to bring about abortion by the introduction of cultures into the vagina and, as a result of their experiments, concluded that in all probability the natural and only route of infection is through the blood stream. They proved that the bacilli have a definite seat of election in the cavity of the yolk-sac.

Sub-cultures were prepared from both the strains supplied by Dr Teacher and 24 hours growths were used for the inoculations.

The vagina of a white rat was smeared with a mixed culture and it was placed with a male.

The female died 2½ months later without becoming pregnant, the cause of death being abscess of the lung. The failure to conceive was not conclusive as the experiment was not conducted during the normal breeding season.
Observations on Wild Rats in England

In the second instance two female rats showing early signs of pregnancy were used and the walls of the vagina were thoroughly smeared with a 24 hours culture of the two strains of this diphtheroid organism. Both of these rats, however, had healthy full-timed litters thirteen and seventeen days later respectively.

It is fully recognised that these experiments were conducted on a very small scale. This was largely due to the extreme difficulty of procuring tame rats from dealers in London during the earlier part of 1921. The results, however, at any rate with the diphtheroid organism, are in accordance with those obtained by Teacher and Burton (Journal of Pathology and Bacteriology, xx, 1915, p. 14) who found that the introduction of cultures of this organism into the vaginas of guinea pigs were negative.

Although the record of these experiments appears scanty the work itself involved a great deal of time and labour and it is unfortunate that nothing definite resulted from it; still, negative results possess a certain value and it seems desirable to draw attention to the matter in case others, perhaps differently situated, may care to carry out further investigations. It was not possible to conduct the enquiry on as large a scale as we originally contemplated, but the failure to recover any efficient organism from wild rats and the paucity of pathological conditions in the genitalia of these animals militated against the success of the work. It is conceivable that in the tropics conditions are different and that a research on these lines might prove more promising.

2. DETERMINATION OF PARASITES IN RATS.

This was taken up as a matter of routine although no very novel results were expected, as the field has already been covered by various observers. At the same time, so far as I am aware, no work on precisely the same lines has been undertaken in this country. Shipley, in 1908, published a useful paper on “Rats and their Parasites,” in which he gave a list of all the various kinds of parasites found on and in rats but this applied to other parts of the world as well as England.

So far as fleas are concerned the most complete account is that by Bacot, 1919. His paper included two lists, one of all the fleas found on rats up to that date in all parts of the world, the other confined to fleas found on rats in Britain. It will be seen (vide infra) that we found only four of the eighteen species he records. Doubtless this is due in part to the fact that the great majority of our rats came from London and nearly all of them from towns. As Bacot points out, rats are probably the true hosts of only seven or eight of the species he mentions.

Newstead and Evans (1921) determined the species of fleas found on rats in Liverpool and identified Xenopsylla cheopis, Ceratophyllus fasciatus, Leptopsylla musculi, Ceratophyllus londoniensis and Ctenocephalus canis. The first-named was found chiefly on ship rats and Leptopsylla musculi was most prevalent on rats from the dock area. We did not find this flea but, on the
other hand, encountered *Ctenophthalmus agyrtes* which they did not come across. This is probably because some of our rats came from rural districts, as was the case with those searched by Nuttall, Strickland and Merriman during their investigations in East Anglia.

To Hirst (1914) we owe information regarding the Acari found on the brown rat in Great Britain and the description of a species which was new at the time he wrote.

The only other papers to which allusion need here be made are those by Moll (1917), "Animal Parasites of Rats at Madison, Wisconsin," which considers only ecto-parasites and helminths, and the Presidential Address of Cleland (1918) to the Royal Society of New South Wales in which the author enters fully into the question of rat parasites but which is merely of the nature of a review of the subject.

At the time Shipley wrote little was known about the intestinal protozoa of the rat but of recent years attention has been directed to the matter. We give a list of six parasites of this class which were encountered by us both in black and brown rats. Shipley's list of helminths is now very much out of date, as much work has been done on the worms of rodents in general and of rats in particular, and it will be found that the present investigation has revealed the presence of a new cestode and has been of interest in other directions.

The study of haematozoa has not been very fruitful but has demonstrated the presence of *Hepatozoon muris* in the leucocytes of the black rat in this country.

Apparently the only work following closely the lines we adopted is that of Splendore, but it deals primarily with parasites of the field vole *Pitymys savii* Selys. Incidentally he mentions the parasites, ecto-parasites, haematozoa and helminths, which rats share in common with this animal. His observations are of an extensive nature and refer also to bacteria, fungi and intestinal protozoa.

**Collection and Technique.**

The rats were for the most part collected from the dealers' shops where they are kept in large cages, each cage containing a number of animals. They had been captured comparatively recently, but as a rule had been in captivity for some hours or even for several days prior to purchase, and were accordingly "sweated." As Newstead and Evans have pointed out in their "Report on Rat Flea Investigation in Liverpool," such sweated rats are apt to lose their fleas, so that it is possible that the number of ecto-parasites, and more especially of fleas, did not quite represent all those originally present on their hosts.

The latter were transferred from the dealers' cages to perforated tin cases, one rat being placed in each tin, and so conveyed to the laboratory where they were chloroformed in the tins and weighed as soon as dead. Any ecto-parasites which had left their hosts in the tins were collected. These proved to be chiefly fleas and were not numerous. Thereafter the fur of the rat was carefully combed with a very fine comb, special attention being paid to the neck region and the point of junction of the limbs with the body on the under-surface of the
observations on wild rats in england

animals. in each case the findings were placed in tubes which were labelled with a number corresponding to that of the host. at a later date the ecto-parasites were determined. a post-mortem examination of the rat was then performed. films were prepared from the heart blood. the alimentary tract and urinary bladder were removed and searched for helminths. specimens of the intestinal contents were examined for protozoa, any macroscopic abnormalities were noted and, if necessary, portions of the internal organs were taken for section-cutting and staining.

ecto-parasites.

(i) the following were found:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laelaps echidinicus Berl.</td>
<td>52.9</td>
</tr>
<tr>
<td>Haematopinus spinulosus Burm.</td>
<td>44.2</td>
</tr>
<tr>
<td>Xenopsylla cheopis Roths.</td>
<td>5.9</td>
</tr>
</tbody>
</table>

on 34 black rats

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laelaps echidinicus Berl.</td>
<td>24.1</td>
</tr>
<tr>
<td>Notoedres notoedres Megn.</td>
<td>0.2</td>
</tr>
<tr>
<td>Haematopinus spinulosus Burm.</td>
<td>11.3</td>
</tr>
<tr>
<td>Xenopsylla cheopis Roths.</td>
<td>3.6</td>
</tr>
<tr>
<td>Ctenophthalmus agyres Heller</td>
<td>0.0</td>
</tr>
<tr>
<td>Ctenocephalus felis Roths. vel</td>
<td>0.2</td>
</tr>
</tbody>
</table>

helminths.

the following intestinal forms were found:

on 444 brown rats

<table>
<thead>
<tr>
<th>Parasite</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Laelaps echidinicus Berl.</td>
<td>24.1</td>
</tr>
<tr>
<td>Notoedres notoedres Megn.</td>
<td>0.2</td>
</tr>
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<td>11.3</td>
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<tr>
<td>Xenopsylla cheopis Roths.</td>
<td>3.6</td>
</tr>
<tr>
<td>Ctenocephalus felis Roths. vel</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hymenolepis diminuta Rud.</td>
<td>28.4</td>
</tr>
<tr>
<td>&quot; nana-fraterna Stiles</td>
<td>1.2</td>
</tr>
<tr>
<td>&quot; longior Baylis</td>
<td>24.9</td>
</tr>
<tr>
<td>Heligmosomum braziliense Trav.</td>
<td>8.6</td>
</tr>
<tr>
<td>&quot; vexillatum Hall</td>
<td>0.2</td>
</tr>
<tr>
<td>Viannia sp. incert.</td>
<td>0.7</td>
</tr>
<tr>
<td>Capillaria annulosa Dujardin</td>
<td>0.4</td>
</tr>
</tbody>
</table>

in addition trichosomoides crassicauda bell was found on three occasions in the urinary bladder but as only a small number of black rats were examined for the presence of this parasite it would serve no purpose to state a percentage.

in 430 brown rats the intestinal worms were as follows:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hymenolepis diminuta Rud.</td>
<td>28.4</td>
</tr>
<tr>
<td>&quot; nana-fraterna Stiles</td>
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<tr>
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<td>0.2</td>
</tr>
<tr>
<td>Viannia sp. incert.</td>
<td>0.7</td>
</tr>
<tr>
<td>Capillaria annulosa Dujardin</td>
<td>0.4</td>
</tr>
</tbody>
</table>

trichosomoides crassicauda bell was found in the bladder of 48.3 per cent. of 333 rats examined.

the unidentified ova (av. measurements 75 µ x 39.7 µ) of an unknown worm were seen in the intestinal contents on three occasions. as no corre-
sponding worms were found it is possible that these eggs had been swallowed by the rats and had passed through their bodies unchanged.

Very often *Cysticercus fasciolaria* of *Taenia crassieollis* Rud. of the cat was found encysted in the liver and in one case the eggs of *Hepaticola hepatica* Bancroft, a nematode first observed by Chaussat in 1850, were found in sections of the liver together with portions of the worm itself (Pl. XXIII, figs. 1 and 2). There is a good account of this parasite of rats by Hall in the *Proceedings of the United States National Museum*, I, 1916, p. 30.

As a result of this work on worms two papers have already been contributed to the literature, one entitled “Observations on Certain Cestodes of Rats” by H. A. Baylis of the British Museum, the other on the “Occurrence of *Heligmosomum braziliense* Trav. in England,” by G. C. Dudgeon. Under the former title the new *Hymenolepis longior* is fully described and illustrated. Although not stated in the latter paper *Heligmosomum braziliense* had previously only been found in Brazil and Australia.

In the duodenal mucus of three rats there was found a number of small transparent worms with blackish granulations in their interior, occurring along with *Heligmosomum braziliense*. These, by reason of their form—corkscrew spiral—and the absence of longitudinal ridges on the cuticle, conformed to the character distinctions of the genus *Viannaia*, but as no sex organs were seen nor any eggs, it was impossible to place them in any genus. It is possible they may be immature and undescribed forms of *H. braziliense*.

Pl. XXIV, fig. 3, shows the contrast between *H. braziliense* and the possible *Viannaia* (Text-fig. 1) as seen in the intestinal mucus.  

1 The above suggestion that the eggs had been swallowed is probably erroneous, as further examination of sections has shown the presence of groups of eggs, situated in the crypts of Lieberkuhn, high up in the small intestine and in various stages of development.
Intestinal Protozoa.

It is only necessary to give a list of those found, with percentage incidence.

In 32 black rats:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entamoeba muris Grassi 1882</td>
<td>25</td>
</tr>
<tr>
<td>Trichomonas muris Galli-Valerio 1910</td>
<td>81.2</td>
</tr>
<tr>
<td>Octomitus intestinalis Grassi 1882</td>
<td>6.2</td>
</tr>
<tr>
<td>Giardia muris Bensen 1907</td>
<td>6.2</td>
</tr>
<tr>
<td>Chilomastix bettencourti Fonseca 1915...</td>
<td>18.6</td>
</tr>
<tr>
<td>Eimeria falciformis A. Scheider 1874</td>
<td>50</td>
</tr>
</tbody>
</table>

In 440 brown rats:

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entamoeba muris Grassi 1882</td>
<td>13.7</td>
</tr>
<tr>
<td>Trichomonas muris Galli-Valerio 1910</td>
<td>12.7</td>
</tr>
<tr>
<td>Octomitus intestinalis Grassi 1882</td>
<td>1.7</td>
</tr>
<tr>
<td>Giardia muris Bensen 1907</td>
<td>6.8</td>
</tr>
<tr>
<td>Chilomastix bettencourti Fonseca 1915...</td>
<td>1.8</td>
</tr>
<tr>
<td>Eimeria falciformis A. Scheider 1874</td>
<td>21</td>
</tr>
</tbody>
</table>

On two occasions in the black rat cysts of the E. coli type were noted. They are possibly the same as those found by Brug. The presence of Blastocystis was noted not infrequently. Some of the above findings have already been recorded in a paper by Stevenson (vide infra).

Haematozoa.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>In 34 black rats per cent.</th>
<th>In 444 brown rats per cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypanosoma lewisi Evans</td>
<td>38.2</td>
<td>19</td>
</tr>
<tr>
<td>Hepatozoon muris Balfour</td>
<td>5.9</td>
<td>10.8</td>
</tr>
<tr>
<td>Grahamella joyeuxi Brumpt 1913</td>
<td></td>
<td>0.2</td>
</tr>
</tbody>
</table>

Dividing forms of T. lewisi, indicating an early stage of infection, were twice seen in the heart's blood, and a peculiar feature of the majority of trypanosomes found in one rat was the presence of a small granule surrounded by a clear halo in the cytoplasm—adjacent and posterior to the nucleus. According to Wenyon this granule has not previously been noted in T. lewisi, and it seems worthy of record owing to the constancy of its occurrence in this particular instance. In some cases it was elongated, in others double, so it is possible that this is evidence of the division of the granule by fission. However, no opinion can be expressed regarding its true nature. Stained with Leishman it appeared of a lilac colour, very different from the deep red of the kinetonucleus.

An illustrated note on "Haemogregarines in Black Rats" was published by G. E. F. Stammers. This, it is believed, was the first record of the occurrence of haemogregarines in the leucocytes of the black rat in Europe.
INCIDENCE OF \textit{LEPTOSPIRA ICTEROHAEMORRHAGIAE} AND MECHANISM OF ITS TRANSMISSION.

The publication in 1919 of the very complete monograph by Martin and Pettit entitled \textit{Spiroch	extepse Ict	extepse h	extepse emo	extepse rragique} has rendered any detailed consideration of the literature superfluous. There have, however, been some important papers since the appearance of the French work and to these, when necessary, attention will be directed.

TECHNIQUE.

At the autopsies when urine was present in the bladders of the rats it was examined by the dark field method. In addition it was customary to make smears from the kidney which were stained by Giemsa, while in a few of the earlier cases portions of the kidney were stained in block by the Levaditi method. As a further confirmatory test on a good many occasions the urine or an emulsion of kidney and liver tissue were inoculated into guinea pigs intraperitoneally.

The results of these procedures were on the whole confirmatory though, as other observers have found, a certain proportion of guinea pigs cannot be infected, either because they are insusceptible or possibly because the particular strain of leptospira possesses no pathogenicity for these animals. In cases where infected urine was employed it is conceivable, as suggested by Uhlenhuth and Zuelzer, that the urine had itself exerted a detrimental effect on the organism, rendering it innocuous. These observers believe that the acidity of urine is a potent factor in weakening or killing the leptospira. There must also be considered the view put forward by Foulerton that infection with \textit{Leptospira icterohaemorrhagiae} can only be definitely determined by guinea pig inoculation. On these grounds he rejects the work of Coles at Bournemouth in 1918. This is doubtless true, but, as pointed out, the inoculation test is by no means a certain one and, as the presence of a leptospira-like organism can after sufficient experience readily be detected in tissue emulsion and in the urine by the dark field method and also in stained smears and sections, and as no other organism morphologically similar is known to infect rats in this country, it is reasonable to assume that in the case of positive findings by these various methods one is actually dealing with \textit{Leptospira icterohaemorrhagiae}. If positive infections with guinea pigs are alone accepted as a criterion of infection there can be no doubt that, for the reasons above stated, cases will be missed.

A record of part of this work has already been published by A. C. Stevenson in a paper entitled "The Incidence of a Leptospira in the Kidneys and of Parasites in the Intestines of One Hundred Wild Rats examined in England." He showed by a comparison of kidney smears and kidney sections that, in the hands of an experienced observer, the smear method sufficed to reveal the presence of leptospira.
The general findings were as follows:

The urine of 154 brown rats was examined and leptospira-like organisms were seen on twenty-one occasions, i.e. in 13.6 per cent. of the cases. The urine of black rats proved invariably negative but it was only possible to make the examination in fifteen instances.

The kidney examinations by smears and sections from brown rats yielded positive results in 22.6 per cent. The few black rats examined in this way were negative.

At first a systematic series of inoculations of guinea pigs either with urine or kidney and liver emulsion was carried out. Twenty-one animals in all were inoculated, two with urine and nineteen with tissue emulsion. The former remained unaffected, of the latter three became infected and died of typical spirochaetal jaundice.

Thereafter it was decided to inoculate guinea pigs only from rats in which leptospira had been found. Five such experiments were conducted, in three cases infected urine being used and in two leptospira-containing tissue emulsion. Of the urine cases one was positive and it is interesting to note that another guinea pig inoculated in a similar manner and with an equal dose of the same urine remained uninfected.

Of the emulsion cases one was positive and here again two guinea pigs were used, one of which yielded a negative result.

These findings tend to show that there may be a true insusceptibility to infection.

Of a total of 40 rats yielding positive results by the inoculation method and by the examination of smears and sections from the kidney, four were from places outside London, two being from Maidstone, one from Colchester and one from Preston. In this connection Dr Stevenson's statement in his paper above quoted, to the effect that a positive rat came from Cheshire, is incorrect. He should have stated Lancashire—the rat to which reference is made being that from Preston.

The investigations of Foulerton on London rats indicated that there was a distinct seasonal incidence. In rats examined by the inoculation method between July 12th and November 18th, 1918, he obtained positive results only during November, a failure which may have been due to guinea pig insusceptibility. In our observations there is also some indication of a limitation according to season in cases where infection was proved by inoculation, the months in which positive results were obtained being October, November and December. On the other hand, if the whole series of positive cases is considered there is evidence that rats harbour leptospira throughout the year.

One record is of special interest as showing how a guinea pig may perhaps act as a carrier without exhibiting any visible signs of disease beyond a transient rise of temperature and thus coming into line with the rat itself.

Rat 215, Rattus norvegicus, female, non-pregnant, weighing 230 grammes, from Cheapside, was found by dark field examination to have leptospira in the urine.
I c.c. of the urine was inoculated intraperitoneally into a guinea pig on January 19th, 1921.

A rise of temperature was noted on January 24th but no other sign of illness was at any time observed. The temperature fell to normal on January 29th and remained normal till February 9th when there was a further slight rise lasting one day.

From this time onwards until the end of the period of observation, i.e. 50 days from date of inoculation, the animal remained apparently in perfect health. It was killed on March 11th and the autopsy showed no signs of infectious jaundice.

A saline emulsion of the liver, lung, kidney and suprarenal was inoculated intraperitoneally on March 11th into another pig, which died on March 22nd with typical post-mortem appearances of the disease after exhibiting the usual clinical symptoms.

There appears to have been no chance of the first guinea pig having become infected subsequent to the inoculation as it was kept in a separate cage, this being the rule in the case of all the experimental animals save those inoculated from one source. It was the custom to keep those together in one cage and it is interesting to note that if one of these developed the disease it did not transmit infection to any of the others.

**Culture of Leptospira.**

For this purpose Wenyon's modification of Noguchi's blood-agar medium was utilised.

Culture was employed chiefly for the purpose of maintaining the strain and thus saving guinea pigs. The leptospira grew vigorously in this medium. In 48 hours at 30° C. the cultures, taken from the heart blood and organs of infected guinea pigs, were found teeming with the organisms, which remained viable from two to three months. In early culture virulence for guinea pigs was fully maintained but after repeated subculture, although a characteristic febrile reaction and the appearance of jaundice followed inoculation, the animals survived.

Even after a year's subculture at weekly or monthly intervals these definite symptoms were forthcoming but, as stated, there was undoubted attenuation of the virus.

Nothing not already recorded by other observers was noted as regards the morphology of the cultural forms.

**Mechanism of Transmission.**

The experiments as regards transmission can be divided into the following groups:

1. Feeding experiments

(a) With natural food infected with leptospira-containing material from guinea pigs dead of the disease. Urine and organ emulsions were used (3 cases).
Observations on Wild Rats in England

(b) With similar infective material administered by the catheter (7 cases).

2. Inunction experiments
(a) With infective material through the skin without shaving the hair (4 cases).
(b) With infective material through the shaved skin (1 case).

These experiments were discontinued when the results of the work of Uhlenhuth and Zuelzer (1921) became available, as the hypothesis they advanced appeared a very reasonable one which was capable of explaining the vagaries of the disease. At the same time there are certain lines of investigation which have not yet been followed and it may be possible to undertake them at a later date.

It is scarcely necessary to give the results of this part of the investigation in detail. Only two positive results were obtained, both in catheter-fed animals. Each of these animals was given 1 c.c. of a saline suspension of the macerated kidneys of two guinea pigs which had died of infectious jaundice. One of the catheter-fed animals was killed 7 days, the other 10 days after injection, and both showed typical post-mortem appearances of the disease. During life both exhibited jaundice and an elevation of temperature (Charts 1 and 2) and in one case there was a characteristic and sudden fall coinciding with the termination of the malady (Chart 1). Had the animal not been killed it would have died within a short time.

4. GENERAL PATHOLOGY.

Pathological conditions, apart from those associated with helminthic and spirochaetal infection were rare. The kidney changes possibly associated with leptospira infection have already been mentioned by Stevenson.

The other diseases encountered were pneumonia, cystic Fallopian tube, cystic kidney, cystic interstitial nephritis, vesical calculus, a condition in the spleen resembling actinomycosis, secondary septic infection of the lymphatic glands and various purulent infections of bacterial origin.
No case of rat leprosy was encountered and the absence of neoplasms was rather surprising.

5. MISCELLANY.

Pregnancies appeared to be commonest from April to June, during which period 39.5 per cent. of female brown rats examined were pregnant. There seems to be a second breeding period from September to November. July and August appear to be off-seasons, as do the winter months from December to January inclusive. The numbers examined, however, were perhaps too small to allow any conclusions to be drawn.

SUMMARY.

1. Attempts to secure a micro-organism which could induce abortion or sterility in wild rats and which might be used in an anti-rat campaign, more especially when plague threatens a community, have not been successful.

2. An account, however, is given of certain micro-organisms producing pathological conditions in the genito-urinary tract of wild rats.

3. A routine determination of rat parasites including ecto-parasites, helminths, intestinal protozoa and haematozoa has been undertaken.

4. It has led to the discovery of a new species of *Hymenolepis* and has shown that *Heligmosomum braziliense* is present in rats in England. It has also demonstrated the occurrence of haemogregarines in the leucocytes of black rats in this country.

5. Work has been done on the incidence of *Leptospira icterohaemorrhagiae* in *Rattus norvegicus* and *Rattus rattus* in England and on the mechanism of its transmission.

6. It has been shown that apparently the guinea pig may act as a carrier of the *Leptospira* without exhibiting any marked symptoms of the disease which the latter produces. There is also some evidence to prove that infection may take place through the alimentary tract.

7. The *Leptospira* was successfully and easily cultivated in Wenyon’s modification of the Noguchi method but in large measure lost its virulence as the result of repeated subcultures. Even after a year, however, it was still capable of producing characteristic symptoms in the guinea pig.

8. General pathological conditions occurring in wild rats have been recorded.

9. A few observations have been made on the seasonal incidence of pregnancy in wild rats.

REFERENCES.


Observations on Wild Rats in England


Fig. 1. Eggs of *Hepaticola hepatica* Hall in liver of rat. Note opercular plugs. $\times$ circa 350 diam.

Fig. 2. Section of rat's liver showing cluster of sections of *Hepaticola hepatica*. $\times$ circa 85 diam.
Fig. 3. Drawing made from photograph showing *Heligmosomum braziliense* in the intestinal mucus of a rat. The small forms may possibly be a species of *Viannaia*. × circa 12 diam.
ON THE LIFE HISTORY OF A NEW GREGARINE: 
PYXINIA ANOBII N.SP. INTESTINAL PARASITE 
OF ANOBIUM PANICEUM L. (COLEOPTERA).

By MARY VINCENT.

(From the Molteno Institute for Research in Parasitology, 
University of Cambridge.)

(With 5 Text-figures.)

This gregarine was found inhabiting the alimentary canal, and especially the midgut, of both larvae and imagines of Anobium paniceum. All the larvae examined, and most of the adult beetles, were found to be very heavily infected, and in some of the larvae almost every epithelial cell harboured one or more of the cephalont stages of the parasite (Fig. 1). The lumen of the midgut of these larvae was densely packed with the freely moving sporonts, and a number of newly formed cysts were visible in the hindgut. In the imago only the cephalont stages were observed, and no sporonts or cysts have been found.

ALIMENTARY CANAL OF HOST.

The alimentary canal of the larva of Anobium is considerably longer than the larva itself, and consequently is much coiled. The foregut is short and passes by the oesophageal valve into the much wider mesenteron. In the midgut three regions may be distinguished. The first is short with its walls...
swollen laterally to form four blind pouches or diverticula; each of these pouches being further partially divided into two. The wall of these pouches shows a number of irregular swellings visible on the surface. The epithelial cells of this region are of two kinds: (1) large cells, somewhat rounded in shape, which contain a number of coarse inclusions, and (2) typical epithelial cells.

These inclusions of the large cells were thought by Karawaiew (1899) to be flagellate organisms, but later it was shown by Escherich (1900) that they are yeast cells: these live in the epithelial cells as symbionts and apparently play an important part in the digestive processes of the larva. In between the yeast infected cells there occur a number of typical epithelial cells, which possess an inner striated margin, absent in the case of the former.

The epithelium of the diverticula was found to be almost free from the gregarine, which seems unable to lodge in the yeast-infected cells. A certain proportion, however, of the simple epithelial cells lying between these had gregarines attached to them (Fig. 2).

The second division of the mesenteron is wide, and it passes by a sharp constriction on the ventral side into the U-shaped much narrower hind-most division. Both these regions of the midgut are lined exclusively by a typical epithelium, and both were very heavily infected with the gregarine.

At the junction of the mesenteron with the hindgut there are six malpighian tubules. The hindgut is a long coiled tube consisting of four divisions.

The midgut of the imago is much shorter than that of the larva. At its anterior end there are similar lateral pouches, but they are relatively smaller in size, and the cells which contain the symbionts are differently shaped from the corresponding cells in the larva.
The cephalont of *Pyxinia anobii* is provided with a long slender epimerite, which penetrates the whole length of the epithelial cell in which it is imbedded. When detached from the gut and examined alive in normal salt solution, the cephalont soon sheds its epimerite. A vacuole is formed at the base of the epimerite, which presently becomes detached, leaving only a very short mobile projection at the anterior end of the protomerite (Fig. 3 C and D).

This phenomenon was observed by Léger and Duboscq (1902) in *Pyxinia mőbuszi*, and it was suggested by them that the cephalont is capable of detaching itself from the host-cell, discarding its epimerite, and subsequently re-attaching itself to a fresh cell by means of its short digitiform rostrum.

The protoplasm of the cephalont is granular and light coloured. The nucleus varies in its position in the deutomerite. In the living specimen it is visible as a somewhat more translucent area. It is spherical in shape and contains one, or sometimes several, deeply staining karyosomes. Secondary karyosomes arise by a process of budding (Fig. 4 B).
The sporont is solitary, and when mature measures on an average between 190 and 220\(\mu\). The largest specimen seen attained a length of 250\(\mu\). The sporont is elongated, and its length is about four or five times its width. The protomerite is roughly hemispherical, but somewhat pointed at the anterior extremity. There is a well-marked constriction at the septum. The deutomerite is cylindrical, tapering away gradually towards the posterior extremity, and ending in a blunt point. The protoplasm is granular, and in the mature specimens very dark in colour.

The nucleus is just visible in the living specimen, more especially if the animal be slightly compressed. It is spherical in shape, and is most often situated towards the anterior end of the deutomerite: its position, however, is variable. The stained preparations show a spherical nucleus with a single large vacuolated karyosome, and a number of chromatic granules (Fig. 4 A).

**Cysts.**

The encystment of the sporonts takes place in the mesenteron of the larva. The cysts are evacuated at an early stage before the nuclei of the two sporonts have divided, and the young cysts may be observed in the rectum. The newly-formed cysts are very dark in colour, and in the living specimens the nuclei of the sporonts are only just discernible.
A section through a newly-formed cyst shows the protomerite and deutomerite of the sporonts still distinct (Fig. 4 D): at a later stage the septa disappear (Fig. 4 E).

Several abnormal cysts were observed in which three sporonts had encysted together; this, however, was quite exceptional. In this connection it is interesting to note that Laveran and Mesnil (1902) found that the encystment of the sporonts of *Pyxinia frenzeli* takes place by two or by three with equal frequency.

The cysts of *Pyxinia anobii* seen in the intestine are spherical, sometimes slightly ellipsoidal, and measure between 70\(\mu\) and 90\(\mu\) in diameter. The cysts were present in great numbers in the excreta of the larvae, and the whole process of spore formation takes place outside the body of the host. After evacuation of the cyst the protoplasm which is to give rise to the gametes, and subsequently to the sporoblasts and spores, shrinks towards the centre, and the cyst wall becomes much thicker. The envelope enclosing the cyst consists of two portions: an outer epicyst, and an inner endocyst. When ripe the spores escape by the rupture of the two integuments: no traces of sporoducts were observed.
The ripe spores are highly refringent barrel-shaped bodies measuring about $7 \mu$ by $3 \mu$ (Fig. 5 D and E).

**Effect of Gregarine on Host Cells.**

The presence of the gregarine seems to be more or less harmless since all the larvae and adult beetles appeared perfectly healthy although they have been reared for the last five years in the same jar and subject to continual infection. Possibly the greater part of the digestive processes of the larva takes place in the foremost part of the mesenteron in the lateral pouches. It is known that the yeasts take an important part in the digestion of the food, and the yeast infested cells are, as stated above, entirely free from the gregarine. This might explain to some extent the ability of the larva to support such a heavy infection of the hinder portion of the midgut.

The action of the gregarine on the cells of the intestinal epithelium is difficult to trace, possibly, however, like *Pyxinia mōbuszi* inhabiting the alimentary canal of *Anthrenus verhasci*, it does not cause atrophy of the host cell. Léger and Duboscq suggest that in this species the epimerite stretches right through the epithelial cell in which it is imbedded, and piercing the basal layer of the cell, absorbs blood from the body cavity of the host.

**Systematic Position.**

This gregarine may be assigned to the genus *Pyxinia* on account of the shape of its epimerite, and the character of its cysts and spores.

It differs essentially from *P. rubecula* Hammerschmidt, *P. crystalligera* Frenzel, and *P. bulbifera* Watson, in the character of its epimerite, and in its much smaller size.

It resembles in many respects *P. frenzeli* of Laveran and Mesnil and *P. mōbuszi* of Léger and Duboscq, but certain characters preclude it from being assigned to either of these species. In *P. anobii* the epimerite is simple, whereas in *P. frenzeli* the epimerite is in two parts: a slender cylinder, and superimposed on the same a short apical style. The shape of the sporont also in the latter is obese, and the spores are much larger ($14 \mu$ by $6 \mu$).

*P. mōbuszi* possesses a simple epimerite, but this is relatively longer than in *P. anobii*. There is also a marked difference in size between these two species: the maximum length of the sporont of *P. anobii* is $250 \mu$, and of *P. mōbuszi* only $140 \mu$.

Acknowledgment: I am greatly indebted to Dr D. Keilin who placed the material for this investigation at my disposal and aided me with valuable suggestions.

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1 "On peut expliquer ainsi pourquoi *Pyxinia mōbuszi* n’entraîne pas d’atrophie. Sa longue trompe n’absorbant sans doute que le sang de l’Anthène, n’enlève aucune nourriture à la cellule intestinale qu’elle traverse, et n’est pour elle qu’une inclusion presque inoffensive.”
A comparison of the different species belonging to the genus *Pyxinia* is given below.

**Pyxinia rubecula** Hammerschmidt.
Sporonts solitary obese.
Measurements not given.
Protomerite large regularly conoidal, a little longer than wide, constriction at septum.
Deutomerite conical widest at shoulder, tapering to a slender pointed extremity.
Nucleus ellipsoidal.
Epimerite situated upon a short neck, urn-shaped, wide mouthed, crenulate on the periphery, with a short, stout conical style projecting upward through the centre.
Cysts spherical 250–280μ in diameter.
Spores bluntly biconical 14μ by 7μ.
Hosts: *Dermestes lardarius* L. larva and *D. vulpinus* Fabr. adult.

**Pyxinia crystalligera** Frenzel.
Sporonts solitary elongate.
Maximum length 750μ. Width not given.
Ratio length protomerite : total length : : 1 : 5 to 1 : 10.
Protomerite spherical in adults.
Deutomerite of adults regularly cylindrical, tapering in posterior third to a long, slender, bluntly pointed extremity.
Epimerite a short sharp rigid style resting upon a small crenulate corona, the whole superimposed upon the cone-shaped protomerite of the cephalont.
Nucleus irregularly ellipsoidal, containing several karyosomes.
Cysts and spores not known.
Hosts: *Dermestes vulpinus* Fabr. and *D. peruvianus* Casteln. adults and larvae of both.

**Pyxinia frenzeli** Laveran and Mesnil.
Sporonts solitary obese.
Length 200μ.
Maximum length of cephalonts 150μ. Maximum width 40μ. Cephalonts only illustrated.
Protomerite (of cephalonts) cylindrical to subglobose, constricted at septum.
Deutomerite subglobose, nearly as wide as long.
Epimerite in two parts, a slender cylindrical base equal in length to the protomerite, and superimposed upon same, and a short, sharp, apical style equal in length to the cylinder.
Nucleus spherical, containing a large karyosome.
Cysts?
Spores 14 by 6μ.
Host: *Attagenus pellio* larva.

**Pyxinia móbuszi** Léger and Duboscq.
Sporonts solitary.
Length 100–140μ. Width not given.
1 Laveran and Mesnil (1900) give no dimensions for the cysts, but Léger and Duboscq (1902) quote diameter of 50μ.
2 Léger and Duboscq (1902) state that the sporocysts of this species found by them were only 8.5μ in length.
A New Gregarine

Protomerite hemispherical, lower margin straight, projecting beyond deutomerite at septum.
Deutomerite regularly cylindrical, ending in a blunt point or in a well-rounded extremity.
Epimerite persistent, a long slender sinuous style, as long as or longer than the whole cephalont itself.
Nucleus spherical, with one karyosome and several chromatic granules.
Cysts spherical, 60–70 μ in diameter.
Spores elongate barrel-shaped, 6–5 μ to 7 μ long.
Hosts: Anthrenus verbasci Olivier (A. verbasci L.) larva.

Pyxinia bulbifera Watson.
Sporonts solitary, long and slender.
Maximum length 850 μ. Maximum width 160 μ.
Ratio length protomerite : total length : : 1 : 5.
,, width protomerite : width deutomerite : : 1 : 1.3
Protomerite cone-shaped, constricted at septum.
Deutomerite tapers gradually from shoulder ending in a blunt point.
Epimerite in two parts, a short, bulbous, crenulate and crateriform base, and a short thick style.
Nucleus ellipsoidal, lying at right angles to the main axis. A single large karyosome present.
Cysts and spores not seen.
Host: Dermestes lardarius L. adult.

Pyxinia anobii n.sp.
Sporonts solitary elongate.
Length 200–250 μ. Width 40–60 μ.
Protomerite hemispherical.
Deutomerite cylindrical, tapering gradually to a blunt point.
Epimerite a long slender style.
Nucleus spherical with one or more karyosomes.
Spores barrel-shaped 7 μ by 3 μ.
Host: Anobium paniceum L. larvae and adults.

REFERENCES.

NOTE ON DRACUNCULUS MEDINENSIS
(GUINEA-WORM)

By MAJOR W. G. RICHARDS, M.B., I.M.S., Retired.

When the 26th Madras Light Cavalry were reconstituted at Bangalore in 1903 the regiment was joined by a couple of squadrons of men from an Imperial Service Regiment in the north, and, after these men had been at Bangalore some months, many developed guinea-worms, our wards becoming filled with cases showing the worms in all stages of delivery. It took many days or weeks to wind out the worms and so many were broken in the operation that the number of men rendered unfit for duty became a serious matter. I consequently made enquiries as to methods employed for hastening the exit of the worm, since its rupture during extraction always led to severe suppuration.

An Indian Hospital Assistant advised me to feed the men with raw Indian sugar while starving and to allow them only sufficient water to keep them on a sugar diet. Some determined men partook of a pound or more of sugar a day. If possible this treatment was continued for a second day, and, in such cases the worm was usually out by the third day, it being found easy to wind out the worm which came away unruptured in all cases wherein the sugar diet was rigidly maintained. In short there were no failures, the worm being extracted in a few days instead of taking weeks to come out. The only cases of dracontiasis we had at Bangalore were among the northern men previously mentioned, and in practically all patients the worms occurred in the legs and feet. The men told me, however, that in the case of water carriers, who carry water in goat skins, the worm sometimes comes out on the back of the shoulder. That the worm occurs in this situation in water carriers is well known.

Dracontiasis, as far as I know, only occurs regularly in step well districts, the worm discharging its embryos into the well water where the larvae penetrate into and develop in Cyclops, which, when swallowed by man are digested, thereby liberating the worm which then attacks the vertebrate host.

In the Salem District in 1913–14, during the cold weather, we had numerous cases of dracontiasis, some men harbouring up to five worms at a time. The worms occurred most commonly in the legs but they also were found in the scrotum and subcutaneous tissue of the abdomen. Whereas the usual native practice of pouring water over the part was tried, the employment of sugar diet proved far better in hastening the elimination of the worm. Cases were
sometimes admitted in which a piece of worm had been retained since the previous year and in which, as soon as the guinea-worm season began, the worms became active and gave trouble. This was no doubt due to the maturing and escape of the embryos which presumably had lain dormant in the worm fragment during the off season. In these cases sugar caused a flare up of the tissues about the worm fragment and made it easy to lay the track open and wash out the remains of the worm, etc.

Whereas the adult guinea-worm’s body usually lies extended beneath the skin, the worms which loose their way to water, so to speak, by becoming lodged in the scrotum or abdominal wall are found coiled in loops and are easily removed by cutting down upon them. Several worms measured at least five feet in length.

Sugar, taken as described, makes the patient almost unbearably thirsty and would appear to cause a condition decidedly uncomfortable to the worm.
TETRACOTYLE SOGDIANA—A NEW TREMATODE PARASITE OF THE FISH, SCHIZOTHORAX INTERMEDIUS, WITH A DESCRIPTION OF ADHESIVE PERITONITIS PRODUCED BY THE PARASITE IN ITS HOST.

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Professor of Zoology.

AND

N. N. ANITCHKOV, M.D.
Professor of General Pathology.

(From the Military Academy of Medicine, Petrograd.)

(With Plate XXV.)

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INTRODUCTION.

In the summer of 1908 one of us (E. N. P.), whilst collecting material in the River Zeravshan in the vicinity of Samarkand (Turkestan), came across a case of adhesive peritonitis in a local fish, Schizothorax intermedius. The abdominal wall of this fish, with a part of the viscera adhering to it, were dissected out and placed in 70 per cent. alcohol where they remained untouched till 1919 when we proceeded to their examination. The results of our investigation are described in this paper. An examination of the excised parts shows a compact accumulation of tissue on the ventral side of the intestinal coils, in the form of a flat patch from which a series of fine strands run to the black peritoneal covering of the lower body-wall. The patch which shows irregular edges is coloured black and is covered with yellowish spots.

STRUCTURE OF THE PARASITE.

For examination, small pieces were cut out from the edge of the patch and either (1) embedded in celloidin or paraffin, cut and stained with iron haematoxylin, eosin, Weigert's haematoxylin, Van Gieson's and Giemsa's stains, or (2) teased and mounted in Canada balsam.
A New Trematode

The preparations in toto show clearly numerous cysts, each containing a Trematode larva belonging to the group of metastatic Distomatids (Monogena), which differ from the group of Digenea by possessing a simple cycle of development. Our larva should be referred to the provisional genus Tetracotyle, which must stand until the complete life-history of the Trematodes included in it is known. The cyst which harbours the Tetracotyle larva is ellipsoidal in shape, 0-658-0-686 mm. long and 0-434-0-490 mm. wide. Close to the anterior end the parasite shows an oval oral sucker measuring 0-034 mm. by 0-077 mm. (Figs. 2, 4, 5, a.). From this sucker originates the oesophagus which branches into two intestinal trunks that are clearly shown only in the middle portion of the larva.

Laterally to the oral sucker are found two cyst-glands composed of numerous unicellular glands (Fig. 6, cd.) which by means of a common biramous duct open exteriorly into two obliquely directed slits. The clusters of both glands are near each other behind the oral sucker (Fig. 2, cd.) and posteriorly they extend almost to the ventral sucker. The secretion of these glands forms the larval portion of the cysts.

The ventral sucker is circular in shape, 0-098 mm. in diameter, and is situated near the middle of the larva. Behind the ventral sucker the dermal cuticle invaginates inside the body forming, in longitudinal section, a T-shaped depression (Fig. 3). On the dorsal side the skin forms two longitudinal invaginations which border a protruded portion of the dorsal wall.

The portion of the body lying between the ventral and dorsal invaginations contains the rudiments of the ovary, testes, and genital ducts, which open into the ventral invagination.

Although we were unable to examine the larva in the extended condition outside the cyst, we believe that the above description is sufficient to enable us to compare the Tetracotyle larva described above with Tetracotyle species previously found in fish, namely: T. ovata, T. echinatum and T. percae. The characteristic features of these four species are shown in the following table:

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<td>Elliptic</td>
<td>Oviform</td>
<td>Elliptic</td>
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<tr>
<td>Dimensions in mm.</td>
<td>0-84 × 0-57</td>
<td>0-5 × 0-6</td>
<td>0-68 × 0-32</td>
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<td>Oral sucker</td>
<td>0-098 × 0-13</td>
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<td>Smooth</td>
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<td>Localisation in host</td>
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The above comparison shows that our species differs from the previously known three species by the following features: (1) the position of the ventral sucker which is anterior to the middle of the body; (2) the position of the cyst-glands in the anterior third of the body and (3) the occurrence of the parasite in *Schizothorax intermedius*, an indigenous fish. These characters form a sufficient basis to create for the above described larva a new species which we have named *Tetracotyle sogdiana*.

**Peritoneum as a Site of Localisation of the Parasite.**

The occurrence of *Tetracotyle* cysts under the peritoneum of *Schizothorax* is by no means exceptional. In fact, in the 17th Book of *Süsswasserfauna Deutschlands* by Lühe, in addition to the species of *Tetracotyle*, the following other Trematodes are mentioned occupying a similar position in their hosts: (1) *Monostomum* sp. occurring on the surface of the pyloric appendages of *Cottus gobio* and on the stomach of *Coregonus albula*. (2) Cysts of embryo *Distomum* found under the peritoneum of *Acerina cerana* and *Perea fluviatilis*. (3) A Distomatid found in a similar condition in *Cottus gobio* and *Nemachilus barbatula* is regarded as a doubtful form.

Amongst other parasitic worms the following were frequently found under the peritoneum of their hosts:

The larvae of *Bothriocephalus* sp. in *Coregonus maraena*, *C. albula*, *Salmo salar*; cysts of larvae of *Triaenophorus nodulosus* in *Coregonus lavaretus*, *Salmo trutta*, *S. fario*, *S. hucho*, *S. salvelinus*; cysts of *Bothriocephalus osmeri* in *Osmerus eperlanus*; larvae of *Pomphorhynchus laevis* (?) in *Osmerus eperlanus* and *Salmo fario*; cysts of larvae of *Tetrarhynchus paleaceus* in *Salmo salar*; cysts of larvae of *Diplostomum cobitidis* in *Nemachilus barbatula*.

Among all the above mentioned parasitic worms, our species is of special interest as it represents the only case where the pathologico-anatomical changes of the host have been investigated in detail.

**Structure of the Cyst-walls.**

We have mentioned above that the cysts containing the parasites are enclosed within a special patchlike thickening of the peritoneum which is attached to the ventral side of the visceral bundle and is connected with the abdominal wall by means of fine adhesive fibres. The sections through the entire patch and through the adjacent portions of viscera show that the cysts (Fig. 7,c.) are surrounded by a connective tissue membrane produced by the inflammatory reaction of the tissue. In some areas these cysts, surrounded by the connective tissue capsule, appear to wedge in between the lobules of the pancreatic glands many of which are seen to be compressed and atrophied or in a state of partial necrosis.

The capsule surrounding the parasite consists of several layers characterized as follows:

(1) The layer nearest to the parasite (Fig. 8,a.) appears in transverse section
in the form of a fairly broad highly refractive and homogeneous band with
a faintly marked concentric stratification. This layer probably represents the
product of secretion of the parasites themselves.

(2) The second layer is well developed in some places, while absent in
others. It consists of weakly straining granular masses derived from broken
up cellular elements (Fig. 8, b.) and showing elongated cavities corresponding
to the outlines of fatty acid crystals dissolved in the process of treatment of
the preparations. In some places, between the cysts, the granular layer fuses
directly with the masses of disintegrated and necrotic pancreatic lobules. The
absence of transitional forms between these two structures indicates, however,
that the granular layer could not possibly be derived from the necrotic
pancreatic lobules. It is more likely that the elements of this layer represent
the necrotic cells of an inflammatory exudate accumulated around the parasitic
cyst.

(3) The third layer, which is the broadest, consists of compact fibrous tissue
with the fibres arranged concentrically around the cyst. This layer is divided
into two strata: (a) the inner and (b) the outer.

(a) The inner stratum is thinner, more compact, with the fibrilar structure
not clearly defined (Fig. 8, c.) and it stains strongly but diffusely with iron
haematoxylin. Between the fibres of this stratum are seen elongated nuclei
(belonging to cellular elements) in a pycnotic state. This stratum seems to
be composed of fibrous connective tissue undergoing necrosis and probably.impregnated with lime.

(b) The outer fibrous stratum has the same structure as the inner one but
its fibrilar structure is well defined; the nuclei of the included elongated cells
show no pycnosis, nor do they stain diffusely with iron haemotoxylin; they
have the appearance peculiar to the nuclei of compact fibrous connective
tissue cells.

Thus the fibrous capsule which surrounds the parasite is quite similar to
those produced in other animals around foreign bodies as the result of inflam¬
matory reaction.

4. Externally to the fibrous layer, there is a more areolar connective
tissue of a lamellar character, which passes directly into the connective
tissue stroma of the pancreas. It contains a fair number of cellular elements
and many pigment cells (Fig. 8, e.) arranged in groups, all elements being
disposed parallel to the layers of the fibrous capsule. The pigment cells may
be either stellate or polygonal whilst the very minute pigment granules
enclosed in them, which are of a deep-black or yellow-brown colour, completely
obscure the cell nuclei. In the outer portions of the areolar layer, as well as
between the separate adjacent lobules of pancreas, in addition to the stellate
pigment cells with black granules, are found groups of smaller, partly polygonal
cells, with yellow-brown granules. The latter are sometimes found irregularly
distributed in the cell protoplasm in the form of large clumps. These cells,
differing considerably from other pigment cells, have probably another origin.
In addition to the pigment cells the outer areolar connective tissue of the capsule contains a small number of the following other cellular structures: (a) small cells with a spherical dark nucleus and basophil protoplasm, and (b) larger cells with a pale peripheral nucleus and vacuolar amoeboid protoplasm. Compared with the cells of higher vertebrates the former cells (a) may be referred to the lymphocytes, while the latter (b) may be regarded as so-called polyblasts (macrophages, histiocytes) of the granulation tissue. It is remarkable that in the protoplasm of the last-named cells, as well as in that of some of the fibroblast cells, separate granules of pigment are encountered, similar to those of the pigment cells. In a few cells of the polyblast type, the accumulation of pigment was so great that it was difficult to decide whether these cells belonged to polyblasts having phagocytised a large number of pigment granules or to the young pigment cells, which probably develop from polyblasts.

Similar cases of accumulation (or production?) of pigment granules by polyblasts have been previously observed by Eberhard (1907) in experimental aseptic inflammation in the turtle (Emys lutaria europaea).

**Development of the Cyst.**

The examination of the histological structure of the connective-tissue capsule formed around the parasites allows us to reconstruct, in its main features, the process of the gradual development of the capsule:

The parasites having reached the pancreatic gland first of all evoked around them an exudative cellular inflammation followed by necrosis of the elements of the exudate (the granular layer of the capsule) and of some of the pancreatic lobules. A granulation tissue was then developed at the expense of the surrounding connective tissue stroma of the pancreas and peritoneum. In some areas this process proceeded on a small scale till the death of the host, since areas with areolar granulation tissue have been observed in the outer layers of the capsule. However, the main mass of granulation tissue formed around the parasite had been already transformed into cicatricial tissue which entered into the composition of the fibrous capsule. This circumstance indicates that the reaction had taken place long before for it is known that in cold-blooded animals inflammatory processes develop very slowly. Owing to cicatrisation and pressure of the cysts, the surrounding lobules of the pancreas have atrophied considerably. The visceral portion of the peritoneum being also involved into this process, pseudo-adhesions between the latter and the parietal portion have been produced, i.e. adhesive peritonitis has developed.

The foregoing shows that the reaction in *Schizothorax* around the parasitic cysts of *Tetracotyle* is similar to that found in other vertebrates and especially in cold-blooded animals as described by Maximow (1906), Eberhard (1907) and others. Our case shows a later stage of the inflammatory process with certain characteristic changes concerning the pigment. But the
most important character in this case is the formation of numerous peritoneal adhesions which have not been hitherto mentioned in the literature in connection with the inflammatory reactions in fishes taking place around a parasitic cyst. It is interesting to note that we have not found the accumulations of eosinophile cells which are so frequently observed in similar conditions in higher vertebrates. It may be concluded that in our case a specially strong inflammatory irritant was present. As such we may consider (1) a very large number of cysts of *Tetracotyle sogdiana* in the pancreas, (2) products of decomposition of the gland, or finally (3) a secondary infection conveyed by the parasite from the intestine of the host itself.

**REFERENCES.**


**EXPLANATION OF PLATE XXV.**

Fig. 1. The abdominal organs of *Schizothorax intermedins* on the inferior wall of which a dark patch with whitish dots is seen; fibres of pseudo-adhesions run from the patch to the pigmented peritoneum.

Fig. 2. *Tetracotyle sogdiana*—general view.

Fig. 3. The same—longitudinal, slightly oblique section. *b.*, ventral sucker; *cd.*, cyst gland.

Fig. 4. The same—part of longitudinal section. Both anterior (*a.*) and ventral (*b.*) suckers visible.

Fig. 5. The same. Surface section through anterior sucker (*a.*) and through cyst glands (*cd.*).

Fig. 6. The same. Longitudinal section through cyst gland; glandular cells (*ed.*) and common duct in the form of a biramous invagination.

Fig. 7. *Schizothorax intermedins*. Section through pancreas (*p.*) between the lobules of which the cysts are disposed (the parasites are not figured).

Fig. 8. The cyst wall of *T. sogdiana*. *a.*, homogeneous layers of the cyst; *b.*, layer composed of broken-up cell elements; *c.*, layers of fibrous tissue around the cyst of *Tetracotyle*; *d.*, outer portion of the layer *c.* more fibrous; *e.*, areolar laminar connective tissue with pigment cells.
A CONTRIBUTION TO THE BIOLOGY OF 

TRYPANOSOMA EQUIPERDUM.

By E. IWANOW,

Director of the Central Experimental Station for the Breeding 
of Domestic Animals, Moscow.

It has hitherto been firmly established from experience gained in the stud-
farms and by experimental investigations (Hertwig, Prince and Lafosse, 
Frasbot, Pench) that Dourine is transmitted almost exclusively through 
coitus. Flies take no part in the transmission of the disease (Laveran and 
Mesnil), however, the possibility of infection through the bite of fleas (Rabino-
witsch and Kempner) or ticks, is not excluded, and was proved experimentally 
(Sieber and Gonder). Experiments conducted on rabbits and dogs (Rouget, 
Schneider and Buffard, Laveran and Mesnil) have shown that T. equiperdum is 
capable of penetrating through the intact mucous membrane of the eye and 
genital organs, but not through the alimentary tract. Mantueffel's experiments 
have shown that T. equiperdum can even penetrate through the intact skin.

It should be noted, however, that infection does not necessarily take place 
in all cases of coitus with known infected animals. According to Nocard and 
Leclainche, an infected stallion infects on the average two-thirds or three-
quartes of the mates covered by him, i.e. one-third or one-quarter of the 
latter remain healthy in spite of coitus with an infected stallion.

Since in nature Dourine is transmitted only through coitus and since 
T. equiperdum is capable of penetrating through intact mucous membranes, 
it was naturally assumed that the infection is introduced together with the 
sperm into the vagina, whence it passes through the mucous membrane into 
the blood. The fact that T. equiperdum was found in the testis of an infected 
animal (Rouget), seemed to support this view. The infection of the male from 
the female also found explanation in the capability of the trypanosome to 
penetrate through intact membranes.

Having worked on the disinfection of the sperm and conducted a series of 
observations on coitus in animals, I found it necessary to verify the facts 
mentioned above. My object was to find whether T. equiperdum passed into 
the sperm, and, if it passed, whether it was capable of penetrating through 
the intact mucous membrane of the female genital tract. For these experi-
ments I used mice, rabbits and dogs.

I found that in mice the contents of the accessory genital glands (vesicula 
seminalis) remained free of T. equiperdum at the time when these parasites 
were abundant in the blood.
Biology of Trypanosoma equiperdum

The experimental mice (four) inoculated subcutaneously with the contents of the vesicula seminalis of mice harbouring the trypanosome in their blood (diluted with physiological salt solution) remained healthy after being under observation for more than a month. On the other hand, the control mice inoculated subcutaneously with saline and the blood of the corresponding mice infected with *T. equiperdum*, showed trypanosomes in their blood after 6–7 days, and succumbed on the 9th–10th day.

Experiments conducted on a dog infected with *T. equiperdum* showed that this trypanosome does not pass into the sperm even at the period of the highest development of the disease.

Mice inoculated subcutaneously with the sperm of the infected dog at different periods of the disease remained healthy (under observation for more than 40 days), whereas the control mice inoculated subcutaneously with saline and blood from the same dog showed trypanosomes in their blood on the 4th day and died on the 7th–8th day. These experiments are illustrated in Table I.

Table I.

_Mice inoculated subcutaneously with the sperm of a dog infected with _T. equiperdum. The presence of trypanosomes in the blood was established by microscopical examination._

<table>
<thead>
<tr>
<th>Infected animal and material used for inoculation</th>
<th>Experimental animal</th>
<th>Result of examination of blood of inoculated animals on days</th>
<th>Results of further blood examinations inoculated mice</th>
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<tr>
<td>1. vi. 1917 Dog's (&quot;Gutik&quot;) sperm</td>
<td>Mouse</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood of the same dog (control exp.)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5. vi. 1917 Dog's (&quot;Gutik&quot;) sperm</td>
<td>Mouse</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood of the same dog (control exp.)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10. vi. 1917 Precipitate of centrifuged dog's (&quot;Gutik&quot;) sperm</td>
<td>Mouse</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blood of the same dog (control exp.)</td>
<td>-</td>
<td>-</td>
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</table>

It was very difficult to obtain the sperm for inoculation into mice on June 10th from the dog "Gutik," as the animal was already very weak. Seven days later the dog died.

The experiments conducted with the view of discovering if _T. equiperdum_ could traverse the intact mucous membrane, were carried out on eleven mice and five rabbits, as follows: A mixture of 1 c.c. of physiological salt solution and five drops of blood (from the tail of a mouse infected with _T. equiperdum_)
was introduced into the vagina by means of a very fine elastic catheter which was lubricated with vaseline. The operation was conducted in two series: (1) care was taken not to produce any excoriation of the mucous membrane (in eight mice and four rabbits), and (2) with the mucous membrane of the vagina scratched (so that blood appeared) with a dissecting needle (three mice and one rabbit).

All the mice of the first series without exception remained alive and showed no trypanosomes in their blood during two months, whilst all the mice of the second series without exception became infected and succumbed after the usual period of infection. In the case of the rabbits of the two series the blood was tested by re-inoculation in mice. Infection occurred only in the rabbit of the second series.

My investigations were carried out in 1917. The strain of T. equiperdum was obtained from the laboratory of Prof. V. L. Yakimov, to whom I wish to express my gratitude. The revolution and civil war in Russia prevented me from publishing these observations in due time, as most of my notes remained in Ascania-Nova which I left in the Autumn of 1917, and where I was unable to return. In the autumn of last year, I succeeded in recovering part of the protocols of my experiments from Ascania-Nova, but some of my papers, which were ready for publication, and preparations, including those of T. equiperdum, have perished.

As my blood-films from infected animals (stained with Giemsa) were not available, and I wished to exclude any doubt regarding the type of trypanosome employed in my experiments, I tried to repeat the experiments. I did not, however, succeed in obtaining a strain of T. equiperdum in Russia. I was able to repeat my experiments only this year, during my commission abroad, thanks to the facilities kindly provided by Prof. Mesnil in his laboratory at the Institut Pasteur. Here I obtained the same results as before. The introduction of T. equiperdum into the vagina of mice (saline and blood) produced infection only in the presence of excoriations in the mucous membrane of the vagina.

On June 30th, 1922, T. equiperdum (five drops of blood from the tail of a mouse diluted in 1 c.c. of saline) was introduced into the vagina of five mice by means of a fine catheter lubricated with vaseline. One of the experimental mice proved to be ill with diarrhoea and died on July 4th, the examination of its blood being negative for trypanosomes. The remaining mice are still living, and have been under observation for more than a month.

The question now arises, by what means is the disease transmitted, and how do the trypanosomes penetrate into the blood, if they are unable to penetrate through the mucous membrane of the vagina, and, finally, how to reconcile the results of observations of other authors with those obtained by myself.

In the coitus of animals, especially such as the horse, there is a whole series of mechanical and physiological conditions, which in themselves are sufficient
to provide the means by which infection with Dourine takes place, without
the necessity of attributing to *T. equiperdum* any specific capacities, which,
it must be said, nobody has yet proved by histological methods.

In horses the sexual act is in very many cases accompanied by more or
less light abrasions with haemorrhage on the mucous membrane of the
genital organs of the male or female or both, even when both horses are in
perfect health. This is due to the fact that in horses this act is very violent,
the size and volume of the male and female organs do not correspond, and
the mucous membrane of the penis is easily scratched by the stiff hairs of
the tail, etc. Having had to deal with hundreds of horses, when working on
artificial insemination, I had abundant material showing how frequently such
light abrasions occur in the vagina and on the mucous membrane of the
penis, and how they pass unobserved during natural coitus. The sponge which
in artificial insemination is introduced into the vagina to absorb the sperm
serves at the same time to demonstrate such abrasions. Each abrasion, how¬
ever small, leaves a trace on the sponge in the form of a spot of blood. In
animals suffering from Dourine the formation of abrasions on the genital
organs during the sexual act must be a still more common phenomenon,
since haemorrhage, oedemas and a swelling of the mucous membranes of the
genital organs are some of the characteristic symptoms of Dourine (cf. Laveran
et Mesnil, *Trypanosomes et Trypanosomiases*, 1912, p. 571). Besides, it should
be remembered, that in animals coitus takes place only when the female is
in the period of oestrum, which, although not always accompanied by abundant
bleeding from the mucous membrane of the uterus, as in man, anthropoids,
dog, is in any case characterised by deep changes in the mucous membrane
of the uterus, viz. this organ becomes swollen, haemorrhagic and the tissues
become areolar.

Thus, during the period of oestrum, the trypanosomes can pass from
the blood stream of the infected female into the genital organs, and, in the
presence of even a slight excoriation on the mucous membrane of the penis,
the infection can be transmitted to the male. Similarly, in an infected male
whose mucous membrane has a tendency to become haemorrhagic, the
chances are considerable for a certain amount of infected blood to be intro¬
duced into the vagina of the female during the act of coitus, and in the
presence of abrasions in the female tract infection is secured. If, on the other
hand, the mucous membrane of the penis remains intact during coitus with
an infected female, or if the mucous membrane of the vagina of a healthy
female remains free of excoriations during intercourse with an infected male,
there is no reason why infection should take place. Neither would we expect
infection to occur, when coitus with an infected male takes place without
any discharge of infected blood. The latter facts easily and simply explain
the cases wherein no infection was produced in one-quarter to one-third of the
total number of mares covered by an infected stallion.

If *T. equiperdum* were discharged with the sperm and were capable of
penetrating through intact mucous membranes, it would be very difficult to explain such facultative infectibility.

I have not repeated the experiments on inoculation of the mucous membranes of the eye, vulva, or of the skin, and I should not be surprised, if infection was produced by such methods, since the mucous membranes of the external organs and even the skin are easily exposed to slight, hardly visible wounds, which, however, are sufficient for the parasite to penetrate. That is why I choose the vagina for my experiments, since this is an organ the mucous membrane of which is protected from external irritations likely to cause wounds (with sand, hay, dust), and at the same time it is accessible for experimental purposes. Of course, in this case it may also happen that the introduction of _T. equiperdum_ into the vagina produces infection, even when the above precautions are taken, _e.g._ if the female is used soon after parturition or with its external genitals injured, or after coitus which has left traces in the form of light abrasions.

In my experiments objection may be taken to the fact that when the catheter is smeared with vaseline and introduced into the vagina, the vaseline may form a coating isolating the mucous membrane. When, however, the infection was introduced into control mice whose vaginal membrane was previously abraded, I also smeared the catheter with vaseline, and this did not prevent the infection and death of the mice.

In the light of my observations on the method of infection with Dourine, in combating this disease, which causes such ravages in the stud-farms not only of Russia, but of Canada and other countries as well, attention should first of all be directed to the methods of preventing possible wounds and abrasions on the mucous membranes of the genital organs of horses during coitus.

This can be attained in the following way. Horses should not be allowed to breed promiscuously in the herd, but should be covered individually under control, which allows of taking care that the external genital organs are clean during coitus, that the penis does not get scatched by the hairs of the mare's tail. The external genitals of the mare should be washed and her tail carefully dressed before the act. Then, to facilitate coitus it is advisable to lubricate the external genitals of the mare and the penis of the stallion with vaseline or oil.
THE OCCURRENCE OF A LEECH (*TROCHETA SUBVIRIDIS*) IN AN ALLOTMENT.

BY R. A. HARPER GRAY, M.A., B.Sc. (Agr.), M.Sc.,
Adviser in Agricultural Zoology, Armstrong College, Newcastle upon Tyne.

In April of this year (1922) a specimen was sent to the Agricultural Department, Armstrong College, by Mr S. Giles of South Shields, along with a note explaining that it had been found “down in the first spit of the soil” in one of a group of allotments there. It was obviously a specimen of a leech, but the specimen was submitted later, to Mr John Ritchie, the Museum, Perth, who kindly identified the species as *Trocheta subviridis*, and who mentioned that “this gives so far as I am aware, a more northern habitat than hitherto recorded. See Parasitology, vol. III, p. 182.”

Apart from the fact that this particular species has been recorded in England only at rare intervals, it is interesting to record the occurrence of a form on land that has been cultivated for several years, seeing that the Hirudinea are associated usually with a water habitat.

A description of *Trocheta subviridis* is given by Harding in his paper on British Leeches¹, to which Mr Ritchie refers, and it appears that up to 1910 it had not been recorded from Scotland or Ireland, and in England the first reliable record (1850) refers to the finding of a single specimen in Regent’s Park. Subsequently it was found “at no place very remote from London.” More recently, however, in 1909, specimens were found at the Withington Sewage Works, near Manchester, where they were occurring in sewage effluent channels, and feeding upon earthworms there.

As is often the case with isolated occurrences of a species, it is somewhat difficult to account for the presence of *Trocheta subviridis* in the South Shields allotment. The allotments lie on a clay bed, and in the one containing the leech, the clay occurs, in places, a few inches below the surface. As there is no drainage system, the land has been trenched to the depth of one and a half spits, and a well sunk in the clay in one corner, into which an endeavour is made to collect water in winter. Notwithstanding this, however, it appears that the land tends to become waterlogged during winter, but in April the soil was quite workable, and the leech was found at a considerable distance from the well.

Subsequent enquiries showed that since 1914 at least twenty loads of night-soil had been brought to an allotment within twenty yards of the one where the leech was found. This appears to have some significance as to its occurrence, when one considers Harding’s reference to examples being found in sewage channels near Manchester, though one would have expected to find Trocheta subviridis in the allotment treated with night-soil, rather than in one situated about twenty yards away. Harding, however, describes the species as amphibious, “frequently leaving the water in order to pursue its prey in moist situations upon land,” so that, owing to the waterlogged condition of the allotments during winter, there would be an opportunity of moving from the well sunk in the clay, or from water in the allotment containing the night-soil.

It may be that the species is not so rare as the few records of its occurrence would lead one to suppose, for, as Harding points out, its superficial resemblance to an earthworm might lead to its being overlooked, and it is well to add a record of its occurrence so far north in England.

Trocheta subviridis is interesting economically in being carnivorous, its food consisting of earthworms, and it is credited with eating insect larvae\(^1\).

\(^1\) Loc. cit. Harding, p. 186.
THE WARBLE-FLIES OF CATTLE. HYPODERMA BOVIS AND H. LINEATUM.

By CECIL WARBURTON, M.A.

Molteno Institute for Research in Parasitology.

(With 3 Text-figures.)

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INTRODUCTION.

The phenomenon of tumours in the backs of cattle from which in due time grubs emerge has been known from very early times. So, also, has the phenomenon of “gadding,” now known to be due to Warble flies, and it is sufficient to cite the familiar and most graphic account given by Virgil\(^1\). Since, however, the origin of the grubs in the tumours was entirely unknown and the “gadding” was usually attributed to members of the Tabanidae, allusions to these phenomena earlier than the 18th century have no value except as indicating the antiquity of the pests.

At the beginning of the 18th century Vallisnieri set himself to discover the insect of which the warble maggots are the larvae, and after much expenditure of time and money he at length succeeded (in 1710) in breeding out a single rather damaged imago, which is described and figured in his *Opere*\(^2\) published by his son in 1733. Therewith begins any exact knowledge we possess on the subject, and though more than two hundred years have elapsed, and the problem has been attacked, somewhat spasmodically, by a host of investigators, there still remain obscure points in the bionomics of these remarkable insects. Nevertheless we have now arrived at a stage where

\(^1\) *Georgics* III. 146-151.
\(^2\) Vol. i. p. 28, fig. 10.
the general course of the life-history is so well established that further research is unlikely to revolutionise the views at present held, though certain lacunae remain to be filled up.

Before 1892 the phenomena of oviposition were entirely unknown, and it was not till 1915 that the long discussed question as to how the parasite obtained an entry into its host was at length definitely settled. It follows, therefore, that the general accounts of the warble-flies which have from time to time appeared', though some of them are quite admirable statements of the facts as then known, are too incomplete to be satisfactory in view of the important discoveries made since they were written. No such general account can claim finality, but whereas hitherto even the broad outline of the mode of life of these insects has been partly conjectural, at present the obscure points are matters of detail, and the time has therefore come when a fresh attempt may be usefully made to gather together the results of a research which has now proceeded for more than two hundred years.

A convenient way will be to state briefly at the outset the facts for which the evidence will afterwards be discussed in detail.

I. Bionomics of *H. bovis* and *H. lineatum* according to our present knowledge.

Domestic cattle, in Europe and N. America, are subject to attack by two species of *Hypoderma* (Diptera, Oestridae)—*H. bovis* and *H. lineatum*. Both species are widely distributed and are common in England, but *H. bovis* is the prevalent species in Ireland and *H. lineatum* in N. America. The flies appear during hot and sunny weather. The date of their appearance naturally varies with the climate and with the nature of the particular season. In England July is the month when most flies are observed, but individuals may appear much earlier and much later. In British Columbia, with a climate similar to that of England, *H. lineatum* has been taken in April, while in Ireland *H. bovis* has occurred as late as September. In the hot climate of Texas in 1892 *H. lineatum* was on the wing in the first days of March (Riley).

*H. lineatum* is the earlier fly by about a month (Hadwen 1914). It follows, therefore, that the fly season may be a long one, though individual flies probably have a very short life. This is particularly the case where both species are present, as in England, where the season may in some years extend from April to September.

Eggs are laid on the hairs of the cattle. *H. bovis*, which only oviposits on hot sunny days, attaches its eggs singly at the base of the hairs; *H. lineatum*, which seems to be less dependent on sunshine, attaches several eggs in series (1–14) to a single hair. In all about 800 eggs may be laid by a female of either species.

The eggs are never laid in the region where the warble tumours subsequently appear. The legs are chosen by preference, especially if the animal

1 Clark, 1815; Joly, 1846; Brauer, 1863; Schneidemühl, 1897; Imms, 1906.
is standing, but *H. lineatum* has been seen to oviposit all along the flank of a sickly recumbent animal, and in the region below the ischial prominence (Hadwen, 1915). Oviposition inflicts no pain on the animal and may be unnoticed, but if the victim becomes aware of the presence of the fly, great uneasiness and even terror are excited and “gadding” results. Wild-eyed with fear, and with tail horizontally outstretched, the animal gallops madly to escape its enemy and takes refuge in the nearest pond or river, where it stands trembling, knee-deep in the water. The terror is infectious, and often spreads to the whole herd. It is much more marked in the case of *H. bovis* than in that of *H. lineatum*.

Young animals are always more parasitised than the older, but yearlings are usually more subject to attack than calves, the preference being: (1) yearlings, (2) calves, (3) older cattle. There is, however, no evidence that eggs are laid more freely on the younger animals, and Hadwen considers the reduction of warbles on the older cattle a case of partial immunity through advancing age.

Under normal conditions the eggs of *H. bovis* hatch in about four days (those of *H. lineatum* taking some days longer), and give rise to minute larvae, about 0.8 mm. in length, armed in every segment with small spines, and furnished with a median piercing tooth, flanked by a pair of well-developed mouth-hooks. The larva crawls down the hair and bores into the skin. In the case of *H. lineatum* it is alleged that the hair follicle is always entered. The process of penetration, which may occupy several hours, causes considerable uneasiness, and gives rise to certain skin lesions, but the symptoms usually disappear very speedily.

The larva has now entered the host at a point (e.g. the lower part of the leg) far removed from its ultimate destination, which is invariably the back of the animal. Several months will elapse before the earliest indication of the formation of “warbles” in the dorsal integument. Taking mid-July as the date of maximum oviposition in England and mid-February as the time when the indications of newly-forming dorsal tumours are the most numerous, we have a rough indication of the average time elapsing between oviposition and the assumption by the larva of its final position, for it is reasonable to suppose that the earliest formed warbles arise from the earliest laid eggs and the latest from the latest. For about seven months, therefore, there is no external sign of the whereabouts of the larva, which is somewhere within its host. It is with regard to this period of the life-history that our knowledge is still incomplete, and especially with regard to the first portion of it, which is not surprising in view of the minute size—less than 1 mm.—of the larva on obtaining entrance.

Now though it is not yet possible to trace accurately the course of a larva throughout these seven months, certain salient facts are well established.

During the autumn and winter months (in England) the larvae, entirely lost sight of since their penetration of the outer integument, re-appear with

1 Carpenter and Steen, 1908.
great regularity in the wall of the oesophagus, in the connective tissue beneath the submucosa. How they reach this position is entirely unknown. Carpenter (1915) suggests that the larva on penetration may enter a small blood vessel and be carried to the oesophagus by the blood stream, but the only basis for this supposition lies in the fact that attempts to find the larva near the spot where penetration was observed were unsuccessful in the few cases in which they were undertaken. The interesting cases, to be discussed later, in which *Hypoderma* larvae have infested man point rather to extensive wanderings in the tissues, and it may be that our ignorance with regard to this portion of the life-history arises simply from the inherent difficulty of tracing so small a parasite.

The larvae in the oesophageal wall, which usually begin to appear in September (in England) and culminate in November, are very different from those which hatched from the eggs, being for the most part of a glassy smoothness, their very minute spines being confined to the regions of the mouth and the posterior spiracles. They are provided with a median tooth and mouth-hooks, and are usually from 6 to 14 mm. in length. There may, of course, be larval stages intermediate between this smooth oesophageal instar and the spiny first instar which hatches from the egg, and in the case of *H. lineatum* one such intermediate form has lately been described by Laake (1920).

From this point our knowledge of the life-history is fairly complete. The oesophageal larvae are always on the move, and may be found with the anterior end, pointed in any direction, but whereas they are at first mostly situated towards the pharyngeal end of the gullet their incidence progresses gradually downwards till towards the time of their disappearance from this organ such as remain are found near the origin of the stomach. Again, for the sake of comparison, speaking only of what happens in England, and neglecting occasional extreme instances, larvae begin to appear in the oesophageal wall in September, are most numerous there in November, and finally disappear in March.

Now these smooth, glassy larvae, averaging about 14 mm. in length when they leave the gullet, are identical with the youngest larvae which are found in the subcutaneous tumours. They proceed from the gullet to their final situation beneath the hide without change of form or noticeable increase in size. There is no invariable route followed in this progress, which takes place always in the connective tissue, but many of the larvae find an intermediate stopping place in the spinal canal. With the same regularity with which larvae are to be found in the oesophagus in autumn, they are to be met with in the spinal canal in the winter.

Probably some if not most of the larvae proceed directly from the oesophagus to the anterior region of the dorsal integument, working their way between the muscle layers. Others have been definitely traced across the diaphragm and upwards along the border of the 9th rib to the spinal canal, which is entered by a posterior foramen. The canal, where the larvae are
found between the periosteum and the dura mater, affords an easy passage
to the posterior region of the back and gives ready access to any part of that
area to which the warble tumours are always confined.

The larva now selects the spot for its final changes, and, entering the hide
from below, begins the formation of a "warble." It quickly undergoes an
ecdysis and becomes fundamentally changed in form. No longer glassy and
semitransparent, it becomes white and opaque, and well furnished on the
ventral surface with minute spines. The mouth-hooks are lost and the posterior
stigmata are markedly increased in size. The shape is somewhat tapering, being
narrowed towards the posterior end. This is the penultimate larval instar, and
it, together with the subsequent history of the insect, has long been perfectly
well known. Migration having now ceased, a change takes place in the walls
of the cavity in which the larva has established itself and a cyst is formed,
but the movements of the spiny larva within cause inflammation in the walls
of the cyst on the products of which the parasite lives. Its next procedure is
to pierce a hole to the exterior towards which it advances tail first. Atmospheric
air being now admitted growth, hitherto so slow, proceeds rapidly. The ad¬
nission of organisms from the exterior changes the nature of the pus formed
in the cyst, and it now becomes dark and foetid. This penultimate larval
instar not only perforates the external hide, but it keeps open and widens
the orifice by the frequent insertion of its narrow posterior extremity. Another
ecdysis then takes place, and the final larval instar is assumed. It is spindle¬
shaped, convex on the ventral surface but flattened dorsally, and furnished
on either side with three longitudinal rows of tubercular ridges, and its spiracles
are notably larger and more conspicuous. At first it is white, but it gradually
assumes a darker hue. It lies in the cyst with its posterior stigmata applied
to the external orifice. In extreme cases the back of a yearling may contain
several hundred larvae in different stages of development, some nearly mature
and others not yet in direct communication with the external air. It often
happens that successive batches of tumours arise in the same animal, and this
may be fairly assumed to be due to different dates of oviposition.

The mature larva now emerges from the tumour through the orifice which
has been formed and maintained during its later stages of development,
though this orifice never approaches in size the normal width of the larva,
and the exit is not made without difficulty. For a day or two before emergence
the larva is observed frequently to protrude its two posterior segments through
the aperture, apparently to widen it still further and to test the possibility
of exit. When the time has arrived for the final effort these two segments are
protruded and inflated to the utmost extent, while the next segment is con¬
stricted and passed through, and this process continues till emergence is com¬
plete. There is a great struggle to pass out the fourth segment (from the
posterior end) but when this is accomplished the rest is fairly easy. Emergence
is believed always to take place fairly early in the morning. In England most
larvae emerge during May. The larva, now free, falls to earth and immediately
crawls away in search of a retreat—preferably a hole in the ground. Here its integument quickly hardens to form a puparium, its colour deepening to a very dark brown or black hue. The pupal period depends on the temperature, but averages about five weeks. The fly then bursts open a triangular operculum on the antero-dorsal portion of the operculum, and the metamorphosis is achieved.

II. The Evidence Discussed.

The Flies.

It is not proposed here to include diagnoses of insects which have long been perfectly well known, though at a later stage it may be useful to give directions for distinguishing the imagos and the different larval instars of the two species. The present account is largely historical.

The poverty of entomological collections in specimens of *Hypoderma* and the difficulty which the earlier investigators experienced in breeding out imagos or in capturing examples in the open are exceedingly striking. It was some years before Vallisnieri succeeded in breeding out the single imperfect specimen which he described in 1710. Réaumur was more fortunate. Finding that the cattle in the open country near Paris were free from warbles but that many in the wooded district near Brie l’Abbaye were badly infested, he obtained his material from that region. Unable to be absent from Paris at the time the larvae left the cattle, he persuaded the Mother Superior to lend him two infested heifers, and had them so closely watched that he was enabled to observe the emergence of several larvae, from some of which he obtained excellent specimens of the fly. His most valuable observations were published in 1738. In 1776 de Geer named a fly which was captured "in the country," and which he considered identical with Réaumur's specimens, *Oestrus bovis*, and in 1825 Latreille founded the genus *Hypoderma*.

Bracy Clark, who published his first paper on the subject in 1797 and whose famous "Essay" was issued in 1815, began his investigations in ignorance of the work of Vallisnieri and Réaumur, being misled by Linnaeus, whom he supposed to be acquainted with their writings. Linnaeus, however, had entirely confused the ox bot-fly with that of the horse, and his *Oestrus bovis* is nothing but *Gastrophilus intestinalis (equi)*, and it was not until much of Clark's research was completed that that writer became aware of the discoveries of his predecessors.

Clark first introduced the method of "sleeving," or fixing muslin bags over the warble tumours so as to capture without injury the larvae as they emerged, and by this means he obtained several specimens of the fly. Joly (1846) based his anatomical studies of the fly on three specimens only, and the material of Brauer (1863) was hardly more plentiful.

Up to 1890 all the observations on ox-warbles were attributed to the species *H. bovis*. It is necessary to bear in mind, that previous to that date the insect concerned in many of the European cases, and probably in all the
N. American cases, was in reality *H. lineatum*. The history of the recognition of this species is curious. In 1875 Brauer received from Colorado larvae taken from a bison which he recognised as different from *H. bovis*, and which he named *H. bonassi*. In 1888 Handlirsch captured some *Hypoderma* flies which were not *bovis* in a field where cattle were pastured, and the following year he, in company with Brauer, found a pupa, corresponding exactly with his *bonassi* larva, from which he bred the same fly, of which the normal host was as yet uncertain. The matter was settled by Brauer in 1890, when the fly was proved to be a cattle parasite and was found to be the one described by de Villers as early as 1789 under the name of *Oestrus lineatus*, of which the bionomics were hitherto quite unknown. Clark seems to have met with it but to have had great doubts as to its identity, as at different dates he named it *Oestrus haemorrhoidalis* var. β (1797), *Oestrus bovis* var. γ *vernalis* (1815), and later, following Leach (1817), *Oestrus ericetorum*.

Since 1890, therefore, it has been clearly realised that there are two ox warble-flies, *H. bovis* and *H. lineatum*, both of which occur, though very unequally, in all the countries from which warble attack has been reported.

The comparative poverty of these flies in entomological collections is largely to be accounted for by their habits. Though the fly season may, by the occurrence of early and late examples, be fairly long, the period during which they are at all plentiful in any district is quite brief, and individual flies are believed to have a very short life. Hadwen (1914–15) twice observed *H. bovis*, which had been captured without injury a few minutes previously, and which had been placed on a calf's back with a net over it to prevent escape, to be seized with a sudden spasm and to die instantaneously. He found the flies very intolerant of captivity. In large cages they quickly batter themselves to pieces, and in small cages Hadwen did not succeed in keeping them alive more than a few hours. Carpenter (1914) also observes that unless transferred at once to a cage in a dark place they damage themselves badly, and that this is especially true of the males.

The fact that *H. bovis*, at all events, is never on the wing except in sunny weather further militates against its capture by entomologists. Moreover both species are very rapid fliers and not easily taken in the open. Nevertheless it is clear that on occasion they may occur plentifully enough, for Riley states that during the first few days of March 1892 in Texas "an old and feeble animal, which had laid down and had not strength to rise, was observed to be attacked by about fifty flies, of which upwards of a dozen were captured."

It has often been remarked, and it was confirmed in these Texas observations that the flies will not venture over water, and while *H. lineatum* was about "the cattle on clear days came to the river about 9 o'clock in the morning, and remained standing in the middle of the river until 5 o'clock in the afternoon... Most of the cattle stood on flat rocks protruding out of the water, so that no part of their body or legs were submerged; but in spite of this fact, while they were standing in the river they were unmolested."
The best observers are now agreed that *Hypoderma* is the sole cause of “gadding” in cattle. Carpenter and Steen (1908) note that “while the presence of numerous clegs (*Haematopota*) does not lead to any visible disturbance among the animals, a calf, the moment he is touched by a warble-fly, becomes frantic and goes off at a bound.” The cause of terror has always been a puzzle, since no pain is inflicted. In the following passage Carpenter (1914) suggests an explanation. “We found that the calves became annoyed and excited and commenced to gad if touched on any part of the legs or flanks, but they did not stir if touched on their backs. This led us to conclude that the mere irritation caused by the fly touching the calf in its persistent attacks is enough to cause the animals’ gadding and terror; they cannot get rid of the fly unless they plunge into a river, or find shady shelter.” Loss of condition and of milk due to “gadding” are among the recognised ill-effects of warble-flies. That they may also cause serious inconvenience is well illustrated by an anecdote of Hadwen (1912) from Alberta. He one day met a cow-boy who seemed to be vastly amused at something, and who, on being asked the cause of his merriment, said: “Well; I came riding by a little lake just now, and I saw some cattle standing in it with their yokes on. A disconsolate settler was sitting on the bank, who said that he was waiting for them to come out with the plough, and that he would not get much ploughing done if the flies did not stop chasing his cattle into the water.” Gadding with a plough is not unattended with danger, and a contrivance is in use in some districts by which the oxen are automatically released from the implement in case of a sudden dash away.

Carpenter and Hewitt published in 1914 (Carpenter 1914) a description of the reproductive organs of the warble-fly. They were apparently unaware of the previous work of Joly in this field.

**Oviposition.**

Though oviposition was not observed in case of either fly till 1892, the egg of *H. bovis* was described and figured by Réaumur (1738). He, and several of his successors (*e.g.* Brauer, 1863) obtained it by expression from the ovipositor of a captured fly. The early view was that the back of the cattle was pierced by the ovipositor, with the infliction of great pain—hence the gadding—though Réaumur could find nothing of a piercing nature in the ovipositor except the minute chitinised points at its extremity. He, indeed, was somewhat sceptical of the extreme suffering of animals so tolerant of the goad. Nor would he adopt Vallisnieri’s view that a poison was inserted with the egg. Though the unsuitability of the ovipositor of *Hypoderma* as a piercing instrument was thus long ago demonstrated, the early view of oviposition was so firmly established in popular belief, thanks largely to Bracy Clark’s “Essay” (1815), that Miss Ormerod (1884–94) only abandoned it towards the end of her investigations. She did, in her later papers, admit that the piercing theory must be abandoned, and held that the flies probably deposited their
eggs on the skin of the back. She advocated, as a most important preventive measure, the smearing of the backs of cattle during the fly season to prevent oviposition. The discoveries of 1892 threw an entirely different light on the matter. An anonymous correspondent\(^1\) communicated to Riley a very complete account of the oviposition of *H. lineatum* in Texas. There the fly was known as the "heel-fly" from its habit of attacking the lower part of the legs of standing cattle, but the actual process of oviposition was studied in the case of an old and feeble animal, incapable of gadding to take refuge in the water. Lying helpless on the ground, it was entirely at the mercy of the warble-flies, and during the first few days of March it was seen to be attacked by about fifty flies.

"The flies were observed to approach very swiftly and deposit their eggs anywhere on the sides, belly, or tail, near the anus, and on the front legs. The flies were left unmolested in many cases until, after remaining on or about the animal from five to ten minutes, they flew off. The eggs were then found without difficulty, usually placed four or six together, and fastened to a hair." Hadwen also observed the oviposition of *H. lineatum* in 1915. We quote the most important passages of his account:

On four occasions *H. lineatum* has been captured by hand on recumbent animals, two by myself and two by my cowman. Each time the fly was resting or ovipositing on the forefoot, which was tucked in under the elbow. When ovipositing, the fly backed up and pushed its ovipositor under the hairs. Numerous eggs were found on this part of the animal, *i.e.* the side of the sternum beneath the elbow—so this seems a favourite spot for egg-laying. On May 2, while watching for flies, I observed one sitting on the ground just behind, and a few inches from the heel of a young heifer. While watching I saw it run backwards like a crab, reach upwards from the ground and oviposit on the hair which hangs down round the coronet. The attitude was reminiscent of the way in which a scorpion curls up its tail... It seems that this site also is a favourite one for oviposition. On May 3... a fly was observed on the ground near a recumbent cow; this insect ran backwards... and oviposited about six inches below the point of the ischium, where the cow's body touched the ground. From this point it laid eggs at intervals all along the side touching the ground, as far forward as the elbow, without touching the animal except with its ovipositor... Numerous eggs have been found also between the point of the hock and the ischium, also on the inside of the legs. On one occasion seven eggs were found on the scrotum of a bull-calf, and at other times they have been encountered on the tail. From these observations it is evident that *H. lineatum* lays its eggs principally when an animal is lying down, and that it does so standing on the ground, thus giving the animal a minimum amount of irritation... The position of *H. lineatum* when ovipositing is always such that its head points in the same direction as the hairs.

In the case of *H. bovis*, oviposition was first noted by Carpenter and Steen in 1907, but the account is meagre and is chiefly important from the statement that the eggs are chiefly laid on the legs. "The fly strikes both at the fore and hind limbs, near the hock, more rarely on the shoulder."

A fuller account was given by Hadwen (1912). "When a warble-fly (*H. bovis*) strikes, it hangs on for a second, evidently holding on with its legs while it fits on an egg."

\(^1\) Now known to be a Mr Schaupp to whom the credit of this important discovery ought to be attributed.
The eggs are in this case laid singly, attached to the base of a hair. "The favourite places for the fly to strike seemed to be the region of the hock in particular, and the back of the knee, occasionally striking as high as the stifle (where eggs were found), and along the flanks to about the same height."

It was thus firmly established by direct observation that the eggs were never deposited by either fly on that part of the body where the warble tumours subsequently occurred, but before oviposition had ever been witnessed, indirect evidence of a very strong nature had been obtained by Carpenter and Steen through a series of remarkable experiments began in 1904 and published in 1908, and designed to test the value of preventive smearing of the backs of cattle, so strongly advocated by Miss Ormerod. All the usually recommended smears were applied in a manner much more thorough than would be possible with any farmer, but the cattle thus treated were no less warbled than the controls. The entire covering of the back with linen or calico during the whole fly season also failed to reduce the average number of warbles. The satisfactory covering of the legs was not found to be practicable, as the animals always succeeded in so damaging the leggings that it was impossible to be confident that the legs had never been exposed to the fly; nor was the experiment on a large enough scale to have great importance. It was found, however, that while four calves with their backs covered developed an average of 10 warbles, four others with their legs protected averaged only 3.5 warbles the following spring under precisely similar conditions.

The facts of oviposition, and the knowledge which had been obtained of oesophageal larvae, were thought strongly to support the view that, as with Gastrophilus, the eggs or newly hatched larvae, were licked off by the cattle and that the parasite entered its host by way of the mouth. Hinrichsen thought so in 1888; Riley gave his adhesion to the view in 1892, and it held the field until 1915.

**Hatching of the eggs.**

Eggs which have hatched are easily recognised by the split distal end. There is no operculum as in the allied genus *Oedemagena*.

Gläser observed the larva in the act of breaking open the egg-shell and making its exit. Approaching its mouth-hooks so that they became parallel, the larva alternately contracted and expanded so as to strike the anterior end of the shell repeatedly with the median spear till at length perforation was accomplished. Into the small aperture thus made it inserted the mouth-hooks, divaricated them, and began tearing at the substance of the shell, which suddenly split open when the operation had been carried on for a short time. The larva then crawled out by the aid of its spiny armature, especially the spines at its posterior end, and proceeded to make its way down the hair.

**Penetration.**

Carpenter, with the aid of various colleagues, endeavoured to test the licking theory by subjecting certain animals to such restraint by tying or
muzzling that they were inhibited from licking themselves during the fly-season. The earlier experiments were invalidated, either through faulty muzzles or from the difficulty of subjecting muzzled and free animals to precisely the same conditions, and for some years the results were conflicting. Improved methods, however, gave a distinct indication that muzzling did nothing to decrease the number of warbles but even tended to increase them, the inference being that such eggs or larvae as were licked off came to nothing. This conclusion was arrived at about 1912, and in 1914 Carpenter and Hewitt remarked that the newly discovered first-stage larva was as well adapted for perforating the skin as the wall of the gullet.

Further negative evidence was obtained by feeding eggs and newly hatched larvae to calves never exposed to fly attack. In 1913 Carpenter and Hewitt fed 16 newly laid eggs of *H. bovis* to each of three calves, and in 1914 a similar experiment was tried with newly hatched larvae, but no warbles resulted.

In 1913 Gläser placed newly hatched larvae on a shaved patch on the skin of a calf, but observed no attempt at penetration. In 1914, Carpenter, Hewitt and Reddin were more fortunate. "Instead of shaving we clipped closely a small patch on the shoulder of a black calf, thus keeping the conditions more nearly normal, and put seven maggots on it. . . . Immediately they were put on the hairs they crawled down them to the skin, and directed their bodies perpendicular to its surface. We soon found that they were disappearing slowly into the skin; four were lost sight of, but the other three were watched cutting the epidermis with their mouth-hooks and occasionally bending the hinder region of their bodies until they disappeared completely. It took them about six hours to get into the skin; possibly hair follicles may have facilitated their entrance." The uneasy movements of the calf made it very difficult to observe exactly the movements of these minute semi-transparent larvae.

In April 1915 Hadwen made several successful experiments with the first-stage larvae of *H. lineatum*, by placing them on small pieces of skin freshly removed from a cow. Their attempts at penetration could be followed at ease under the microscope, and though the condition of the skin of course rapidly deteriorated, despite the arrangements to keep it constantly moistened, many succeeded in half burying themselves, and a few entirely disappeared. On May 7 a piece of skin underlying three hairs to which twelve eggs were attached was removed from a cow. At the foot of one of the hairs a small droplet of clear serum was exuding, which kept increasing in size. By doubling the skin and squeezing, Hadwen expressed two larvae, one of which made determined efforts to re-enter the hair follicle. Carpenter, Hewitt and Reddin had a similar experience.

As to the actual procedure of the larva, Hadwen writes:

Upon emerging from the egg the larva crawls actively along the hair to the skin. It is apparently aided in this by a sticky exudate with which it is covered, and which seems also to serve the purpose of preserving it from drying out. Upon reaching the root of the hair it begins to work with its mouth-parts. In no instance was a larva observed to penetrate the skin otherwise than by a hair follicle. The adherence of the larva to the hair keeps it in proper position for penetration and provides it with a *point d'appui*. 
Thus in the case of both flies it has been demonstrated that direct entrance is obtained at the spot where the eggs are laid. That this is the only mode of entrance is not proved, though much negative evidence in the case of H. bovis tends to show that that fly, at all events, seldom or never obtains entrance by way of the mouth. It is perhaps worth noting, that muzzling experiments have not been applied to the case of H. lineatum, and that the eggs of that species are laid, as with Gasterophilus equi, in full view and much more accessible to the tongue than those of H. bovis, which are deposited singly and concealed at the base of a hair. Though in all probability the life-history is similar in the two cases, the possibility of licking in the case of H. lineatum cannot be altogether excluded.

The Larva within the Host.

The two last larval instars were well known to the earliest investigators, since they could be extracted from the open tumours. The antipenultimate instar was not properly described before the appearance of Brauer's monograph (1863) though Clark (1815) evidently saw it, for he observes that "when young, the larva is smooth, white and transparent." In 1884 Hinrichsen began a series of observations the results of which he did not publish till 1888, and his discoveries heralded a great advance in our knowledge of the bionomics of the warble-fly, though their interpretation was held to support a theory now discredited, namely that the parasite obtained an entrance to the host by way of the mouth. He found larvae which he doubtfully ascribed to H. bovis in the wall of the gullet and in the spinal canal, and he suggested that the cattle obtained the newly-hatched larvae from the grass and that these bored their way into the oesophageal wall where they spent about five months, proceeding thence by way of the spinal canal to their ultimate situation in the back. The lead given by Hinrichsen was followed by Curtice (1890), Horne (1894), Ruser (1896), Schneidemühl (1897), Koorevaar (1898), J. P. Koch (1903), Jensen (1903), des Gayets and Vaney (1912), Gläser (1912–14) and Hadwen (1916), and though the "licking" theory had to be relinquished in 1915 when Carpenter proved direct penetration, Hinrichsen's view of the passage of the larva from the oesophagus to the back was thoroughly confirmed.

All these investigators agreed that larvae identical in structure with the earliest stage found in the warble tumours and little inferior to them in size, occurred with regularity in the oesophageal wall soon after the fly season, and in the spinal canal shortly before their appearance in the back.

Koorevaar tried the experiment of introducing freshly obtained oesophageal larvae under the skin of a small dog, but the larvae were apparently not ripe for their final migration, and though on dissecting the animal a few weeks later he succeeded in accounting for every one of the 36 larvae employed, little was proved beyond their extensive wandering. Hadwen (1914–15) was more successful. He introduced twelve oesophageal larvae under the skin of the back of a calf born in September and therefore under no suspicion of having been struck by the fly on its own account. Four warbles resulted.
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thus proving clearly the identity of the larvae. It appears that only the largest oesophageal larvae are ready to proceed at once to form warbles, and that those less advanced continue their wanderings—sometimes returning to the oesophagus when experimentally placed beneath the skin.

Ordinarily the course of a migrating larva is betrayed by no track, but on occasion sepsis has arisen and a greenish trace is visible, as has been noted by Ruser and by Hadwen. The oesophageal larvae experimentally inserted without disinfection always mark their course by a distinct track, and are therefore particularly instructive. The tracks, whether occurring naturally or experimentally, are always in the connective tissue.

It might be supposed that the larvae in the oesophageal wall would all be found to be travelling in the same direction, but this is far from being the case. Their orientation is quite irregular and they clearly spend a considerable time in this organ, moving in any direction. It is, however, observed that they are at first chiefly congregated towards the pharyngeal end of the oesophagus and that they progressively descend, the last remaining larvae being always near the entrance to the stomach.

The choice of the oesophagus for a prolonged stay on the way to the back is of great interest. As Hadwen remarks, there are two points of supreme importance to the parasite: it must avoid organs where the reaction to its presence would be so great as to endanger its life or that of its host, and it must provide against the possibility of encystment. Encystment is avoided by constant motion, and the oesophageal wall is admirably suited for its purpose, as the reaction is slight unless the larvae are very numerous.

There appears to be no invariable route from the oesophagus to the back. Occasional larvae found outside the wall of the oesophagus (Carpenter, etc.) seem to indicate a direct passage to the anterior region of the warble area, but the general course is to proceed along the gullet to the diaphragm. One route thence has been definitely traced by Hadwen (1915), the greenish track of the larva being visible the whole way. The larva had entered the diaphragm, passing under the pleura enfolding the termination of the oesophagus, and had then struck downwards and outwards, following the radial arrangement of the muscle fibres. This brought it to the spot where the diaphragm meets the ninth rib, and it proceeded up the posterior border of this rib, following the course of the blood vessels, and arrived at the spinal canal, which it entered by a posterior foramen.

This is the only course absolutely demonstrated, but it is clear that any of the ribs upon which the diaphragm impinges might be utilised, and it is quite possible, and Hadwen considers it probable, that many larvae do not use the ribs at all, but take a shorter course directly up the diaphragm.

The remarkable observations here briefly described have removed many of the obscurities which so long enveloped the larval life of Hypoderma, but they throw no light on the period between oviposition and the arrival of the larva in the gullet.
Hypoderma larvae have often been found attacking man and their behaviour in such cases is very instructive. In 1886 Prof. W. M. Schoyen collected a number of instances, which had occurred in Norway during the previous hundred years, and though at that date our knowledge of the larvae of the two species was very defective, Schoyen states in a letter to Riley in 1891\(^1\) that he had himself seen many of the larvae in question and that they were undoubtedly *Hypoderma* larvae "sine dubio—*H. bovis*." He continues: "As a rule they have undertaken long ramblings under the skin, always in upward directions, previous to their appearance through an opening in a tumour on the upper part of the body (head, neck, shoulders, etc.). All of them lived in this manner for months and came out in the course of the winter months (February or so) but were always still much too young to be hatched. However I have no doubt at all that they belong to *H. bovis*, as it is especially in those persons who take care of cattle in the summer months that such grubs are to be found during the winter. It is evidently the smell of cattle which attracts the bot-fly to them."

Two cases are so fully authenticated that a more detailed account of them may be given. In 1889 Dr Elizabeth R. Kane communicated to Riley the following facts\(^2\). On February 22 Dr S. D. Freeman of Smethport, McKean County, Pa., was called in to see a small boy three or four years old. He was at first unable to go but sent his assistant, who found the patient’s ear greatly swollen and threatening erysipelas, though there were no constitutional symptoms. Dr Freeman was again sent for a few days later. On Feb. 28 the ear swelling had subsided, but a red line of inflammation went up under the eyelid and then down the cheek which the doctor lanced, and from which he extracted a living grub about \(\frac{1}{2}\) inch long. He was told that the eye had been entirely closed for 24 hours.

The mother who called the grub a "pollywog," said she had first noticed it five months previously. It was then near the sternal end of the right clavicle. Thence it passed, appearing as a tiny lump followed by a red track, down and up the chest, down one arm as far as the elbow, then up and over the shoulder and part of the back, from which it had travelled to the ear, never crossing the median line. Sometimes it had "pointed," and seemed about to come out, but it presently resumed its wanderings. Until the last few weeks the child had shown little uneasiness, but of late he had been unable to sleep. The mother believed that the grub travelled at night, for she never noted any advance during the day time. Dr Freeman writes: "I have positive knowledge of its movements, having just seen the track over the scapula, then up the neck to base of ear which was enormously swollen, from there to the outer corner of the eye, which was entirely closed, then to the middle of the cheek where it was plainly felt, and the opening made and expelled." The grub was sent to Riley who in the then state of knowledge was unable to identify it with certainty, but doubtfully attributed it to *H. diana*, which infests deer.

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\(^1\) *Insect Life*, iv. p. 275.

\(^2\) *Ib*. ii. p. 238.
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Fortunately it was kept, and on re-examining it in 1892 Riley had no difficulty in recognising it as belonging to *H. lineatum*.

The other case concerned Gläser himself in the course of his investigations. He was experimenting in June 1913 with a female of *H. lineatum*, which oviposited on some hairs which he held behind it. The same insect laid an egg on his trousers. The eggs on the hairs hatched in twelve days; that on his trousers hatched in eight days and the grub began boring into the skin of his leg. In $1 \frac{3}{4}$ hrs. it had disappeared leaving a small round red spot. Four or five days later it could be felt through the skin, and seemed to be about $2 \frac{1}{2}$ mm. long. Early in September its whereabouts was indicated by swellings on the hip and the abdomen, and at the end of September Gläser judged, from a pain in swallowing, that it was at the lower end of the gullet. Thence it rapidly proceeded upwards, and in October he extracted it from his mouth. It was a typical oesophageal larva 7.5 mm. in length.

### III. Injuries to Cattle.

The most obvious injury to cattle inflicted by warble-flies is reflected in the hide-markets, badly warbled hides being useless as leather. Every warble tumour of the current year means a hole in the leather—usually in the most valuable part, near the middle of the back—and though the openings of the tumours of previous years close up and no perforation remains, the new tissue is of a weaker consistency and a scar is left. To estimate the annual loss from this source only it would be necessary to collect statistics from all the hide-markets, and the matter would be very complicated, for the hides are graded and the percentage of attacked animals differs in each grade, and varies greatly in different localities. Miss Ormerod collected the opinions of a large number of hide-merchants. A fair sample of the replies she elicited is that received from Messrs. Richard Markendale and Co. of Manchester. In 1888 the firm handled 250,740 hides of which 83,580 were warbled, and they estimated the year's loss through warbles at £16,716.

Other ill-effects of warble-fly attack which have long been recognised are loss of condition and of milk by “gadding,” and the deterioration of the flesh of the back at the time the larvae are entering the hide, an oedematous condition known to butchers as "licked beef," necessitating the cutting away of considerable portions as unfit for human food. Massive infestation may be fatal, and it is probable that in a great many cases warble-fly alone was responsible for death attributed to various causes.

The investigations which have led to a fuller knowledge of the life-history of the fly have revealed several other lesions for which it is accountable. Penetration, for instance, may have serious results, as Hadwen shows in the following passage:

Following the penetration of the larvae down the hair follicles, serum is exuded which dries on the surface of the skin, completely covering its pores. The subsequent skin lesions

1 *Twelfth Report, p. 125.*
are, I believe, entirely due to the introduction of bacteria.... In some instances no further symptoms have been noticed except perhaps a slight thickening of the skin and a gluing together of the hairs. In other cases there has been intense irritation and a dermatitis has been set up.... Sometimes the inflammation is more extensive, and large oedematous areas have been noticed, extending into the deep tissues. In the outbreaks of previous years a number of bad sloughs have occurred, some of them on the upper part of the udder, and on the thighs. Other parts where serious lesions have been encountered are in the line from the point of the ischium and the patella, and some few patches on the flanks, becoming more numerous until they reach the sides of the sternum, under the elbow. Some quite large lesions have been noticed on the tail.... Seeing that the penetration of the larvae produces a definite disease, I would propose the name 'hypodermal rash.'

In the case of *H. bovis*, Carpenter, Hewitt and Reddin¹ noticed a rash of small pimples on the day following penetration, but the lesions were not serious. *H. lineatum* might be expected to be much more formidable in this respect, because of the number of larvae which must enter near the same spot. It has been suggested by several writers that anthrax germs may thus find an entrance to cattle.

Des Gayets and Vaney, and Jensen, have especially studied the lesions caused by the larvae in the oesophageal wall, and have met with cases of stenosis of the gullet caused by them.

Lesions in the spinal canal are difficult to verify, but there is little doubt that at this stage of their journey considerable harm may be done to cattle by *Hypoderma* larvae. Signs of inflammation in the cord are generally noticeable in their neighbourhood, and Moussu told Hadwen (1916, p. 11) that he had seen nervous symptoms in cattle which he attributed to this cause.

There are indications that the first appearance of the maggots in the dorsal integument is the occasion of especial constitutional disturbance. Mr K. J. Mackenzie tells me that he has often observed a sudden rise of temperature in animals at the time when warble tumours first become recognisable to the touch as small hard swellings, and that the temperature falls as suddenly after the formation of the external aperture. It is a common experience that animals that have done badly during the winter and early spring, and then develop many warbles, pick up at once after the warble season.

There is an acute but usually transient and not fatal cattle disease prevalent in the spring in some localities and known as rose fever, which has recently been investigated by Brodersen. There is extensive oedema, especially on the eyelids, muzzle and lower jaw, and often on the udder and in the region of the anus. The disease has usually been attributed to mistakes in feeding, but Brodersen finds that it frequently follows immediately on the operation of expressing *Hypoderma* larvae, and he thinks it due to the toxic effect of grubs crushed beneath the hide. In this connexion Jensen recalls his experiments of 1903, when he injected under the skin of a calf the extract made in sterile salt solution, of two larvae taken from the gullet of a cow and obtained, about an hour later, symptoms characteristic of rose fever.

¹ *Journal xv. 1916.*
IV. *H. bovis* AND *H. lineatum* DIFFERENTIATED.

The Flies.

Though diagnoses of flies already well known would be out of place in the present paper, it may be useful to give the characters by which the two species can be distinguished at the various stages of their life-history.

Many writers have indicated the most obvious differences between the imagos (e.g. Hadwen, 1916) which may be thus tabulated:

<table>
<thead>
<tr>
<th>Character</th>
<th><em>H. bovis</em></th>
<th><em>H. lineatum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average length</td>
<td>14 mm.</td>
<td>12-7 mm.</td>
</tr>
<tr>
<td>(pinned specimens)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thorax</td>
<td>Dense yellow hairs in front, hiding longitudinal markings, and contrasting strongly with black mesothoracic region</td>
<td>Uniform covering of mixed black and white hairs, leaving longitudinal marks visible (hence <em>lineatum</em>)</td>
</tr>
<tr>
<td>Wings</td>
<td>Veins dark brown; alulae with reddish brown border</td>
<td>Veins black (or nearly), alulae uniformly white</td>
</tr>
<tr>
<td>Legs</td>
<td>Comparatively smooth, with few hairs</td>
<td>Rougher, and more hairy,— especially tarsi</td>
</tr>
<tr>
<td>Abdomen</td>
<td>Terminal hairs yellow</td>
<td>Terminal hairs orange-red</td>
</tr>
</tbody>
</table>

The Eggs.

The eggs of both flies are long-oval, white bodies about 1 mm. long, provided with a bi-lobed foot or clasper for attachment to a hair. "The egg of *H. lineatum* is relatively longer and more parallel-sided than that of *H. bovis*, while the latter has a shorter and broader foot" (Carpenter and Steen). *In situ* the eggs are easily recognised because of the different habit of oviposition of the two flies. *H. bovis* deposits them singly at the base of a hair; *H. lineatum* attaches several eggs to the same hair.

The Larvae.

There is a difficulty as regards the nomenclature of the larval instars, because we are still uncertain as to their number. This has led to confusion in the past, for different authors allude to the same instar by different numbers, according to the state of knowledge at the time. We now know the first-stage larva and also three final stages in the case of both flies, and in the case of *H. lineatum* Laake has recently shown that the oesophageal larvae hitherto considered as all of the same instar, present two different forms, the younger spiny, the older smooth. This gives for *lineatum* five instars at least and it is likely that a revision of all the oesophageal larvae of *H. bovis* will reveal a similar state of things. Add to this that there is no certainty that moults do not occur between penetration and arrival in the gullet, and it is clear that any numbers applied to the later instars are purely provisional. The only safe way, however cumbersome, seems to be to allude to the later instars as ultimate, penultimate, antepenultimate, without allotting definite numbers to the stadia. These three instars all occur in the hide, but the first of them—the antepenultimate as we term it—is the form in which the larva leaves the gullet to take up its final position, and includes, therefore, the larger oesophageal
larvae, the larvae of the spinal canal, and those which first appear in the warble tumours. The smaller, spinous oesophageal larva has not yet been demonstrated for *H. bovis*, but in all the other instars Laake has indicated characters by which the two species may be differentiated.

**First-stage Larvae.**

The mouth armature and the spines on the terminal segment of the body serve to distinguish the two species in this stage. The differences are best indicated by diagrams. In the mouth-hooks, note in *bovis* the bifid anterior limb and the blunt posterior limb of the crescent, which is not closely applied to the median spur. In *lineatum* the hook is more crescentic with both limbs sharply pointed, and it touches the median spear tangentially.

![Diagrams of mouth-hooks for *H. bovis* and *H. lineatum*](Laake24, III. and Laake22)

The spines on the last segment of *H. bovis* are much larger than in *H. lineatum*, and it is particularly noticeable that in *lineatum* the two flattened spines flanking each spiracle are at least twice the size of the others, while in *bovis* they are not conspicuously larger.

**Antepenultimate (?? third stage) Larva.**

At this stage the mouth-hooks, which can be observed in cleared specimens, present much the same differences as served to distinguish the species in instar 1.

![Diagrams of mouth-hooks for *H. bovis* and *H. lineatum*](Laake11 and Laake15)

In this "smooth" larva the middle segments are unarmed in both species, and there is nothing characteristic about the spiracles.

**Penultimate (?? fourth stage) Larva.**

At this stage the species are best differentiated by the spiracles. Each spiracle is composed of a varying number of "discs" or rings. In *H. bovis*
The Warble-flies of Cattle

these are about 30 in number, and are compactly bound together in a black mass. In *H. lineatum* there are about 20 yellow, loosely bound discs in each spiracle.

*Ultimate († fifth stage) Larva.*

The ripe larva which leaves the warble tumour has a complicated spiny armature best represented by the ingenious form of diagram devised by Brauer. Though there is considerable variation in individuals, there is this constant difference between the species, that on the ventral (convex) side *H. bovis* has the two last segments entirely destitute of spines, while in *H. lineatum* the last segment alone is naked.

Laake points out that the species at this instar may also be distinguished by the spiracles, and may be recognised while the grub is still in the warble. In *H. bovis* the reniform spiracle is somewhat funnel-shaped, the central boss being depressed, while in *H. lineatum* the whole surface is level.

REFERENCES.
The papers not consulted in the original are marked with an asterisk.


Vallissleri, A. (1710). *Opere (published 1733 by his son), 28, fig. 10.

De Villers (1789). *Ent. Lin. iii. 349.*
THE DISCOVERY OF THE COCCIDIA.

By CLIFFORD DOBELL, F.R.S.

Students of the history of Protozoology are generally agreed that the earliest recorded observations on the Coccidia are those of Hake (1839). Bütschli (1882, p. 490) mentions his work, though he did not see it; and it has been cited by Leuckart (1879), Labbé (1896), and several other writers on the Sporozoa—including myself (1919)—as the first publication in which coccidia are recognizably described.

Dr Thomas Gordon Hake was a practising London physician. His book is now extremely rare, and has probably been seen by but few protozoologists. In this work he described the lesions caused by *Eimeria stiedae* in the rabbit's liver, and gave an account, accompanied by admirable figures, of the unsegmented oocysts of this parasite. But Hake's description—though it appears to be the first in which any stages of the coccidia themselves were described—is not the first account of coccidial lesions in the rabbit: for I find that in the previous year (1838) Robert Carswell—at that time Professor of Pathological Anatomy at University College, London—published a coloured picture of a rabbit's liver showing the lesions caused by *E. stiedae*. It is to be found in the section devoted to "Tubercle" (last section of the book), on Plate II (illustrating this section), Fig. 6; but the lesions shown in the bile-ducts—which are partly dissected out—are described in the accompanying letterpress as "a beautiful illustration of the seat of tuberculous matter in the liver of the rabbit." The lesions are, however, to my mind clearly those of coccidiosis—not of tuberculosis.

In his interpretation of his own findings Hake was no happier than Carswell. For him the coccidial lesions were not tuberculous, but constituted a "carcinoma of the bile-ducts"; while the unripe oocysts which he found in them were described as being "a new form of the pus globule," though they were also called more objectively "ovate corpuscles." Nevertheless, in spite of his errors Hake probably deserves the credit of being the first to

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1 Diesing (1851, p. 16) has supposed that sporozoan parasites were seen by Redi in Crustacea: but Bütschli, Léger, and other authoritative writers on the Sporozoa, have rejected this supposition; and after a careful study of the original work of Redi (1684) I consider their conclusions fully justified. At all events, I think there can be no doubt that the things which Redi found in crabs were certainly not coccidia—whatever else they may have been.

2 In Carswell's book the pages are not numbered, nor are the plates numbered consecutively. I trust, however, that the particulars given above will suffice to enable anybody to find the figure in question. The book itself is rare, and not readily accessible. Carswell is mentioned by some of the writers on the Coccidia, but usually without any exact reference to his work.
describe coccidia, in a recognizable manner, in any printed work. It is not my aim, in the present paper, to dispute his claims in this respect: but I hope to be able to show that another and a greater man discovered the "ovate corpuscles" long before Hake was born; and not only discovered them, but left a record of his observations—a record which was written nearly 250 years ago, but which has never yet, I believe, been read aright, and has certainly never yet been printed.

It is well known that the celebrated Dutch microscopist Antony van Leeuwenhoek (1632-1723), the discoverer of the Protozoa, never published his earliest letters—though his real reasons for not doing so are still unknown. In both the Dutch and the Latin printed editions of his collected works the first epistle is that which he himself called his "28th Letter" (April 25, 1679): and a note by the publisher informs us that "the previous 27 letters written by the author, he could not hitherto resolve to make public by printing: and thus the 28th is the first that is here printed." Yet these early letters are not lost. The original manuscripts, written in Dutch, have been carefully kept by the Royal Society, to which body they were addressed: and as they are all dated, it is not difficult, for anybody who will take the trouble, to ascertain their true sequence and the distinguishing numbers which Leeuwenhoek himself probably assigned to each. Moreover, some of these letters have been partly printed, in the form of English or Latin abstracts, in the early volumes of the Philosophical Transactions: but these are fragmentary and incomplete, and convey a very imperfect idea of the originals; while at least eight have never been printed at all, and their contents have, to the best of my belief, remained entirely unknown to the scientific world from that day to this.

During the last few years I have made a careful study of these early unpublished letters, and I have found many very interesting observations recorded in them. I hope to be able to say more about these letters on a future occasion; but in the present note I wish to direct special attention to only one of them—a letter (entirely unpublished hitherto) which is dated October 19, 1674, and which is, according to my reckoning, the "7th Letter" in the whole series. It is not very long—not long, that is to say, for Leeuwenhoek—for it consists of only five large and rather closely written pages. It is addressed to Henry Oldenburg—then Secretary of the Society—and contains a number of microscopic observations on bile, fat, sweat, etc. The observations on bile are in some respects so remarkable that I think no apology is needed for publishing them. As Leeuwenhoek's Dutch would not be readily intelligible to most modern scientific readers, I have—though not without reluctance—translated the passage in question bodily, and as literally as

1 See the Dutch works of Leeuwenhoek, II Deel, ad init. (Register der saaken).
2 I should state that there are three exceptions; for the first letter of all, and two others which I believe to have been the 17th and 27th, are missing. The first was partly printed, however, in the Philosophical Transactions for 1673. The others are, I believe, wholly and irrecoverably lost.
I can, into English. I will now give this translation, and will make my comments upon the observations afterwards.

From Leeuwenhoek’s 7th Letter, October 19, 1674.

The bile of an ox was examined [i.e. with the microscope] by me on the 1st instant [i.e. 1 Oct. 1674], and therein I beheld some few globules that floated in the liquid; but [I saw them] only when I set the bile in a continual motion before my sight, for it would else have been impossible for me to perceive the globules in it, owing to their fewness in the bile that I was examining. But afterwards, examining the bile of another ox, I found that the globules were of a heavier matter than the liquid that they floated in; wherefore I drew off the bile from the bottom of the gall-bladder, and then found that there were many hundred times more globules in this bile than in that which I had taken from the upper part of the gall-bladder; and there were, besides, some corpuscles which, to my eye, looked as big as ants’ eggs. These had the figure of an egg [i.e. a hen’s egg], only with this difference, that whereas an egg is more sharply pointed at one end than at the other, yet these corpuscles were equally pointed at both their ends: and moreover these corpuscles were composed of globules joined together, and had a yellow colour, except several which were somewhat whitish; but notwithstanding, they were so transparent that you could see the body of one through that of another. And this transparency making me wonder whether they were not, in fact, little vesicles filled inside with liquid, I took some of these corpuscles out of the bile with a fine hair; and examining them on the hair, I perceived two which seemed to be bent in, just as though you had filled a bladder with air and then put your thumb on it, so as to make a dent in it: whereupon I was the more firmly persuaded that these corpuscles were filled with some sort of liquid. Afterwards, examining more biles from oxen, I found them the same as before; only with this difference, that one bile would contain more of the oval corpuscles than another.

In the bile of two calves I find, furthermore, some very little globules floating, and very many irregular particles of divers forms; among others, some like little floating clouds, all consisting of very little globules joined together. On seeing these irregular aggregated particles, I judged them to be joined or stuck together through no other cause than because the bile had got cold, and was without motion. In the bile of a third calf there were a few oval corpuscles.

Moreover, in the bile of sucking lambs I find there are very little globules, and some, though very few, bright particles, which are a bit bigger; besides irregular particles, of divers figures, and also composed of aggregated globules. The bile of a yearling sheep I find to be like that of sucking lambs, only with this difference, that in this bile there are also oval corpuscles of the bigness and figure of those that I remarked in ox-bile.

I have examined the bile of two young rabbits: that of the first was inclined to a purple colour, and in it I beheld very many globules, and irregular particles made up of aggregated globules, which were of various red colours: and this diversity of colour I conceived to be due to no other cause than that some of these compound particles, being made up of more globules, were denser than the rest. In the other bile the irregular particles were fewer, but there were more globules and the colour was a light red.

1 Words in square brackets are added by me in order to preserve the sense or in explanation of expressions used in the original.

2 This is a usual form of expression with Leeuwenhoek. He means that the objects looked, under the microscope, about as big as “ants’ eggs” look to the naked eye. At a later date he published a remarkable account of ants, from which it is clear that he was well aware that the ant’s “egg” is not really an egg, but a pupa. See Letter 58, Sept. 9, 1687.

3 It seems probable that these globules were red blood-corpuscles. Leeuwenhoek had described these—from his own blood—in another letter written earlier in the same year (April 7, 1674).
Further, I examined the bile from three old rabbits. The [bile of the] first contained a very few small globules, but very many oval corpuscles of a figure like those that, as I have said, I saw in the bile of an ox. In the bile of the two other rabbits there was nothing but globules, and irregular particles composed of globules joined together; though the thin matter [= liquid constituent] of one was much thicker and more viscid than that of the other, and there were some little clouds floating through it.

I have further examined the bile of fowls, turkeys, etc., and in it I also found very little globules floating, and irregular particles composed of globules joined together.

I would invite the reader's attention especially to the concluding paragraphs of the foregoing extract, wherein Leeuwenhoek describes his observations on the bile of rabbits. It will be observed that he examined altogether five of these animals—three old and two young ones. In the latter he found nothing but "particles" and "globules" (called deeltgens and clootgens in the original) which were, so he supposed, probably formed by precipitation and aggregation on cooling, and which were observed floating singly or clumped into irregular flakes. But in one of the three old rabbits he found, in addition to the "globules," many "oval corpuscles" (eijrond deeltgens) like those which he saw in the bile of oxen and sheep. I do not think there can be much doubt as to the correct interpretation of these discoveries. The egg-like bodies in the gall-bladders of sheep and oxen were undoubtedly the eggs of trematodes; while those in the bile of the rabbit were no less certainly the oocysts of Eimeria stiedae. Let us consider these interpretations in more detail.

From Leeuwenhoek's account of the oval structures in the bile of the ox it appears to me certain that he was dealing with the eggs of Fasciola hepatica L., or some similar trematode. Their shape—resembling an "ant's egg," but not a hen's egg—and their yellowish colour; their transparency under the microscope; their vesicular constitution, and their contained globules; their specific gravity—greater than that of the bile in which they floated; the fact that they possessed resistant but not inflexible shells—as is indicated by the experiment with the hair;—all these characters convince me that these "oval corpuscles" were the eggs of liver-flukes. The distribution of these corpuscles in the various samples of bile examined is also most significant. It will be seen that Leeuwenhoek found them regularly in the bile of (adult) oxen, but in only one calf out of three examined: he found them in a year-old sheep, but not in lambs; and he did not find them at all in the bile of the birds which he studied. Moreover, he tells us that they varied in numbers in the different specimens of bile which he obtained from oxen. The observations are, therefore, exactly accordant with what we should expect if the objects which he found were the eggs of liver-flukes.

I do not know whether these observations are the first ever made on the eggs of trematodes, but I think they probably were. The worms which lay these eggs were discovered, however, at an earlier date: for I find them mentioned in the work of Redi (1668), who tells us they were called "bisciole da' macellai," and gives us (p. 190) a picture of one (Fasciola hepatica) from
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the liver of a sheep. They were also known—at a later date, at all events—to Leeuwenhoek; for the anatomist Bidloo (1698) addressed a small work\(^1\) to him in which they were described and figured in some detail, and Leeuwenhoek himself afterwards wrote a letter on the "Worms observ'd in Sheeps Livers" to the Royal Society, who published a short English abstract of it in their Transactions in 1704 (Letter dated November 3, 1703, Phil. Trans. xxiv. 1522: not in Latin or Dutch collected works).

But I am not now concerned with the discoveries in the bile of oxen and sheep save in so far as they throw light upon the objects which Leeuwenhoek found in the bile of the rabbit. He says that these objects were like those which he saw in the bile of the ox; and consequently, if my interpretation be correct, they were like the eggs of a liver-fluke. Now as everybody knows, the gall-bladder of the rabbit very frequently does contain bodies which are, superficially, extremely like the eggs of Fasciola hepatica—namely, the oocysts of Eimeria stiedae Lindemann. In size, shape, and general appearance these are so like eggs that they were long known—before their nature was ascertained—as "egg-like corpuscles" or "oviform psorosperms." Davaine (1860), for example, devoted considerable space to the discussion of these structures and concluded that they were probably "the eggs of helminths"; and a direct proof that they are not the eggs of worms was not obtained until long afterwards, when it was shown that they are the cysts (oocysts) of a sporozoon living in the bile-canal system within the substance of the liver. E. stiedae\(^2\) is now known to every elementary student of zoology, but our knowledge of it, and of its life-history, was acquired only after many years of study, and by the labours of many men. It is unfortunate that Leeuwenhoek said so little about what he found in the rabbit's bile: but his bare statement that the "bodies" in it were like those which he found in oxen—of which he gives an easily recognizable description—suffices, in my opinion, to place the matter beyond all reasonable doubt. Anybody who will take the trouble to examine the contents of the gall-bladders of a few rabbits can readily confirm his observations.

It will be noted that Leeuwenhoek says he found the "oval corpuscles" in the bile of one old rabbit out of three examined; but that he found none in two young ones. He does not say whether the rabbits were wild or tame, but one may perhaps infer that they were wild ones which had not long been kept in captivity; for tame rabbits are often more heavily infected, and his observations seem to indicate that he was dealing with comparatively clean stock—some of the old ones but none of the young having acquired infection. But the numbers are obviously too few to warrant any definite conclusions.

\(^1\) An English translation of this memoir was incorporated by Hoole in his Select Works of Leeuwenhoek, vol. i, part 2, 1798. There is also a Dutch version, published at Delft in 1698; but this last I have not seen. The Latin version is also to be found reprinted in Bidloo's Opera Omnia Anatomico-chirurgica, Lugd. Bat., 1715.

\(^2\) The parasite is commonly called "Coccidium oviforme" or "C. cuniculi" in the elementary text-books.
on this point, and so far as I can discover Leeuwenhoek nowhere published any further observations on the bile of rabbits, though he records many other observations made upon these animals—both wild and tame. I mention these points for the following reason. It might be said that had Leeuwenhoek really studied a rabbit suffering from coccidiosis, then he ought to have noted the lesions—visible to the naked eye—in its liver. To this I would reply that what evidence there is points to the conclusion that his animals were not heavily infected, and in such animals it often happens that no superficial lesions are visible to the naked eye, even though oocysts can be found in the gall-bladder. The fact that Leeuwenhoek did not record any abnormality in the liver of the animal from which he obtained the “egg-like bodies” does not, therefore, weigh against the interpretation here advanced. The recorded findings are, on the contrary, quite consistent with my conclusions.

If the interpretations here advanced are correct, then it must be concluded that Leeuwenhoek discovered and described the oocysts of *Eimeria stiedae* as long ago as 1674. It is true that he did not know what they were: but then neither did any of the earliest observers of the Coccidia. The fact remains, none the less, that he discovered them: and if this be admitted, then it must also be admitted, I think, that these observations are among the first ever made upon the Protozoa. It is true that Robert Hooke described and figured the shell of a foraminiferan about ten years earlier—calling it a “small Shelled-fish” (see Hooke (1665), Obs. xi, p. 80, and Scheme V, Fig. x). Nummulites—large fossil shells of Foraminifera—were also known to Strabo the geographer, and doubtless to others among the ancients: but none of these writers can properly be said to have “discovered” the Protozoa. Leeuwenhoek’s famous letter describing free-living protozoa in various waters is generally allowed to be the first in which the living animals were described. It was written in 1676 and partly published in the *Philosophical Transactions* in 1677, but describes a number of discoveries made in 1675. Yet his discovery of the oocysts of *E. stiedae* appears to have been made still earlier, and the observations on this organism are, so far as I have been able to ascertain, among the first which Leeuwenhoek ever made on Protozoa of any sort. Consequently, they are probably—if we except Hooke’s foraminiferan shell—the first microscopic observations on the Protozoa1, the first on any parasitic protozoon2, and the first on any species of the Coccidia.

The conclusions which I draw from the foregoing considerations are, therefore, as follows. The Coccidia were discovered by Leeuwenhoek, who made a brief but recognizable reference to the oocysts of *Eimeria stiedae* in 1674: but he did not know what they were, and his observations were never published. The first published account of the oocysts of this parasite

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1 There are, I believe, a few observations on free-living protozoa in a slightly earlier letter (Sept. 7, 1674), but these are not generally known, and the letter has not yet been printed in full.

2 Leeuwenhoek’s observations on *Giardia intestinalis*—which I have discussed in detail elsewhere (Dobell, 1920)—are dated 1681; and the better known letter on the ciliates of frogs was written in 1683.
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is probably that of Hake (1839), though he also was unable to interpret his findings correctly; while the first published description and figure of the lesions of hepatic coccidiosis in the rabbit are probably to be found in the work of Carswell (1838), who likewise misinterpreted what he saw.

Leeuwenhoek’s observations seem to me so remarkable, and to possess such interest for all students of the history of Protozoology, that I have felt it my duty—and a very pleasant one—to rescue them from the oblivion in which they have already lain all too long: and their publication, even at this belated hour, will serve, I hope, still further to enhance the reputation of one who is rightly regarded as the Father of Protozoology.

REFERENCES


HAKE, T. G. (1839). A Treatise on Varicose Capillaries, as constituting the structure of carcinoma of the hepatic ducts, and developing the law and treatment of morbid growths. With an account of a new form of the pus globule. 4°, London.


LEEUVENHOEK, A. VAN. See, in addition to the works to which particular reference is made in the text, Opera Omnia [Latin works], 4 vols., sm. 4°, Lugd. Bat., 1722: Werken [Dutch works], 4 vols., sm. 4°, Leyden en Delft, 1686–1718.

LEUCKART, R. (1879). Die Parasiten des Menschen, 2 Aufl., 1 Bd. 8°, Leipzig u. Heidelberg.


— (1684). Osservazioni intorno agli Animali viventi che si trovano negli Animali viventi. 4°, Firenze.
ON THE LIFE-HISTORY OF MELITTOBIA ACASTA, WALKER; A CHALCID PARASITE OF BEES AND WASPS.

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(From the Zoological Laboratory, Cambridge.)

(With Plate XXVI.)

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I. INTRODUCTION.

(a) Material.

Melittobia has attracted the attention of a number of entomologists since it was first described under the name of Cirrospilus acasta by Francis Walker in 1839 and a number of papers have been published contributing facts with regard to its life-history.
Life-history of Melittobia acasta

The insect first came under my notice in 1918 when I was watching the habits of and making experiments with certain solitary bees and wasps in my garden in Cambridge. I had started there a small colony of *Odynerus spinipes* by bringing home pieces of an old mud wall containing larvae from a ruined cottage at Histon and I had attracted various other wasps and bees by providing glass and elder tubes arranged on shelves attached to my garden fence.

On the 30th May of that year I noticed that a minute Chalcid was swarming over one of the pieces of the mud wall and later freely entering tubes which were being occupied by bees and wasps and, in the case of glass tubes so occupied, I noticed that numbers of this minute insect became sealed up in the cells constructed by the bees or wasps.

At first I thought that these imprisoned individuals had been caught accidentally in the cells and that they would quickly perish but, on watching them, I noticed certain acts which caused me to form a different opinion.

I therefore began to investigate the life-history and by the autumn of that year I had worked it out and it was not until I was describing some of the curious habits of this little parasite to my friend Dr Keilin that he referred me to Malyshev's paper which he very kindly translated for me. I then found that, although in the main my observations agreed with those of the Russian author there were various points upon which I differed from him and I therefore decided to work out the life-history again and to make some further experiments.

It was not until January 1921 that I was again able to obtain the necessary material as I had allowed all my stock to die out after completing my observations in 1918.

(b) Technique.

In order to watch the life-history of and to experiment with this minute insect I made a number of cells by cutting pieces of glass tube of about 8 mm. diameter gauge into 3 inch lengths and fixing these horizontally to small pieces of cardboard by means of thin wires (Pl. XXVI, fig. 9). Into the middle of such a piece of tube a wad of cotton wool was pushed and rammed tight, the wad being about half an inch long. Each end of the tube plugged with a similar wad gave me two cells to each tube. I found that these cotton-wool plugs were sufficient to prevent the escape of the insects under ordinary circumstances since, if the female is placed under conditions which give her suitable food and suitable material for the reception of her eggs, she makes no effort to escape. If however females were retained in such a cell without food they frequently attacked the plugs and when they were numerous—as when a whole brood hatched out and was left in a cell, they frequently bit their way out. It is curious to notice that under these circumstances, the central plug was seldom, if ever, attacked and presumably the insects were guided to the end plug by air currents through the wool. The cardboard to which the tubes were attached made them stable and upon it numbers and dates referring to the imprisoned insects were written.
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The food-material, in the form of resting larvae and of pupae of various species, was placed in the cell and every movement of the parasite on its host could be watched under a Zeiss binocular dissecting microscope. The tube could be rotated so as to cause the host larva or pupa to roll over so that *Melittobia* eggs and larvae could be seen and even counted, if not numerous, without removing the host from the cell. As a rule however the cell was opened and the host tipped out whenever a count of eggs and larvae of the parasite was to be made and at these times the parasite sometimes remained in the tube and sometimes came out upon the host. In the latter case she frequently remained upon the host the whole time that her eggs and larvae were being removed and usually showed no signs of fear or annoyance even when pushed out of the way by the brush or needle.

Occasionally the cells required cleaning as the glass became dulled by the excreta of the female.

The cotton-wool plugs seemed to permit the passage of all necessary air and in every way these cells were most convenient to handle. Most of my experiments were made with the larvae of species of *Odynerus* and of *Osmia rufa* but, as will be seen later on, the latter was, for some unknown reason, not an ideal host, so that most of my results were obtained from experiments with the wasp larvae.

II. The Hosts of *Melittobia*.

*Melittobia* is parasitic upon a number of kinds of bees and wasps. Howard (1892) gives a list of 14 different genera which are attacked by it. Malyshev (1911) adds three more and Girault (1912) adds another two. It was also known to be parasitic upon various flies of which it attacks the pupa.

Experiment however shows that almost any larva or pupa of bee or wasp is attacked by it under laboratory conditions and even beetle larvae and pupae served as food for both imago and larva.

But although almost any insect larva or spider in a quiescent condition—such as those paralyzed and stored up by various fossorial and Eumenid wasps, or larvae about to pupate—seemed to be regarded as suitable for oviposition and as food for the resulting larvae, in many cases such larvae failed to mature, mainly, I believe, because the food material dried up and the parasites consequently starved.

The following is a list of the genera of hosts previously recorded:

- Anthophora
- Osmia
- Chalicodoma
- Heriades
- Anthidium
- Bombus
- Megachile
- Ceratina
- Prosopis
- Stelis
- Odynerus
- Trypoxylon
- Agenia
- Pison
- Sceliphron
- Chrysis
- Monodontomerus
- Lencopsis
- Musca and other fly pupae.
Life-history of Melittobia acasta

I have experimented with the following hosts:

- *Chrysis ignita* Ichneumonid parasite of larvae of *Hypera variabilis*
- Paralyzed larvae of *Hypera variabilis*
- Tineid caterpillars
- Spiders
- From cells of solitary wasps
- *Osmia rufa*
- *Megachile spp.*
- *Odynerus spinipes*
- *antilope*
- *callidus*
- *Vespa* sp.
- *Trypoxylon figulus*
- *Crabro* sp.
- *Psen* sp.
- *Ichneumonid parasite of larvae of Hypera variabilis*
- *Paralyzed larvae of Hypera variabilis*
- *Tineid caterpillars*
- *Spiders*
- From cells of solitary wasps

In every case, excepting the paralyzed spiders and larvae from the cells of solitary wasps, I have succeeded in rearing the parasite on these hosts but, as will be seen later, in one case, that of *Osmia rufa*, the food seemed very unsatisfactory (v. Chap. XI).

III. MALE AND FEMALE CHARACTERS.

The male and female imagines are very different from one another. The former (Plate XXVI, fig. 1) is of a dark brown colour, even reddish, with minute wings quite useless for flight. The anterior pair, which are the larger, are only about one-third the length of the insect and, when lying at rest on the dorsum, only reach just beyond the first visible abdominal segment, the posterior pair not extending more than two-thirds of the way across this segment.

The head bears on each side, instead of the usual compound eyes, a single ocellus and there are also three ocelli in a group in the usual position on the vertex.

The antennae are very peculiar. They consist of what are apparently eight segments but the apical one is two or three segments compressed to form a club. The scape consists of one very large segment which forms about half the total length of the antenna. This large segment is broad and triangular in shape, attached to the head by one angle, the flagellum being attached to it at one of the others, the inner one when the antenna is viewed from above. On the under side of the scape at its distal end and between the two angles is a hollow space (Plate XXVI, fig. 1 a, b).

The female (Plate XXVI, fig. 2) is darker in appearance, being usually pitchy brown, has compound eyes and the normal chalcid elbowed antennae. The wings, which overlap when at rest, extend almost to or slightly beyond the apex of the abdomen, this depending upon whether the latter is full of eggs or otherwise.

The only published figures, so far as I can find, are those of Graham Smith (1915–16 and 1918–19) who illustrates male and female in both his papers, but as the figures in the two papers are so different and as, in my opinion, they do not give a correct idea of the insect, I have made drawings for this paper.

Waterston (1917) gives figures of certain parts of the adult insects such as the male antenna and the metasternum and first abdominal segment on a larger scale than in my drawings.
IV. Life-history.

(a) General statement.

Various facts in the life-history have been given by different authors but there are only two accounts which are in any way complete, those of Malyshev (1911) and of Graham Smith (1915–16 and 1917–18). The former author deals with the species as a parasite of Odynerus, a genus of solitary diplopterous wasps, while the latter deals with it as a parasite of certain Diptera. Malyshev's observations and experiments go farther than those of Graham Smith but in one or two important points they are incomplete. In view of the fact that Malyshev's paper is in Russian, I offer no excuse for giving here a detailed account of the life-history.

So far as I have observed, the imagines first appear under natural conditions some time after the middle of May and they can be found in their haunts as late as September. Under laboratory conditions, by keeping the species in an incubator at summer temperatures, it is possible to continue to produce successive generations throughout the winter, and this fact has enabled me to complete and repeat certain experiments which otherwise would have taken perhaps six or seven months (see Chap. XII, "Winter rearing of Melittobia," p. 367).

Although occasional over-wintering pupae are to be found the species normally winters in the larval condition and although, as I have said, the imagines may appear in May, overwintered larvae may remain as larvae until June, the imagines not appearing until July, even when kept in the laboratory. These hibernating larvae are not always full-grown but as many batches of such larvae have completely finished their food supply before commencing hibernation, they either complete their growth by feeding upon their neighbours in the mass or perhaps in some cases pupate prematurely.

It might also be reasonably assumed that any food left over would be unfit for consumption by the time the larvae became active again but this is not necessarily the case as the host larva remains alive for a long time during the process of "deflation."

Under natural conditions, shortly after emergence, the females, having mated, break out of the cells in which they have developed. At this stage they show a definitely positive heliotropism. Such individuals, released in front of a window in the laboratory, move directly towards the light and, if confined in a horizontal glass tube closed at one end, they will remain in the tube if the open end is turned away from the source of light. If such a tube is turned round, the females at once stream out towards the light.

This positive phototropism lasts for a considerable time but it is apparently eventually overcome by a chemiotropism when the insects seek the cells of their hosts, primarily for nourishment.

The first tendency of the female on entering the cell of a host is to feed, although oviposition upon the host may commence very soon.
But females can delay oviposition apparently almost indefinitely, even when their abdomens are swelled almost to bursting point, if the host is not in a suitable condition or, as will be seen later, if mating has not taken place. Thus I have had females laying within 24 hours of emerging from the pupal stage, when placed upon suitable material, and I have had other females which had not oviposited 50 and even 60 days after emergence, when placed in a cell with an unhatched egg of the host-species, these females having waited for the host to reach the full grown larval condition before commencing oviposition.

(b) The egg. (Plate XXVI, fig. 5.)

The eggs, which are hyaline and almost transparent, vary somewhat in shape and in size. The smallest eggs measured were about 0.33 mm. in length while the largest were about 0.37 mm. They are elongated and taper somewhat towards one pole and measure about 1 mm. across at the widest part. Some taper more strongly than others and as these were scarcer I assumed at first that they were destined to produce males, but experiment showed that this was not the case and that there is no visible difference between male and female eggs.

The egg is not firmly fastened to the host but adheres very slightly as if it were just damp. It is quite easily lifted off by means of a needle point or fine brush but as a rule it will not fall off if the host is turned over. If, however, in the process of turning over the host, the egg comes in contact with the glass cell it adheres more readily to the latter.

Incubation varies from 2 to 9 days and the emergence of the larva from the egg is almost impossible to determine. The chorion is extremely thin and the larva apparently begins to feed through it, as I have observed is the case in some other hymenoptera, for instance *Trypoxylon figulus*. Sooner or later this thin "shell" splits and contracts.

(c) The larva. (Plate XXVI, figs. 6 and 7.)

The newly emerged larva is transparent and is composed of a head and thirteen segments. The tracheal system shows only four pairs of spiracles, one on the mesothorax and one on each of the first three abdominal segments, though the branches from the lateral trunks in the three or four succeeding abdominal segments indicate where, at a later stage, additional spiracles will appear.

The larva remains in the first stage from two to six days or even longer and then a moult takes place and five additional pairs of spiracles appear, one on the metathorax and the others on the fourth to the seventh abdominal segments. I have never actually seen a moult take place but, by examining numbers of larvae 0.60 to 0.70 mm. in length, it is possible to find individuals about to moult in which the additional spiracles are faintly visible subcutaneously.
A second moult takes place before the final larval stage is reached but no further change takes place in the number of spiracles.

The full-grown larva attains a length of about 1.7 to 1.9 mm. and is usually smooth, the constrictions between the segments having disappeared during growth.

It has been said that the larva feeds without puncturing its host but this does not seem to be strictly accurate. The larva possesses a minute pair of mandibles which, in the first stage, are more or less triangular in shape, the free angle being sharply pointed; but in larger larvae this point is greatly lengthened (Plate XXVI, figs. 3 and 4). I imagine that these points are driven in subcutaneously and serve to hold the mouth of the parasite to the host and at the same time allow the "blood" of the host to pass out. The minuteness of the punctures can be gathered from the fact that, on the removal of a feeding larva the spot at which it has been feeding is not recognisable, even under high magnification. On the other hand, if a large number of feeding larvae are gently removed from a host the surface of the latter appears as if it were gently perspiring, though of course this might be due to exudation of fluid from the mouths of the larvae at the moment of removal.

The larva is full grown, under the most favourable conditions of food and temperature, in eight or nine days, and only then does it begin to produce excrement. This habit of not producing excrement until feeding has ceased seems to occur in a large number of Hymenopterous larvae though there are others, such as Osmia, Megachile, Sapyga, etc., which begin to produce faecal pellets when about half grown.

Shortly after completing this cleansing process the Melittobia larvae become pupae.

It is interesting to note that the larvae take the colour of the host upon which they feed, as do other "blood" sucking Hymenoptera, e.g. Odynerus, Chrysis, etc. By feeding the Melittobia larvae upon the salmon-red coloured larvae of a small Crabro sp., the larvae became salmon-red. Fed on larvae of Odynerus spinipes the larvae are yellow, while if fed upon larvae of O. antilope they are creamy white and this colour depends upon the blood colour of the host, which in turn depends upon the blood colour of the beetle- or moth-larva or spider upon which it fed. By feeding larvae for a few days on Crabro and then transferring them to Odynerus, the colour of the parasite changes from salmon-red to yellow or cream.

As soon as the larva begins to cast out excrement, the colour begins to disappear and all Melittobia larvae, however they have been fed, ultimately become a pale transparent creamy-white colour.

(d) The pupa.

As soon as the pupa appears it is possible to decide the sex of the individual since, in the female pupa, the compound eyes are visible, though at first without colour, while no eyes are at first visible in the male pupa. In the
course of time the eyes of the female pupa become red and shortly before the emergence of the imago, the whole pupa becomes black while that of the male becomes reddish brown. The duration of the pupal period under the most advantageous conditions is about seven days.

But both larval and pupal periods vary very considerably in their duration under different conditions. So far as I know, the species normally hibernates in the larval stage and such larvae, as I have already mentioned, may not pupate until the following July, so that we have the shortest larval period as eight days and the longest as about eleven months. The pupal period also varies between about seven days and perhaps ten months.

V. The Habits of the Female.

(a) Food and method of feeding.

The female feeds upon the blood of her host which she obtains by puncturing with her ovipositor. A hungry female walks about upon the host, be it egg, larva or pupa, tapping it with the apices of her antennae until she selects a suitable spot. She then bends the apex of her abdomen downwards and thus brings the point of her ovipositor into contact with the surface of the host (Plate XXVI, fig. 8a). The ovipositor is then released from its sheath by the abdomen straightening again and the insect by a steady or, may be, a slightly jerky pressure of the body forces the apex of the ovipositor through the "skin" of the host (Plate XXVI, fig. 8b). The stylet may be driven in deeply, even to its base, or it may merely penetrate but, after a short interval, it is withdrawn and the insect rapidly moves backwards, feeling about with her antennae until the puncture is discovered, when the mouth is at once applied to it (Plate XXVI, fig. 8c). After sucking for a short time, the insect moves away and may at once repeat the process at some other spot.

One would scarcely expect that the egg or young larva of a bee or a wasp could suffer this treatment and survive and yet I have had many examples of eggs punctured, even so that a globular effusion of "blood" appeared upon the surface, without the vitality being, to all appearance, affected. I have placed as many as 15 Melittobia females in a cell with a newly hatched larva of Osmia and have allowed them to feed freely upon the host for a fortnight, and yet ultimately these heavily taxed hosts have pupated and emerged as adults.

In the same way Melittobia females placed in cells with older host larvae produced no ill-effects so long as they were only feeding and I am therefore quite satisfied that the feeding habits of this parasite are not necessarily injurious to the host.

(b) Preparation of food for the larvae.

On the other hand, once a Melittobia female lays an egg upon its host, whether the latter is in the egg, larva or pupa stage, the host is apparently doomed. This seemed at first a most extraordinary thing. It was difficult to
believe that the mere fact of the parasite egg being in contact with the body of the host could cause the destruction of the latter and the experiment of removing eggs from one host and placing them upon another with which no *Melittobia* female had come into contact proved that the parasite egg itself in no way affected the host.

This point puzzled me for some time as it seemed that the only explanation was that the *Melittobia* possessed some subtle instinct by which it knew when a host was moribund and that, in such a case, the parasite oviposited on what was really an unsuitable host. What seems to be the real explanation came to me accidentally. With a high power binocular microscope, I was watching a *Melittobia* female puncturing a mature *Odynerus* larva and I happened to notice a fluid oscillating in the semi-transparent ovipositor. Gradually the ovipositor was buried to its base in the host and after an interval of about two minutes it was withdrawn and I was surprised to see the insect move away without making any attempt to find or to suck the puncture. This, then, appeared to be a different process from the feeding one and I made a number of experiments with ripe female *Melittobias*, i.e. females which had already begun to oviposit, isolating individuals in glass cells each with a host suitable for oviposition, the host in every case being a resting larva of either an *Osmia*, *Odynerus* or a *Chrysis*.

By watching these females it was easy to establish the fact that they frequently made punctures without afterwards sucking them and occasionally it was possible to imagine a fluid passing down the ovipositor into the wound.

As soon as I saw a host larva punctured in such a manner, I transferred it to a separate cell and left it, with the object of seeing whether it would ultimately become a pupa and emerge in the normal manner. In some cases these larvae may have been thus punctured only once and in other cases they may have been punctured a number of times but they were always removed from the parasite before the latter laid any eggs upon them. All but one of these larvae, numbering thirty-eight, failed to develop.

In another set of experiments, involving twenty *Odynerus* larvae in the resting stage, I allowed *Melittobias* to lay a few eggs upon them, always removing the eggs before they hatched and removing the females after a few days and in all these cases again the *Odynerus* larvae failed to pupate.

It seems therefore that the *Melittobia* female punctures her host for two different purposes, one in order that she may feed upon its "blood" and the other in order that she may inject something so as to stop the development of the host for the benefit of her offspring.

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1 I have used the word "imagine" here because it is extremely difficult to make the observation, it being necessary to get the parasite in a particular position with regard to the light and, of course, to see the ovipositor before it is driven in to its base. The only case of which I am absolutely certain that I saw a movement of fluid in the ovipositor from parasite to host was one in which a female was puncturing a fly puparium but, as in the case of fly puparia the parasite lays its egg on the fly pupa contained within the puparium, I may have been watching the passage of eggs.
Malyshev (1911) had observed the puncturing in connection with oviposition and he regarded its function as twofold; first to paralyse the host and secondly to preserve it, but here, I think, he went too far. He only mentions one case, in which he saw a larva of *Odynerus antilope* receive three punctures in the beginning of September and he remarks that it remained soft and fresh, without showing any other sign of life, until the following May, when it dried up. Had this larva not been punctured at all it would have remained soft and fresh until the following May when it would have pupated. There seems to me to be no need to imagine any preservative effect, apart from which there is good reason for believing that there is none, since in every case where eggs were laid upon eggs or immature larvae of a host, these host eggs and larvae quickly collapsed and showed all the usual signs of decay.

It is sufficient then to regard this preparation of the host by the injection of some paralysing fluid as similar to that seen in those solitary wasps which store up living food for their progeny.

So far as my observations go, this "stinging" by *Melittobia* always takes place at least once, and sometimes frequently, before any eggs are laid, although Malyshev asserts that oviposition may be commenced before any "stinging" takes place, though this is less usual.

(c) Oviposition.

*Melittobia* is in all cases ectoparasitic and the statement of Malyshev that, under certain circumstances, it is an endoparasite is misleading. Thus he speaks of it as becoming endoparasitic when it oviposits through the cocoon of an hymenopterous host or the puparium of a fly.

I have opened numbers of fly puparia after having observed *Melittobias* puncturing them but in no case did I find any eggs except on the enclosed pupa.

When a female is about to lay her eggs she moves over the host, tapping with her antennae until she finds a suitable spot. Such a spot is usually, though not always, upon the upper side of the host larva or pupa as it lies in the cell, whether it be dorsum or venter uppermost and, if there is any choice of position, of which I am doubtful, the head end is more favoured than the apex of the abdomen.

Having selected the spot the female brings the apex of her abdomen into contact with the surface of the host and, having fixed the apex of the ovipositor in the chitin, the apex of the abdomen withdraws to its normal position. So far the action has been that of a feeding or a "stinging" female but, for oviposition, the ovipositor is not driven into the host, merely remaining fixed by its apex.

The abdomen can now be seen slowly expanding and contracting until, after a few such movements, a slight bulge appears at the base of the ovipositor on its anterior side and this bulge rapidly passes downwards and the egg suddenly shoots out near the apex. It is a most extraordinary sight and looks
like a conjuring trick to see the comparatively enormous egg appear from the exceedingly fine tube.

The egg shoots out more or less horizontally and lies on the surface of the host, its long axis, as it lies, corresponding more or less closely with that of the Melittobia. Sometimes one of the middle legs seems to be used to guide the egg as it emerges.

Within about half a minute another egg may be laid alongside the first and as many as 7 or 8 eggs may be laid in one spot within 5 or 6 minutes. A large number of eggs may ultimately become massed together at one spot or the female may scatter her eggs over the surface, but where she is ovipositing through a cocoon or puparium the eggs are almost invariably laid in heaps.

VI. THE HABITS OF THE MALE.

(a) General habits.

I have said that, as a rule, the males emerge first in the cells. These males are very active; they creep over and amongst the larvae and pupae, even seizing them in their jaws and thus moving them about, though I have never seen a male injure a larva or pupa.

Two males, on meeting, usually engage in mortal combat, though when a cell is very full of emerging females the males are so busy that they seem to pay less attention to one another.

So far as I can find the males do not feed.

I have paid but little attention to the length of life of the male, but in those cases where a male has been introduced into a cell containing a female, the former has usually succumbed within seven days. In these cases, however, I have no record of the age of the male when he was introduced into the female cell but in one case I found a male alive twenty days after introduction, the female having died—or having been killed by the male—within 24 hours after his introduction. It is possible that inability to fulfil the sexual function may prolong life as in the case of the female. (See section upon "Longevity of the Female," p. 363.) Smith, F. (1853) says that the male usually lives about seven weeks.

The killing of the female by the male was, unfortunately, a not uncommon occurrence and on several occasions stopped experiments of some importance. Whether or not this is a phenomenon caused by the experimental conditions it is, of course, impossible to say.

(b) Courting.

Courting is a strange phenomenon in that a special contact is made between the antennae of the male and female as a preliminary to copulation. The male mounts upon the back of the female and moves forward, seeking for her antennae, the apices of which he ultimately succeeds in getting into the pocket on the underside of the long scape segment of his antennae (v. Plate XXVI, fig. 1a and b).
Coitus is very rapid and the male immediately seeks for another mate. He quickly decides whether a female is willing or unwilling and wastes very little time over the latter.

VII. THE NUMERICAL PROPORTION OF THE SEXES.

There seems to be no reliable way of determining the proportion of the sexes in a family, since the male and female eggs are indistinguishable, as I have already said, and the larvae are more or less cannibals, so that sometimes quite large numbers of individuals disappear during the larval period. By counting pupae one gets more reliable results than by counting the imagines since the males in the latter stage destroy one another, and, under laboratory conditions, also sometimes destroy females.

Graham Smith gives results of counts of imagines which range from less than one to more than fifty males per hundred females but, since a female may lay more than a thousand eggs and his figures are all for small batches, they give us no reliable criterion.

By counting pupae I found that, in any one batch of eggs, the males ran from about one to four or five per cent. of the females.

Now, as will be shown later, a female normally lays two, and perhaps three, batches of eggs and she mates with a male before each bout of egg-laying. If she is un fertilized or if, after laying a batch of eggs she is not permitted to mate again, she can only lay male eggs, that is, the eggs can only develop into males. Presumably therefore unfertilized eggs produced males and fertilized produce females, as in many other Hymenoptera. As therefore she produces both male and female offspring after mating and before she has exhausted her supply of spermatozoa, it is reasonable to conclude that she can control the flow of spermatozoa from her spermotheca and thus determine the sex of her offspring—as Fabre has shown is the case with certain species of Osmia and Chalicodoma (v. Bramble Bees and Others, Chaps. IV and V). It might also be concluded from the fact that males usually emerge first in a brood, that the first eggs laid by the female are male eggs, but it may be that males develop more rapidly than females, a point I have so far omitted to investigate.

If therefore the sex is determined by the mother at the time of oviposition the proportion may well vary for each female according to circumstances.

VIII. FECUNDITY.

I have already mentioned that oviposition may commence within twenty-four hours of the emergence of the female but it is often, perhaps usually, a very prolonged process.

Malyshchev states that the females lay 200 to 300 eggs and perhaps more during four or five weeks but he does not say how he obtained his figures. I have, however, obtained results in my experiments which seem to indicate that the females are much more prolific than this author has shown.
In order to test the fecundity of individuals I placed in separate cells five freshly emerged females which had mated, supplying each with a resting Odynerus larva and, at intervals, removing from the latter the eggs and larvae, keeping a record of the totals for each female. In this way I found that the egg-laying capacity of the females varied considerably, the total number of eggs laid by each being: 961, 529, 1217, 1086 and 598. It will be noted however that the lowest total exceeds considerably the figures given by previous observers.

The eggs and larvae were removed and counted, usually at intervals of seven days after the first few weeks, and at the end of six or seven weeks the females had almost ceased to lay eggs. The few eggs laid by each at this period were placed upon fresh hosts and allowed to hatch out in order that pupae might be obtained to determine the sex. They all proved to be males, indicating that the mother had come to the end of her supply of spermatozoa. A male was therefore introduced into each of the cells containing the females and, during the following week, the number of eggs laid in four out of the five cases ran into three figures.

Although the number of individuals experimented with is too small to justify any definite statement, these experiments seem to indicate that the female normally lays about half her eggs after her first mating, the totals being 439, 270, 680, 348 and 597 and, in three out of the five examples, she had exhausted her egg-laying capacity after the results of a second mating, the numbers of eggs in the second broods being 522, 259, 333, 738 and 1 respectively.

The last few eggs of these second broods were allowed to hatch and the larvae reared in order to test their sex and, with the exception of a single individual, all were males, so that, at the same time as the female exhausted her supply of eggs she had exhausted her second supply of spermatozoa.

The following table (p. 362) gives details of all the results referred to above:

As to the rate of oviposition I have made no daily counts and at first I adopted a somewhat rough method of estimating which gave some very divergent results. Thus in August, in 17 days, out of a batch of four females the lowest yield was 152 eggs while one female gave 213 eggs. Of another batch of six females in the same month, one laid 92 eggs in 12 days while another laid 218 in the same period. These figures give averages per day of 9, 12-5, 7-6 and 18-1.

In December I began to go into this question more carefully, using the five females already referred to, and Table II shows the days upon which the counts of eggs were made and the average number of eggs laid per day as worked out from these counts. This table thus shows that the productivity of the female, when at her best, may reach 31 eggs per day and that she is at her best from 7 to 14 days after mating. I shall refer to the fecundity of the unfertilized female later on.
Table I. Showing the number of eggs laid by fertilized females kept in the incubator at a temperature of about 21°C. (about 70°F.). On the dates given all eggs and larvae were counted and removed.

<table>
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D = ? dead. The dark lines indicate mating dates.

Table II. Showing the dates upon which eggs and larvae were counted and removed and the average number of eggs laid per day by each female.

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D = death of the female Melittobia. The dark lines indicate mating dates.
IX. Longevity of the Female.

Table I, already referred to, shows that, in the cases of the four females which produced second broods, the length of life varied from twelve to sixteen weeks, i.e. 84 to 112 days, and that, during all this time, they were actively ovipositing. Graham Smith (1918–19) records having seen one female ovipositing over a period of 48 days and another over a period of 37 days but it is possible that his observations were upon females which had no chance of a second mating and had therefore ceased to lay eggs because they had exhausted their supplies of spermatozoa.

I have several other records of ovipositing females which survived for periods exceeding 90 days and it seems, therefore, that, under normal conditions, this would be about the average length of life of the female.

If, however, unfertilized females are prevented from mating, the length of life is enormously increased, eight such females having lived as follows: 211, 204, 225, 223, 209, 195, 202, 202 days. This will be referred to further in the next chapter where full details are given in Table III, p. 365.

X. The Unfertilized Female.

I have already mentioned that, under natural conditions, the males never leave their natal cells, so that mating takes place there: and I believe that it is not until after mating has taken place that the females become positively heliotropic. I have never, under natural conditions, come across an unfertilized female; that is, every female which has been allowed to emerge from the pupal stage in the cell where it grew up mates before leaving that cell. It is quite easy, however, to obtain unfertilized females by simply removing female pupae from the cells and allowing them to hatch out without a male having access to them.

Several authors mention the results of experiments with unfertilized females. Such a female, when placed in a cell with suitable food material will, sooner or later, lay a small number of eggs—it may be only one but is more usually four or six, though occasionally there are a few more. The female takes great interest in these eggs and in the larvae which hatch from them, patting them with her antennae and returning to them again and again. She takes, perhaps, greater interest in the resulting pupae and may even be said to show excitement when one of them is about to become an imago. All the resulting imagines are males and she mates with the first one to emerge and within a very short time commences to lay freely, twenty or thirty eggs sometimes being deposited within twenty-four hours of the appearance of the male.

At first sight this habit of the unfertilized female of producing her own mate when another is unobtainable seems a somewhat extraordinary phenomenon and yet, from what I have shown by experiment, it appears to be
Life-history of Melittobia acasta

a normal phase in the life-history of the insect after she has exhausted her first supply of spermatozoa and preparatory to producing her second brood. It is mainly conjecture, but I believe that the female imprisoned in the host's cell develops a desire to break out of the cell only when she has her spermatheca full, so that once the female has produced her first brood she waits patiently for her second mating and then passes on to another cell to produce her second brood. My only evidence in support of this belief lies in the fact that in my glass cells the female was often to be seen on the cotton-wool plug after her second mating especially when the host larva within the cell was already fully stocked or was almost reduced to an empty skin.

It is interesting to notice that inbreeding is the rule with this species, the first mating being between brother and sister and the subsequent ones between mother and son. Exceptions to this rule may occur when two females together enter a host cell and both produce a brood but as in such a case the two females must frequently come from the same cell, they will usually be sisters and the second mating would in this case be between nephew and aunt.

I made a number of experiments with a view to seeing what an unfertilized female would do if I prevented her from mating by removing her eggs or larvae so that no male could develop, and these experiments have given somewhat interesting results.

In the first place, as I have already mentioned, the life of the female is greatly prolonged, the ninety days, which I believe to be about the average life of the normal female, being, in a number of cases, more than doubled and in my eighteen experiments averaging 174·8 days.

In the second place, whereas under normal conditions no imago is to be found after about the end of October, unfertilized females remained alive all through the winter, withstanding at times temperatures below freezing-point and only died in the second week of March after living 202 days—in fact, longer than many similar females kept at summer temperatures in the incubator.

In the third place the female can be induced to lay many more male eggs under experimental conditions than she would probably do under natural ones—although it must be remembered that we have no definite knowledge of the number of male eggs laid by a female under natural conditions.

Of eighteen cases, the average number of eggs laid per female was 35·6, one individual laying as many as 93 eggs, others 70 and 61, while one individual only laid 7 eggs in 131 days. In all these cases the experiment was kept going until the female died, so that the number of eggs obtained from each female was the total number she laid during her life. The following Table (III) gives the details of these experiments.
Table III. The unfertilized female. Oviposition induced by frequent removal of eggs.

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<tr>
<th>Date</th>
<th>No. of days</th>
<th>August</th>
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The two top transverse columns refer to the month and the day. The next refers to the number of days' interval since the previous inspection.

M1 to M9 are the 9 unfertilized females used in this experiment and the transverse columns show the number of eggs laid by each of them and removed at short or long intervals. M1 to M7 were placed in an incubator at a temperature of about 21°C. (70°F.) on December 6th. D = death of the female Melittobia.
XI. **Osmia rufa** Larvae and Pupae used as Hosts.

I have already said something about the apparently indefinite variety of hosts which *Melittobia* will attack but I have had one very curious experience in this matter.

In a large number of my earlier experiments the larvae and pupae of the red *Osmia* (*O. rufa*) were used because it was one of the commonest species at my garden fence and, although *Melittobia* eggs were laid in the normal way upon the resting larvae and pupae in the laboratory, the eggs often failed to hatch out and frequently the larvae died before they were full grown, in fact it was rather exceptional to rear a *Melittobia* from egg to adult using this species as the host.

At the time that my garden fence was infested with the parasite I opened more than 130 cells of *Osmia rufa* which had been constructed in tubes placed upon shelves attached to the fence and in only one case did I find any sign of the *Melittobia* and in that one cell were two half-grown larvae. And yet in the laboratory the resting larvae and pupae of the *Osmia* were readily accepted by the parasite which fed and oviposited upon them—but the majority of the brood died.

Another interesting point in the relationship of *Osmia rufa* to *Melittobia* is that in the number of cases in which I have allowed one or more of the parasites to enter the cell of the growing larva, the parasites have invariably perished when the *Osmia* larva began to spin its cocoon, being caught amongst the outer threads—and yet *Melittobia*, in similar relationship with any of the three species of *Odynerus* with which I have worked, always survived and destroyed its host.

In a number of cases I placed *Melittobia* females in cells with *Osmia rufa* pupae enclosed in cocoons and in almost every case the parasite failed to destroy the bee. In these cases it was evidently because the *Osmia* cocoon was too tough for the parasite to penetrate, since pupae removed from their cocoons were invariably oviposited upon and in such cases the female *Melittobia* lived for many weeks, feeding upon the blood of the pupa, while those placed with *Osmia* cocoons died after three or four weeks, evidently from starvation.

This is the only host which so far I have found, upon which *Melittobia* does not really flourish and it seems as if there is some quality in the blood which has a deleterious effect upon the parasite.

That the toughness of the cocoon should be an additional safeguard is worthy of remark since Malyshev suggests that some species may escape the parasite because of the nature of the building materials used in the construction of the cells. Thus he mentions some of the *Heriades* which build the cell-walls of resin in which *Melittobia* gets caught and he suggests that *Odynerus alpestris* possibly escapes to some extent for the same reason.

These few facts serve to emphasise the peculiarities of the relationship between parasite and host and to give an idea of the apparent triviality of
the things which make all the difference in that relationship. Another example
of this is to be seen in the life-history of the fossorial wasp *Sapyga quinque-
punctata* which I have recently investigated.

In this case the usual host of this parasite in my garden in Cambridge
was the Blue Osmia (*O. aenea* (*caerulescens*)) while *Osmia rufa* was immune
and it appeared that the latter species owed its immunity to the fact that
the pollen paste stored in the cells by the mother bee was too dry for the
larvae of the parasite. Eggs of *Sapyga* were occasionally laid in the cells of
this species but the larvae always perished and, in laboratory experiments,
where the *Sapyga* eggs were placed in the cells of *O. rufa*, the result was always
the same. If, however, the pollen paste was made suitably moist by the
addition of honey or even treacle—the latter, as experiment showed, being
quite useless as a food for the *Sapyga* larva—the parasite could be brought
to maturity without any difficulty.

**XII. Winter rearing of *Melittobia*.**

I have already said that by keeping the species during the winter in an
incubator at a summer temperature 20°–22° C. (about 70° F.) it was possible
to raise successive generations.

I first tried this because I was held up in the autumn in the middle of
experiments which I was very anxious to continue and I therefore placed
batches of hibernating larvae in glass cells in an incubator early in December.

Different batches of larvae behaved differently. Some responded within
a few days to the warmth and began to void excrement preparatory to
pupation; others remained apparently quite unaffected by the warmth for
much longer periods. Moreover a whole batch of larvae did not necessarily
behave in the same way and a few extreme examples will make this point clear.

**Batch 1.**
- Placed in incubator 10. xii. 21
- Some casting excrement 26. xii. 21
- A few pupae 31. xii. 21
- A few imagines 5. ii. 22
- Mostly pupae, a few larvae 15. i. 22

**Batch 2.**
- Placed in incubator 10. xii. 21
- One pupa 24. xii. 21
- All pupae 31. xii. 21
- One pupa and the male appeared 6. ii. 21
- Many still larvae 19. ii. 22

**Batch 3.**
- Placed in incubator 10. xii. 21
- One pupa and the male appeared 6. i. 21
- About half have pupated 5. ii. 22
- Many still larvae 19. ii. 22

<table>
<thead>
<tr>
<th>Pupation period</th>
<th>31 days and upwards</th>
<th>50 days and upwards</th>
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During the summer, the normal pupal period is only about seven days
and females hatched in the incubator at summer temperatures gave rise to
generations which had pupal periods of about the normal summer duration
and yet in the case of those hibernating larvae which pupated in the incubator,
the pupal period varied from 18 to 57 days in duration. Now a long pupal
period is not an innovation to the species, since over-wintering pupae do occur
under natural conditions and therefore the explanation of the long pupal
period in the case of these incubated hibernating larvae seems to be that the
effects of the incubator are gradual and that it takes some time before the
insect reacts to the increased temperature, or it may be that the reaction of
Life-history of Melittobia acasta

A larva or pupa to change of temperature is slight but that of the imago is more marked, since once the imago appeared she behaved exactly like a summer individual and passed on her activity to her eggs, larvae and pupae.

The apparent irregularity of the action of increased temperature on different individuals in the same batch (e.g. 21 to more than 67 days in Batch 1) is perhaps to be accounted for on the assumption that the individuals in a batch differ considerably in age.

XIII. MEANS OF DISPERSAL OF MELITTOBIA.

In the course of the season there may be two to five and even more generations of Melittobia so that, but for the cannibalism amongst the larvae and the poor means of distribution available for the species, this parasite would be a very serious menace to a very large number of species of bees and wasps.

Yet, as a matter of fact, in the Cambridge district I have only succeeded in finding one old clay wall of a few square yards in extent from which I have been able to get my supplies of material and even from this wall, after three or four years, there are still Odynerus spinipes emerging, although what used to be a very large and flourishing colony is now reduced to very small numbers.

Now the females can apparently only fly an inch or two at a time and, for the most part, they do not use their wings. I have never lost a specimen by letting her run about upon the laboratory table when I have wished to clean out her glass cell.

These females run about fairly rapidly and are active and it is quite easy to see how they might quickly overwhelm a colony of a host species once they reached it but it is the passage from one colony to another that is difficult to understand.

Walker mentions that the species occurs "on windows" and although I have not so far found it in such situations, this suggests to me a possible means of dispersal. I cannot imagine Melittobia deliberately walking into a house and then becoming positively heliotropic, but, when wandering about the haunts of solitary wasps and bees it may occasionally mount upon these, and thus be transported to other haunts whereby it would occasionally be bumped off against the window-pane.

XIV. SUMMARY.

Melittobia is a chalcid, ectoparasitic upon a number of species of Hymenoptera and upon the pupae of certain flies. The insect was bred in the laboratory and the life-history is described in detail.

A number of experiments were made with regard to possible hosts and as to feeding and reproduction and certain points are of some interest.

For instance, the male apparently does not feed but the female uses her ovipositor with which she punctures the eggs, larvae or pupae of various insects, afterwards sucking the blood which oozes from the puncture. Such eggs, larvae or pupae, in spite of repeated punctures, may continue to develop.
Before beginning oviposition upon the surface of a suitable host, the female punctures the host one or more times with her ovipositor and apparently injects an anaesthetizing fluid, since insect larvae and pupae, upon which eggs have been laid, very rarely develop farther and yet remain fresh for a considerable time extending to many months.

With regard to reproduction, inbreeding appears to be normal, mating usually taking place between two individuals of the same brood and later between the female and one of her own offspring. Virgin females, unable to find a male, lay a few eggs, which always produce males, the first of which to emerge mates with the female.

The female is very prolific and may lay as many as twelve hundred eggs in two or three batches, mating taking place before each batch is laid. The last eggs of a batch are almost invariably male eggs, indicating that the female has exhausted her supply of spermatozoa.

A virgin female, whose eggs are removed so that she cannot rear a mate, may lay as many as ninety eggs during her life-time, her length of life being more than doubled, and such a female will survive through the winter, even withstanding frost, whereas fertilized females apparently all die in October or November at the latest.

All the eggs of a virgin female are apparently capable of developing and all such eggs produce males.

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XV. Bibliography.


Morley, C. (1910). *Catalogue of Chalcididae* [synonymy of *M. acasta*].


Life-history of Melittobia acasta

EXPLANATION OF PLATE XXVI.

Fig. 1. *Melittobia acasta* ♀. Dorsal view. *sp.* = spiracle of first abdominal segment. *cx3* = base of coxa of third pair of legs attached at posterior end of the pleural suture, the epimeron not being separated by a suture from the first abdominal segment. *a* and *b* = dorsal and ventral views of antenna.

Fig. 2. *Melittobia acasta* ♂. Dorsal view.

Fig. 3. Third stage larva—ventral view of head showing mouth (*m.); head skeleton (*t.*); mandibles (*m.*), retracted within mouth but capable of being protruded, and salivary ducts (*sd.*). *Ant.* = antennae.

Fig. 4. Third stage larva; mandible (ventral view).

Fig. 5. Egg. Two eggs which produced females and two which produced males.

Fig. 6. First stage larva; lateral view.

Fig. 7. Second stage larva; lateral view.

Fig. 8. The female feeding upon its host. Diagrammatic figures made from sketches showing the insect placing the ovipositor in position, (*a*): drawing in the ovipositor, (*b*): licking the puncture (*c*).

Fig. 9. Glass cells used in breeding *Melittobia*. *C.* = plugs of cotton-wool. The tube is held down to a piece of cardboard by wires.
ON A NEW CILIATE, *BALANTIDIUM OVATUM*, SP. NOV., AN INTESTINAL PARASITE IN THE COMMON COCKROACH (*BLATTA AMERICANA*).

By EKENDRANATH GHOSH, M.Sc., M.D.,
Professor of Biology, Medical College, Calcutta.

(With 1 Text-figure.)

The parasite herein described was found in the intestinal contents of *Blatta americana* at Calcutta. A single specimen was observed.

**Diagnosis:** Body elongately ovate, wider posteriorly than anteriorly, slightly less than twice as long as its greatest diameter and broadly oval in transverse section. Anterior end rounded and slightly bent to the side of the peristome. Posterior end abruptly tapering to a point. Side with the peristome slightly depressed in front and convex behind. Side opposite to the peristome convex. Peristome small, tubuliform, about one-fifth the body-length, directed backwards and medianwise and slightly twisted from right to left.

![Text-figure](image)

The peristome has an undulating membrane running along its postero-lateral portion and a row of stout cilia in its anterior portion, this row being continuous with the long anterior body cilia. Body surface smooth. Body cilia long; a row of long and somewhat stout cilia at the anterior end. Ectoplasm generally thin, but thick near the anterior and posterior ends of the body. Endoplasm densely filled with coarse granules and presenting a comparatively light area opposite to the peristome. Macronucleus broadly oval, situate in the middle of the body. A large contractile vacuole is seen posteriorly, with an oblique anal canal opening in front of the posterior end. Length 0.085 mm.

This parasite forms the second example of *Balantidium* found in the intestine of the cockroach. It differs from all the known species in the presence of an anal canal in connection with the contractile vacuole. It is distinguished from *B. blattarum* (*Parasitology*, xiv. 15–16) in its shape, the position of the undulating membrane, and in the thinness of the ectoplasm.
SUR LES ACANTHOCÉPHALES DE L’EIDER
(SOMATERIA MOLLISSIMA L.).

PAR LE DR N. KOSTYLEV.

(Travail du Laboratoire de M. le Professeur E. Pavlovsky
de l’Académie Militaire de Médecine de Pétrograde.)

(Avec 5 Figures dans le Texte.)

La littérature ne nous offre que des renseignements fort embrouillés sur les Acanthocéphales parasites intestinaux de l’eider (Somateria mollissima L.). Ces parasites furent découverts par Phipps en 1774–1775 et décrits par lui sous le nom de Sipunculus lendix. Cependant, de la description et surtout des figures de cet auteur on voit bien qu’il ne s’agissait point de Sipunculides mais de vrais Acanthocéphales (Lühe, 1905). Plus tard, d’autres auteurs ont rapporté cette espèce comme synonyme d’Echinorhynchus polymorphus Brems.


En 1916 Van Cleave a décrit une nouvelle espèce d’Acanthocéphale: Filicollis botulus sortie de l’intestin de l’eider dresser (Somateria dresseri) et plus tard il la retrouva dans l’intestin de l’eider commun (Somateria mollissima). Cette espèce, d’après la description et les figures de Van Cleave, possède un corps épais, sacciforme, partagé par une rainure peu profonde en deux parties inégales dont l’antérieure chez les mâles est criblée de petits aiguillons, tandis que chez les femelles les aiguillons sont remplacés par des tubercules. La longueur du corps atteint 20 mm. Le cou, très long, se termine par un rostre ovale portant 16 rangées longitudinales de crochets à 7–8 crochets par rangée. Les œufs (0,071–0,083 mm. × 0,003 mm.) sont elliptiques à membranes concentriques. Plus tard Van Cleave (1920) a décrit une nouvelle espèce d’Acanthocéphale: Filicollis arcticus qui diffère de F. botulus par son armure particulière du rostre et par les dimensions plus considérables de ses œufs.

L’examen des Acanthocéphales des collections du Musée Zoologique de l’Académie des Sciences et de l’Académie Militaire de Médecine m’a révélé la présence de 3 espèces d’Acanthocéphales parasites de l’eider: (1) Filicollis
botulus Van Cleave, 1916; (2) une nouvelle espèce que je nommerai Polymorphus phippsi; et (3) Echinorhynchus pupa v. Linstow, 1905.

Filicollis botulus Van Cleave répondrait bien à la description de l'auteur; je dois, cependant, faire une petite correction: tandis que d’après Van Cleave les femelles portent à la surface de leur corps des tubercules au lieu d’aiguillons, j’ai trouvé, par contre, chez les mâles ainsi que chez les femelles des aiguillons cachés dans les tubercules avec leurs pointes saillant vers l’extérieur.

Polymorphus phippsi n. sp.
(Figs. 1, 2, 3, 4c.)

Je dédie cette espèce à l’auteur qui découvrit le premier Acanthocéphale parasite intestinal de l’eider. La dénomination lendix doit disparaître parce qu’on ne sait pas au juste laquelle des deux espèces, F. botulus ou P. phippsi, a été découverte par Phipps¹.

Je passerai maintenant à la description de cette nouvelle espèce:

Le corps de ce parasite est allongé, rétréci dans sa partie antérieure, séparé de la partie médiane élargie par une petite rainure et se rétrécissant de nouveau vers l’extrémité postérieure; cependant cette dernière est souvent tirée en dedans et dans ce cas le corps paraît saciforme et allongé. Cette forme de corps est surtout propre à la femelle. La longueur atteint 15 mm., les mâles sont plus petits que les femelles. La partie antérieure du corps à une certaine distance est criblée de petits aiguillons qui sur la partie ventrale s’étendent plus loin en arrière que sur la partie dorsale. Antérieurement le corps passe en un cou sans armure; de 0,73 mm. de longueur et dont le diamètre à la base est de 0,27–0,33 mm. Le rostre de forme ovale (0,47–0,7: 0,39–0,47 mm.) est pourvu de 16 rangées longitudinales à 6 crochets par rangée. Un spécimen était trouvé portant sur son rostre seulement 14 rangées longitudinales de 5–6 crochets par rangée.

Les variations du nombre des crochets sur le rostre sont représentées dans le tableau suivant:

<table>
<thead>
<tr>
<th>Exemplaire No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nombre de crochets dans chaque rangée longitudinale</td>
<td>14</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Nombre de rangées longitudinales de crochets</td>
<td>5–6</td>
<td>5–6</td>
<td>5–6</td>
<td>6</td>
<td>7–8</td>
<td>5–6</td>
<td></td>
</tr>
</tbody>
</table>

La dimension des crochets et de leurs racines dans chacun des rangées longitudinales depuis le sommet à la base du rostre variait de la manière suivante:

<table>
<thead>
<tr>
<th>Crochets No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longueur (mm.) de chaque crochet dans la rangée longitudinale</td>
<td>0,0607</td>
<td>0,0714</td>
<td>0,0714</td>
<td>0,0643</td>
<td>0,0607</td>
<td>0,0536</td>
</tr>
<tr>
<td>Longueur (mm.) de sa racine</td>
<td>0,025 (?)</td>
<td>0,0643</td>
<td>0,0714</td>
<td>0,0643</td>
<td>0,0428</td>
<td>rudiment</td>
</tr>
</tbody>
</table>

¹ Ochanine, Codes des règles internationales de nomenclature systématique. St Pétersbourg, 1911, p. 11, § 36.
La poche du rostre, à parois musculaires doubles, commence à l'extrémité postérieure du premier et atteint une longueur de 0,14-0,21 mm. La longueur des lemnisques atteint parfois jusqu'à 2 mm.

L'appareil génital mâle se compose de deux testicules de 0,96-1,2 : 0,64-0,72 mm. de longueur, placés très près l'un derrière l'autre dans la partie élargie du corps, et de 4 glandes supplémentaires prostatiques d'une forme allongée et cylindrique. Les œufs qui se trouvent en grande quantité dans la
cavité du corps des femelles sont allongées et atteignent une dimension de 0,1178-0,1321: 0,0224-0,025, et même de 0,1392 mm. Leur membrane médiane est détirée et élargie vers les pôles. Les œufs moins développés, mais dont les trois membranes sont déjà marquées, atteignent \( \frac{p}{3} \) de la longueur d’un œuf mûr.

Il est évident que l’espèce mentionnée est complètement indépendante du *Felicollis botulus*; outre la différence du rostre et de son armure la forme et la dimension des œufs présentent aussi des particularités.

Les raisons pour lesquelles je rapporte cette espèce au genre *Polymorphus* sont:

(1) Les 4 glandes prostatiques de forme allongée et cylindrique chez les mâles;

(2) la forme très caractéristique pour le *Polymorphus* des œufs avec une membrane tirée vers les pôles;

(3) la rainure qui sépare la partie antérieure du corps, criblée d’aiguillons, de la partie postérieure.

Étant donné que l’espèce en question fut à plusieurs reprises confondue avec *Polymorphus minutus* il est bien opportun de donner ici les caractères différentiels des ces deux espèces:

<table>
<thead>
<tr>
<th>Nom de l’espèce</th>
<th><em>Polymorphus minutus</em></th>
<th><em>Polymorphus phippsi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Longueur du corps</td>
<td>3-10 mm.</td>
<td>3-15 mm.</td>
</tr>
<tr>
<td>Rostre</td>
<td>de forme ovale,</td>
<td>ovale</td>
</tr>
<tr>
<td></td>
<td>allongée</td>
<td></td>
</tr>
<tr>
<td>Nombre de crochets</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d’une rangée longitudinale</td>
<td>7-10</td>
<td>5-7 (rarement 8)</td>
</tr>
<tr>
<td>œufs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( 0,110:0,013 ) mm.,</td>
<td>( 0,1178- )</td>
</tr>
<tr>
<td></td>
<td>( 0,091- )</td>
<td>( 0,1392:0,0224- )</td>
</tr>
<tr>
<td></td>
<td>( 0,095:0,0182 ) mm.</td>
<td>( 0,025 ) mm.</td>
</tr>
</tbody>
</table>

Il faut ajouter, qu’il existe encore une espèce, *Ech. campylurus* Nitzsch (parasite de *Stercorarius skua* Brünn.), qui ressemble à *Polymorphus phippsi* par le nombre de crochets sur la trompe et par la forme de l’appareil génital mâle. Pourtant d’après l’étude de Lühe on voit que les racines des crochets de la trompe d’*Ech. campylurus* ont une forme tout-à-fait différente de celle de *Polymorphus phippsi* et il est aussi à remarquer que la femelle et donc les œufs d’*Ech. campylurus* restent encore inconnus.

En 1920 Van Cleave décrivit l’espèce *Felicollis arcticus* pris dans les intestins du *Somateria spectabilis*. En terminant cette description il ajoute qu’en 1905 v. Linstow avait présenté une étude sur le *Ech. pupa*, parasite du même hôte; cependant ce travail n’étant pas suffisamment complet, il est impossible de se rendre bien compte de la position systématique de cette espèce. Le Musée Zoologique de l’Académie des Sciences de Pétrograde possède encore les exemplaires originaux qui avaient servi de base à la description de v. Linstow et j’en ai fait une étude minutieuse autant que la quantité et la qualité des spécimens me l’avaient permis.
Echinorhynchus pupa von Linstow. (Figs. 4b, 5.)

Des quatre spécimens présents il y en a deux qui ont la partie antérieure du corps arrachée; chez les deux autres elle est tirée en dedans. Les deux premiers et l’un des derniers ont le corps allongé, sillonné de rides transversales et longitudinales; les dernières sont moins bien marquées. La longueur du plus grand des 4 spécimens (celui qui à la partie antérieure du corps arrachée) atteint 15 mm. sur 2 mm. d'épaisseur. Les deux spécimens non endommagés ont 7 mm. de longueur et 3,5–4,0 mm. d'épaisseur. Chez l’un d’eux, à la suite d’une réduction ir régulière de la musculature, la partie antérieure du corps est considérablement élargie, ce qui lui donne une ressemblance apparente avec le Corynosoma semerme Forss. Cet exemplaire correspondait mieux que les autres à la description de v. Linstow. Cet auteur observe que le corps d’Ech. pupa est tordu en forme de limaçon: cependant ce caractère n’est pas général, on ne le trouve que chez deux exemplaires qui présentent une certaine torsion du corps, due probablement à la réduction de la musculature longitudinale; cette torsion du reste n’est pas assez prononcée pour dire que leur corps est tordu en spirale ou en forme de limaçon. La surface de la partie antérieure du corps porte une certaine quantité de petits aiguillons (la description de v. Linstow n’en fait point mention).

Après avoir extrait le rostre avec sa poche, je constatais qu’il était planté sur un cou de 0,94 mm. de longueur. Sa moitié antérieure est tirée en dedans et dans cette position il a l’aspect d’un cylindre de 0,36 mm. de diamètre. L’armure du rostre se compose de 18 ou 20 (?) rangées longitudinales de crochets (selon v. Linstow il n’y en a que 18), dont chacune montre environ 8 crochets. La poche du rostre, 2,1 mm. de longueur, possède une paroi musculaire double. Les lemnisques présentent l’aspect de lamettes plates de 2 mm. de longueur. Les œufs sont allongés, de forme ovale; leur dimension est de 0,125 : 0,036–0,136 : 0,039 mm.

L’espèce en question appartient sans aucun doute au même groupe d’Acanthocéphales auquel Van Cleave a rapporté F. botulus et le F. arcticus. Pour les distinguer les uns des autres d’une manière plus nette je présente ci-dessous le tableau suivant:

<table>
<thead>
<tr>
<th>Nom de l’espèce</th>
<th>Hôte</th>
<th>F. botulus Van Cleave</th>
<th>F. arcticus Van Cleave</th>
<th>E. pupa v. Linstow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longueur du corps</td>
<td>Jusqu’à 20 mm.</td>
<td>Jusqu’à 20 mm.</td>
<td>Jusqu’à 20 mm.</td>
<td>Jusqu’à 20 mm.</td>
</tr>
<tr>
<td>Rostre</td>
<td>Ovale</td>
<td>Ovale</td>
<td>Impervible de juger de la forme du rostre car sa partie antérieure est tirée en dedans</td>
<td></td>
</tr>
<tr>
<td>Nombre des rangées longitudinales des crochets</td>
<td>16</td>
<td>22</td>
<td>18–20</td>
<td></td>
</tr>
<tr>
<td>Nombre des crochets dans chaque rangée</td>
<td>7–8</td>
<td>7–8</td>
<td>Près de 8</td>
<td></td>
</tr>
<tr>
<td>Dimension des œufs</td>
<td>0,071–0,083 ; 0,03 mm.</td>
<td>0,126–0,03 mm.</td>
<td>0,125–0,036 mm.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0,155–0,041 mm.</td>
<td>0,136–0,039 mm.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Remarque. La partie antérieure du corps de ces trois espèces est couverte de petits aiguillons. La description de l'appareil du rostre de Ech. pupa est fondée sur l'étude d'un seul exemplaire.

Cet examen nous révèle une grande ressemblance entre les espèces F. arcticus et Ech. pupa et on pourrait peut-être conclure qu'elles sont identiques et appartiennent à la même espèce. Je m'abstiens pourtant de trop insister là-dessus car d'un côté je n'a pas eu l'occasion d'étudier le F. arcticus personnellement, et de l'autre côté mes études sur le Ech. pupa étaient faites sur un exemplaire dont le rostre était à moitié tiré en dedans, ce qui m'a enlevé toute possibilité de bien voir son armature.

Pour conclure je tiens à ajouter que les trois espèces mentionnées, d'après les données de collections, sont des espèces arctiques, appartenant au littoral de l'Europe, de l'Asie et de l'Amérique du Nord.

Le Fillicollis botulus van Cleave a été trouvé dans l'intestin du Somateria mollissima des régions: 1. l'île Spitzbergen (No. 55); 2. à l'est du golfe de Kokoueff (No. 57, No. 53); 3. Mourman (No. 98).

Le Fillicollis arcticus van Cleave a été trouvé dans l'intestin de Somateria spectabilis de l'Amérique du Nord.

Ech. pupa v. Linstow (des intestins de Somateria spectabilis) à l'est de la presqu'île de Taimir.

Polymorphus philippi n. sp. (hôte Somateria mollissima): 1. du détroit de Behring (No. 87, No. 88); 2. presqu'île des Tshouktschis; 3. Mourman.

RÉSUMÉ.
1. On trouve dans les intestins de l'eider (S. mollissima) deux espèces d'Acanthocéphales: F. botulus van Cleave et Polymorphus philippi n. sp.
2. Il est impossible de définir à laquelle des deux espèces correspond la dénomination de Ech. lendix Phipps.
3. La dénomination de Polymorphus philippi est adoptée étant donné que les règles de la nomenclature zoologique exigent la suppression de toute dénomination servant à la fois à deux espèces différentes.
4. Le Fillicollis arcticus van Cleave et Echinorhynchus pupa v. Linstow sont probablement identiques.
5. D'après les travaux de van Cleave on peut conclure que les Acanthocéphales de l'eider (Somateria mollissima et S. spectabilis) dans l'Amérique appartiennent aux mêmes espèces que ceux d'Europe et d'Asie.

INDEX BIBLIOGRAPHIQUE.
RECENT PROGRESS IN OUR KNOWLEDGE OF PARASITIC WORMS AND THEIR RELATION TO THE PUBLIC HEALTH.

By WILLIAM NICOLL, M.A., D.Sc., M.D., D.P.H.
(London School of Tropical Medicine.)

Ten years ago I made an attempt to summarize briefly the advances which had been made in our knowledge of parasitic worms during the preceding few years. The interval has witnessed much upheaval and interruption of scientific labour, but nevertheless a very considerable amount of work on the subject has been accomplished. The nature of this does not appear to have been influenced to any exceptional extent by the war.

During this period a terse but useful summary of the more outstanding recent parasitological work has been published by Faust who deals with the subject mainly from its zoological aspect; while the chief publications of medical interest have been succinctly reviewed by Leiper. In the following notes I propose to deal with the subject in somewhat greater detail particularly in its relation to the public health.

One might have anticipated that in a country such as France the preparation and publication of scientific papers would have been grievously hampered by war conditions but that was certainly far from being the case, for French workers, indeed, are excelled only by British and Americans in the quantity and quality of their output of helminthological literature. Russia and Germany are, as might have been expected, a considerable distance behind, while the only other countries in which any outstanding work on parasitic worms has been done are Brazil and Japan.

Britain has certainly been fortunate in possessing such workers as Leiper, Beddard, Baylis, Boulenger, Clayton Lane (India) and T. H. and S. J. Johnston (Australia), but there has been until quite recently a comparative dearth of younger helminthologists. America has been much better supplied in this respect for, in addition to men of such well-established reputation as Cort, Hall, Ransom and Faust, others such as Van Cleave, Cooper, G. A. MacCallum and Larue have produced work of a high order.

The Frenchmen who, in my opinion, have contributed most largely to the science of helminthology have been Seurat, Dévé, Railliet, Henry and, as a physiologist, Fauré-Frémiet. The Russians who appear to have done most useful work are Skrjabin and Cholodkowski, while Germany has been most ably represented by Fülleborn, Martini and Fibiger. Travassos in Brazil and Yoshida in Japan complete the list of the most outstanding names. This list,
however, does not include the names of many able contributors whose work will be referred to later.

In referring to Faust's review I am struck with the fact that he makes no mention of such names as Hall, Fülleborn, Baylis, Clayton Lane, Dévé and Fibiger, while including the names of others whose claims even to be regarded as helminthologists are, to say the least, obscure. We may take it, however, that this is, as may be, a matter of opinion.

There can be no doubt that during the past ten years America has drawn well ahead of all other countries, with the possible exception of Britain, in the matter of helminthological work. This might be attributed to some extent to the war which did not upset American economic conditions so seriously as it did those of Europe. Another reason perhaps is that, helminthologically speaking, America is a new and largely untouched terrain. Its great faunistic wealth implies a correspondingly great variety of helminth forms. The chief reason, however, is that Americans have been the first to realize to the full the great importance of helminthology in veterinary medicine and agriculture. The work of Hall and of Ransom, for instance, has been devoted essentially to this side of the subject. Another department in which Americans have taken a foremost place is that of bibliography, in which connection the names of Stiles and Hassall are outstanding. It is somewhat gratifying to find that the subject of veterinary helminthology has begun to receive a little more of the attention which it greatly needs in this country and the colonies. It has, of course, for some time held a fairly prominent position in Australia thanks largely to the occurrence there of worm nodule disease in cattle but even there it has not to any great extent been organized as it properly should be.

In view of their great prevalence and economic importance it is not surprising that Nematode parasites have received considerably more attention than any other group of parasitic worms. In consequence, we find that such men as Seurat, Hall, Fülleborn, Baylis, Leiper, Railliet and Henry have devoted the greater part of their attention to this group. Cestodes have been dealt with most extensively by Beddard, Harvey Johnston, Hall and Skrjabin, while the chief work on Trematodes has been done by Cort, Faust, Yoshida, Leiper, S. J. Johnston and myself.

The bulk of zoological literature relating to parasitic worms deals naturally with structure and classification. The elaboration of means of accurate identification and ready classification is one of the primary objects of all zoological work. Neglect of this has led in the past to a certain amount of confusion in experimental investigation. The identification of adult forms is in the main a comparatively easy task. The identification, however, of larval forms, particularly of many Nematodes, is much more difficult. On that account reliable work on larval structure and development is of the utmost importance, especially from the point of view of preventive medicine. It is only when the salient features of the life-histories of these parasites are accurately known that we can hope to attain administrative control over the diseases to which
Parasitic Worms

they give rise. In no department of preventive medicine is the statement that prevention is more useful than cure of greater applicability than in Helminthology, and no more striking instances of this could be wished for than such diseases as Ancylostomiasis and Bilharziasis.

The importance of attention to minute structural and anatomical detail is well instanced by recent work on the Sclerostome parasites of horses. Where not many years ago only a dozen or so ill-defined and much-confused species were recognized, now there exists a large family with numerous genera and species. While this involves a much extended and possibly more cumbrous nomenclature it yet creates a greater simplification and precision of detail.

On that account one must welcome such work as that of Beddard which though inclined to be prolix in certain respects is yet of the greatest fundamental value. His work is probably amongst the best which has been done on Cestodes in this country. Another contribution on Cestode structure which appears to be of considerable importance is that of La Rue on the Cestode family Proteocephalidae. Although this work is to a large extent revisionary it constitutes a valuable summary of our knowledge of a somewhat unusual group. Of a similar character is Douthitt’s monograph on the Anoplocephalid family of Cestodes. This is of great importance to those who may be concerned with the tapeworm parasites of horses.

A useful summarizing work is that of Krause on the Hemistomes, a group of Trematodes which is of considerable zoological interest though not of much pathological importance. Of equal merit and of much greater economic importance is Stunkard’s monograph on the Polystomidae, Aspidogastridae and Paramphistomidae of North America. Of some interest, too, is the embryological work of Lepeskin on the ovogenesis of Zoogonus mirus, a peculiar ovoviviparous Trematode parasite of Mediterranean fishes. This parasite lends itself particularly well to such study and has been the subject of previous similar work by von Hofsten and others.

These constitute perhaps the most extensive single works on Cestode and Trematode anatomy. On Nematode structure the work is considerably more voluminous but one can deal here with only a very restricted selection. Perhaps the most outstanding monograph is that of Martini on the anatomy of Oxyurus curvula, the large threadworm of the horse. Martini’s histological work is, of course, well known and this monograph constitutes a fitting successor to his earlier work on Ascaris. Of somewhat similar nature is Deinke’s histological researches on the nervous system of Ascaris megalacephala. The work of Neveu-Lemaire on the reproductive organs of the Metastrongylidae is also deserving of special mention as is that of Zacharias on the finer structure of Ascaris from a cytological standpoint. Seurat’s monograph on the parasitic Nematodes of Northern Africa (1920) and Khalil’s revision of the Nematode parasites of Elephants (1922) are two of the most important recent systematic works on Nematodes.

Of more practical importance to the economic helminthologist is Hall’s
review of the Nematode parasites of rodents, a subject which has acquired extraneous medical interest as a result of Fibiger’s investigation on rat cancer.

*Ascaris lumbricoides* has formed the subject of a fairly important work on chemical physiology by von Kemnitz, but this is of little intrinsic interest to helminthologists.

At the present time the life-history and mode of development of parasitic worms are most important subjects of research and discussion both from a zoological and from a medical point of view. The complicated life history of some of these worms is at times baffling and requires much patience and persistence in its elucidation.

Without doubt, during the past decade several discoveries of the very highest importance have been made. Chief amongst these must be placed the work on Bilharziasis. This subject has called forth a greater bulk of literature than any other in Helminthology. Ten years ago we owed a large part of our knowledge and opinion on the subject to Looss but he, curiously enough, formed the conclusion, based apparently on negative observations and experiments, that no intermediate host was concerned in the life-history of *Bilharzia (Schistosoma) haematobia* and that the larval parasite after its escape from the eggshell was able to re-infect the human subject in the miracidial or first larval stage by direct penetration through the skin. Such a conclusion was, needless to say, opposed to all previously acquired ideas of the life-history of digenetic Trematodes though the fact that the Schistosomes are so sharply differentiated structurally from all other digenetic Trematodes lent colour to the supposition that their life-cycle might also be radically different. The subject, however, was of such great economic and medical importance that this conclusion could not be accepted without very much more definite and irrefutable evidence. Such evidence has been accumulatively forthcoming from various quarters, particularly Japan and Egypt.

It is impossible, in a brief summary, to enter into full details of this voluminous work. A recent review of current knowledge and progress in the matter has been written by Fairley (1919) but the most comprehensive account is contained in Leiper’s original reports on the work of the Bilharzia mission in Egypt (1915–1918). This report will probably take rank as the greatest helminthological classic since Looss’s monograph on *Ancylostoma*. Though it be true that some of Leiper’s results were forestalled by Japanese investigators (e.g. Miyairi and Suzuki, 1914) and that he may be indebted to them for suggestive ideas, yet the credit of the completed work is undoubtedly to a large extent his. It has been shown definitely that an intermediate molluscan host plays a necessary part in the life-cycle of the parasite. The important feature remains, however, that infection can take place through the intact skin. The chief point for consideration is the interpolation of the intermediate host for, from a prophylactic point of view, this affords a useful if not particularly vulnerable point of attack in the life-cycle. Further reference will be made to this.

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Bahr and Fairley (1920) have confirmed Leiper’s opinion that *Schistosoma mansoni* and *S. haematobium* are distinct. They found that the molluscan intermediate host, *Planorbs*, can only be infected with *Schistosoma mansoni*, while *Bullinus*, another intermediate host, is not susceptible to infection with that species. Moreover, the cercariae from *Planorbs* give rise to the rectal form of Bilharziasis while those from *Bullinus* produce the urinary form.

Another important Trematode, the life-history of which has been recently elucidated, is *Fasciolopsis buski*. Our knowledge of this we owe to Nakagawa (1921) who has found that the miracidial larva encysts in species of snails of the genera, *Limnaea*, *Planorbs* and *Segmentina*, especially *Planorbs coenosus* and *Segmentina largillierti*. The cercariae, like those of *Fasciola hepatica*, encyst on grass.

Amongst other works on Trematode life-histories mention must be made of Faust’s valuable work on American and South African forms, Cort’s studies on North American larval Trematodes and Jegen’s monograph on *Collyriclum faba*, a Monostome parasite of singing birds.

The most important work on Cestode development is that of Janicki and Rosen (1917) on the life-cycle of *Dibothriocephalus latus*, a work which has evidently given rise to some controversy between the collaborateurs. Rosen, in a later publication (1918, 1919), maintains that the first intermediate hosts of the broad tapeworm are the Copepod Crustaceans, *Cyclops strenuus* and *Diaptomus gracilis*. In addition he found that *Cyclops* also functions as the primary intermediate host of *Ligula simplicissima*, a common tapeworm of aquatic birds. Galli-Valerio (1919) has also added some notes on the development of the broad tapeworm. Another tapeworm of the same group, *Schistocephalus solidus*, has been shown by Nybelin (1919) to pass its larval stage also in species of *Cyclops* (*C. serrulatus* and *C. bicuspidatus*).

Perhaps the most interesting work on this group of tapeworms, however, is that of Okumura (1919) on *Sparganum mansoni*, a not uncommon tapeworm parasite, in its larval stage, of man in the East. According to Okumura the onchosphere or first larval stage of this parasite develops in the Copepod, *Cyclops leuckartii*. When this is ingested by a mouse or a frog the onchosphere penetrates the intestinal wall and develops into the plerocercoid stage in the body cavity. This plerocercoid, when swallowed by a dog, develops into an adult tapeworm in the dogs’ intestine just as the human *Sparganum* does when similarly ingested, and the adult tapeworms resulting from such infections are, according to Okumura, identical.

The interesting subject of the life-history of *Hymenolepis nana* has been taken up by Joyeux (1920). He has confirmed the belief that this tapeworm infects without the intervention of an intermediate host but he was unable to infect rats by means of eggs from human cases thus apparently proving that the human parasite is specifically distinct from the morphologically similar *Hymenolepis murina* which normally infects rats. In 1911 Minchin and Nicoll found a cysticercus larva in the rat flea (*Ceratophyllus*...
fasciatus) which they considered to be the larva either of *Hymenolepis murina* or of some yet unrecognized species. Such a species has now been discovered by Baylis (1922) and named *Hymenolepis longior*.

Of some interest is the record by Parisot and Joyeux (1920) and Blanc and Caminopetros (1921) of the discovery of the larvae of two tapeworms of the cat (*Dipylidium trinchesei* and *D. chyzeri*).

In contrast to the meagre amount of work on Cestode life-history is the large volume of interesting researches on Nematode larval development. Most prominent amongst this is Looss’s monograph on the life-history of the hookworm. This work, however, is now so generally known and has been so extensively reviewed and commented upon that no further criticism appears necessary. One point nevertheless, of some importance has arisen, namely the alleged heterogenesis or alternating life-cycle of *Ancylostoma*. Surprising as it may seem to anyone with a working acquaintance with hookworm development this point has been raised anew by more than one observer but in view of the very large consensus of confirmation of Looss’s work it is to be hoped that the matter has been finally settled.

Of more recent date is the work of Fauré-Fremiet on the embryological development of *Ascaris*. Extending over a number of years this work was published in the form of a large monograph in 1913.

The most important work on Nematode life-history is, possibly, that on the life-history of *Ascaris*, investigations which probably rank next in importance to those on *Bilharzia*. The names most closely associated with this work are those of Stewart, Ransom, Foster and Yoshida. Stewart, experimenting with rats and mice, found that the infecting larvae pursued a migratory course in the body of the experimental animal somewhat similar to that followed by the larvae of *Ancylostoma*, namely a passage through the intestinal wall, transference to the lungs *via* the blood stream and a migration thence *via* the trachea into the intestine again. The fact that these larvae, after they had again reached the intestine, did not develope further there but were passed out with the intestinal contents led Stewart at first to form the hypothesis (abandoned later) that rats and mice constituted a sort of intermediate host for the Ascarids. (It may be noted that no species of adult *Ascaris* has ever been recorded as a natural parasite of rats or mice.) This view was not accepted by Ransom and Foster (1919) who have demonstrated that a similar migratory course is pursued by larval Ascarids in infecting their natural host, *e.g.* *Ascaris lumbricoides* in the pig. It is thus evident that there is some resemblance between the invading stages of *Ascaris* and *Ancylostoma*, the essential point of difference in the life-history, however, being that *Ascaris* has no external free larval life.

In addition to his work on the life-history of *Ascaris* Ransom has added considerably to our knowledge of the life-history of two other Nematode parasites, namely *Habronema muscae* and *Gongylonema scutatum*, both of which are of decided importance in veterinary helminthology. In the case of
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the former Ransom has demonstrated that the larval Nematode found as long
ago as 1859 by Carter in the common house-fly in Bombay and named by him
Habronema muscae is actually the larval stage of a parasite of the horse,
closely allied to a similar horse parasite, Spiroptera microstoma described in
1866 by Schneider. The occurrence of this larval Nematode in the house-fly
and the stable-fly (Stomoxys calcitrans) has also been recorded in Australia by
Harvey Johnston (1912).

The life-history of this parasite is one of the most remarkable which has
been brought to light within recent years. It furnishes yet another instance
of the important part which house-flies play in the transmission of disease.
So far as one can judge from observation and experiment the life-history
appears to proceed on the following lines. The eggs or larvae of Habronema,
occurring in the horse manure, are ingested by the larvae of the house-fly.
During the metamorphosis of the fly the larval worms find their way into the
Malpighian tubules and thence in the adult fly they migrate into the proboscis.
When the fly applies its proboscis to an open sore or to the lips or conjunctivae
of the horse the larval worms escape from the fly’s proboscis and invade the
abraded tissue. Thence the worms migrate through the body of their host and
eventually find their way into the intestine. Infection with this worm is thus
curiously correlated with the occurrence of “summer sores.” We owe the eluci¬
dation of this remarkable life-history chiefly to Railliet and Henry (1915), van
Saceghem (1917), Hill (1918), Teppaz (1919) and Roubaud and Descazeux (1921).

The most recent discovery of prime importance to human medicine is that
of the life-history of Loa (Filaria) loa. This is the result of some excellent
experimental work by A. and S. Connal (1922) in Lagos. Following one of
Manson’s intuitive suspicions they devoted attention to the Tabanid flies,
Chrysops silaria and C. dimidiata in which they have been able to demonstrate
that the worm passes its larval life. The larvae develop in the abdominal
muscles and connective tissue of the fly and eventually in their infective state
migrate to the fly’s proboscis.

The life-history of another worm of the Spiroptera group, Gongylonema
scutatum, a parasite of sheep and cattle, has also been dealt with by Ransom
and Hall (1915, 1917) who found that the larvae live in dung-beetles of the
genera Aphodius and Onthophagus and in the croton bug (Ectobia germanica).
The allied parasite, Gongylonema pulchrum, of the pig also develops in the
croton bug. The first observations on the larval host of worms of this group
were made by Leuckart (1867) and Marchi (1871) in the case of the rat para¬
site, “Spiroptera” obtusa, the larva of which was found to occur in a beetle,
Tenebrio molitor.

Another strikingly able paper is that of Veglia on the anatomy and life-
history of the Nematode, Haemonchus contortus. The developmental history
of this important parasite of sheep is worked out in great detail and the result
is a model of what such experimental investigations should be. It is un¬
doubtedly one of the best pieces of work of its kind.
Martin has published an interesting paper on the conditions of embryonic development of parasitic Nematodes in general, while Kautzsch has dealt at length with certain anomalies in the development of *Ascaris*. With regard to "free living" Nematodes the most economically important work appears to be that of Stift on the life-history of root Nematodes.

Turning now to what is, from a medical point of view, the subject of greatest interest, namely the relation of parasitic worms to disease we find that quite an extensive literature has accumulated, much of it being of the very highest order. First in point of importance and by reason of the large amount of excellent research work done in regard to it is Bilharziiasis. While not having quite such a widespread distribution as the hookworms or the filarial worms the *Bilharzia* species are the cause of some of the most serious pathological conditions originated by any worm parasite, and the gravity of such conditions is accentuated by the fact of their comparative intractability to medical treatment.

Although the disease has been known apparently from very ancient times it is only within the past seventy years that its causal agent has been definitely recognized. The worm is thus a slightly more modern acquaintance than its rival *Ancylostoma duodenale* though both, so far as may be judged from historical evidence, were familiar to the people of ancient Egypt some thousands of years ago (Khalil).

Much as one may feel tempted to enter on a detailed discussion of the administrative measures, necessary or advisable for the control of this disease, it seems to me that the conclusions reached by Leiper sum up the position sufficiently clearly. These have been quoted by more than one reviewer but their administrative importance justifies further repetition. They are that (1) *transient* collections of water are quite safe after recent contamination; (2) all *permanent* collections of water such as the river Nile, canals, marshes and birkets are potentially dangerous, depending upon the presence of the essential intermediate host; (3) the removal of infected persons from a specified area would have no effect, at least for some months, in reducing the liability to infection (i.e. of other persons), as the intermediate hosts continue to discharge infective larval forms for a prolonged period; (4) infected persons cannot reinfect themselves or spread the disease directly to others. They could only convey the disease to those parts of the world where suitable local molluscs were present to act as intermediate hosts; (5) infection actually takes place both by the mouth and through the skin; (6) infection in towns is acquired from *unfiltered* water which, in addition to filtered water, is still supplied even in Cairo though it is delivered by a separate system of pipes; (7) eradication can be effected without the cooperation of infected individuals by destroying the molluscan intermediate hosts.

In these respects Leiper's conclusions differ radically from those of Looss, the pivotal point being, of course, the *infective* stage of the worm (*cercaria* instead of *miracidium*). They are, undoubtedly, of the very highest importance.
to all sanitarians who have to deal with the problem of Bilharzia prevention and eradication.

In the nature of constructive administrative policy Leiper adds: “If a campaign against bilharziasis were commenced on the lines here proposed it is evident that the whole scheme should be under the charge of a medical zoologist who should be attached, not solely to the Public Health service as in Ancylostoma campaigns, but also to the Department of Irrigation. In this way, only, could the full and continuous effect of the present administrative control of the Nile water be brought to bear upon the Bilharzia-carrying molluscs so as to ensure their permanent eradication from lands now heavily infected and their exclusion from new areas about to be reclaimed.”

Of practical local measures which might, with advantage, be adopted Leiper suggests that unfiltered water from potentially infected sources such as canals, ditches and birkets would be rendered safe (1) if stored beyond the survival period of the cercariae, i.e. for at least forty-eight hours; (2) if heated to 50° C., a temperature at which the cercariae are immediately killed; (3) if previously treated with chemicals which are lethal to the cercariae, e.g. chlorinated lime, benzoic acid, etc.

Other noteworthy and important contributions to our knowledge of Bilharziasis are those of Katsurada (1913), Looss and Kartulis (1913), Miyagawa (1913), Miyairi and Suzuki (1914), Ogata (1914), Narabayashi (1914), Cawston (1918), Liston and Soparkar (1918), Lutz (1920), Bahr and Fairley (1920) and Cort (1921). Of these the last mentioned is of most interest. Cort has been able to supply in the case of Schistosoma japonicum, in a fairly complete form, a knowledge of the course pursued by the parasites from their entry into the body till they reach their ultimate habitat in the portal veins. After penetrating the skin or mucous membrane the larvae are carried by the veins or lymphatic vessels to the heart, whence they are conveyed to the lungs. Thence there appear to be two alternative courses. The first is via the pulmonary veins back to the heart and thence to the hepatic-portal system. In the second and possibly the more frequent course the larvae penetrate the substance of the lungs. Thence, via the posterior and anterior mediastina, they migrate to the peritoneal cavity where they bore their way into the liver and so reach the hepatic-portal venous system. It is evident that in this complicated migration pathological effects may be produced in the lungs, liver, etc., in addition to the more characteristic pelvic and urinary symptoms.

Interesting serological work in relation to Bilharzia infection has been carried out by Fairley (1919) who has prepared a specific antigen from the liver of molluscs infected with Bilharzia cercariae. More recent work on the same subject has been published by Le Bas (1922).

It is a matter of some significance that indigenous cases of Bilharziasis have recently been recorded in Europe (Southern Portugal) by França (1921) and others, the intermediate host there being a subspecies of Planorbis corneus.
The next most important subject is Filariasis in its multiplicity of forms. This has called forth almost as voluminous a literature as Bilharziasis, but no one species has received such exhaustive treatment as *Schistosoma haematobium*, nor is the intrinsic value of the work comparable with that done on *Bilharzia*. The most considerable contribution is probably that of Manson-Bahr (1912) on Filariasis and Elephantiasis in Fiji. Fülleborn (1913) gives an up-to-date account of human Filariasis in its various aspects and in the same year he published an exceedingly useful monograph on the differential diagnosis of Microfilariae. Meinhof (1913) has dealt pretty thoroughly with the morphology of *Loa loa* and its clinical manifestations. A new human microfilaria (*M. nuda*) has been observed by Rodenwaldt (1914) in Togoland while Biglieri and Araos (1917) have also described a new human Filaria (*F. tucumani*) from South America. La Cava (1916) has recorded the first autochthonous case of elephantiasis in Europe caused by *F. bancrofti* in the province of Treviso, Italy, while Skrjabin (1917) has described a new species of ocular Filaria (*Loa extracocularis*) from man. Yorke and Blacklock (1917) have made useful observations on the periodicity of *Microfilaria nocturna* and Ruiz Arnim (1916) contributes a lengthy monograph on primary tropical lymphectomy. Wirth's paper (1917) on the Filariace of horses is also rather useful.

The interesting subject of Filariasis in dogs (*Dirofilaria immitis*) has recently received renewed attention with the result that much light has been thrown on the life-history of this dangerous parasite. For this we are indebted chiefly to Fülleborn (1912) and Saisawa (1912) who have shown that the worm passes its larval stage in the mosquitoes, *Stegomyia fasciata* and *Anopheles maculipennis*. More recently Breinl (1921) has found that the worm may also complete its larval development in the dog-fleas, *Ctenocephalus canis* and *C. felis*.

Bernard and Bauche (1913) have further concluded that *Stegomyia fasciata* also serves as the intermediate host of the subcutaneous Filaria of the dog (*Dirofilaria repens*).

Ancylostomiasis has, until recently, not attracted quite so much attention during the past decade as it did previously. This is in part due to the fact that Looss in 1911 set the stamp of his authority on many points that had for some time been controversial and in part to the fact that effective means of treatment have been elaborated and carried into practice in various parts of the world. In spite of this the disease remains and will remain one of the most serious with which sanitarians, particularly in the Tropics, have to deal.

Fülleborn (1914) has contributed an interesting and useful paper on the biology and mode of infection of *Ancylostoma* and *Strongyloides*, while Baermann (1917) has recorded his extensive experience in Java and has dealt with preventive measures at some length. Not the least important part of Baermann's work, however, lies in the fact that he devised an efficient method of isolating hookworm larvae from samples of soil. This method was adopted and elaborated by the American Hookworm Commission in the West Indies. The work of this Commission (Cort, etc., 1921–2) is unquestionably the most
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far-reaching which has been accomplished on the subject since that of Looss in Egypt.

One of the most interesting biological facts revealed by the work of the Commission is that hookworm larvae commonly live in an unensheathed state in the soil. Hitherto it has generally been believed that the unsheathing takes place at the moment the larvae penetrate the skin of their host, though the occurrence of unsheathed mature larvae in experimental cultures must be a matter of fairly common observation. The report also deals with several matters of administrative importance.

Another species of hookworm, Ancylostoma ceylanicum, is dealt with by several authors and its geographical distribution forms the subject of a paper by Ihle (1918).

The matter of next greatest economic importance appears to be Oncho
cerciasis (worm nodule disease in cattle). This is a subject our knowledge of which is of comparatively recent date. Although the disease has been recognized for many years scientific interest in it dates only from the past twenty years. Curiously enough it is in Australia, the land of imported cattle, that the condition has given rise to most discussion and in consequence the bulk of the work has been done by investigators in Australia. The most considerable contribution to the subject is that of Sweet (1915) who in the course of a tour round the world visited most of the countries in which the disease is known or suspected to occur. Her paper forms therefore a very useful summary of the geographical distribution of the parasite and contains many interesting observations on local conditions as they may affect the spread of the infection. Previously (1912) Harvey Johnston published a summary of existing knowledge on the subject at that time. The most important experimental work, however, is that of Burton Cleland (1914) who attacked the problem in an energetic manner but without solving the mystery of transmission. I have personally (1914) carried out some experiments on the nodules both in vivo and in vitro without any striking result except to demonstrate that the larvae can and do penetrate the thick capsule of the nodule in which they are enclosed and thus get into a situation in which a potential intermediate host may reach them. No intermediate host has, however, been discovered so far and practically no definite clue is available to point the way to a possible life-history. The fact that similar worms cause disease in man (Onchocerca volvulus) and in horses (O. cecutiens) gives the subject an economic interest which is not confined to cattle. Rodhain and van den Branden (1916) and Robles (1919) have made interesting contributions to our knowledge of the human form of Onchocerciasis which has received further treatment at the hands of Brumpt (1920), Desoil (1920), Montpellier and Lacroix (1920), and most recently Ouzilleau, Laigret and Lefron (1921). Further remarks on cervical Oncho
cerciasis of horses have been published by Railliet (1919).

Of much greater medical interest and importance is hydatid disease (Echinococciosis). This is another disease which has afflicted Australia in
perhaps undue proportion, but although a considerable Australian literature on the subject exists most recent work has been done in Europe, chiefly by Dévé. In the course of a very extensive series of experiments he has dealt with a great variety of aspects of the disease both primary and secondary. Most recently he has been especially concerned with experimental echinococcosis of bones and with the disease as it manifests itself in infants, both subjects which appear to be of importance from the point of view of comparative pathology. He has also dealt to some extent with the matter of treatment.

The subject of serological diagnosis of hydatid disease has also engaged a fair amount of attention. This practically dates from the original work of Weinberg and of Welsh and Chapman in 1908. Since then, however, Weinberg (1913) has further elaborated the work which is of great importance as, in addition to its bearing on hydatid disease, it raises the whole question of serum diagnosis in the case of helminths. It is obvious that the method is of significance in the diagnosis of such helminth infections as are not readily diagnostable by ordinary clinical methods.

Another important contribution to our knowledge of echinococcosis appears to be that of Mita (1918) but this work I have been unable to consult.

The occurrence of the adult *Taenia echinococcus* in dogs in Friesland forms the subject of a monograph by Tenhaeff and Ferwerda (1919).

A subject which has recently come into prominence is that of rat cancer (*Spiroptera* tumours) with which the name of Fibiger is most intimately associated. He and his colleague, Ditlevsen, conclude that *Spiroptera* or *Gongylonema neoplasticum*, as they name the worm, is capable of giving rise to intestinal growths of a true malignant or cancerous character, with metastases. According to Fibiger the larvae of this parasite occur encysted in cockroaches (*Periplaneta americana*), with which he claims to have infected rats. It should be noted that a number of worms (e.g. *Oesophagostomum* spp., etc.) cause tumour formation in the intestine of their host but such formation is not necessarily of a malignant character. Bulloch and Rohdenburg (1918), moreover, showed that similar tumours could be produced in rats by introducing a spiny glass ball into the stomach.

Compared with that on Bilharzia recent literature relating to the lung-flukes (*Paragonimus*) is extremely scanty. The most important contributions are those of Ward and Hirsch (1915), Yoshida (1916) and Nakagawa (1916). The first-mentioned consists of a revision and correction of existing morphological knowledge of the species of *Paragonimus*. Nakagawa was the first to discover the intermediate hosts of the parasite, namely the freshwater crabs, *Potamon obtusipes* and *P. dehaanii*. Yoshida added two other species of crab, *Sesarma dehaanii* and *Eriocheir japonicus*. In these crabs the parasites occur in their encysted cercarial stage, and an earlier intermediate host of the sporocyst stage, probably a mollusc, yet awaits discovery. Yoshida (1916 a) followed the course of the infecting larvae in their final host (dog and cat) experimentally.
and found that they penetrated the stomach or intestinal wall, thus reaching the peritoneal cavity, whence by penetrating the diaphragm and pleurae they reached the lungs. According to Yoshida, therefore, the worms, unlike Bilharzia, reach their ultimate destination, the lungs, by their own migratory efforts, without the aid of the blood vascular system. He does not entirely account for the occasional occurrence of the worms in the brain though he states that "some of the worms may proceed cephalad, taking their course through the loose connective tissue along the oesophagus or the blood vessels." Later experimental work of Yokogawa and Suyemori (1921) on intracranial infection has failed to solve this point so that for the present we must accept Yoshida's supposition as a working hypothesis.

We have now dealt with what are generally considered (from a pathogenic and public health point of view) to be the most important parasitic worms. There remain, however, several others to which at least passing reference must be made. Of these Trichuris trichiurus (Trichocephalus dispar) has probably attracted most attention. It is a worm which in itself is possibly of second-rate pathogenic importance but which as a predisposing agent in serious intestinal affections may play a more definite part than has been generally believed to be the case. There appears to be quite strong evidence to associate it with a form of enteritis (Garin, 1912) and it is certainly suspect as a predisposing factor in cholera (Guerrini, 1915), and dysentery (Brau, 1914). An extensive monograph on the worm has been written by Christoffersen (1913).

There appears some ground for supposing that Trichuris like Ascaris and Ancylostoma pursues a complicated route of invasion in its host, but the only evidence so far reported is that of Neshi (1918) who recorded the finding of a few larvae of T. depressiusculus (the whipworm of the dog) in the lungs within twenty-four hours after the ingestion of Trichuris eggs.

Also, according to the observation and opinion of Yokogawa (1921), another allied worm, Trichosomoides crassicauda, the common parasite of the urinary bladder of rats, has a similar migratory course via the lungs, but the route of the larvae from the lungs to the bladder has not been traced. Yokogawa, however, considers that von Linstow’s observation in 1874 of young worms in the kidneys and ureters is suggestive that the worms enter the bladder by way of the kidneys. Further investigation, however, is necessary to complete our knowledge of the migratory course in the case of this parasite. Its importance as a pathogenic agent is evidenced by the fact that it may give rise to papillomatous growths in the bladder and apparently also in the kidneys (Löwenstein, 1912).

Allied to Trichuris but of much greater economic importance is the measles worm (Trichinella spiralis). Its frequency and dangerous nature are notorious in many parts of Europe. A comprehensive account of the structure and pathogenicity of this parasite is given by Stäubli (1913), but further researches on the toxic properties of the worm have been made by Flury (1913).
With regard to *Ascaris* the only important work is that on its life-history which has already been discussed. Two matters of some medical interest however may be mentioned, namely, the association of *Ascaris* with reflex epileptiform attacks (Francavigna, 1915) and with erythema nodosum (Montel, 1916).

Researches on *Strongyloides stercoralis* have not been numerous but they include the important work of Fülleborn (1914) on the biology and mode of infection of the parasite. It is not altogether remarkable that this worm should have to some extent confused the issue in regard to *Ancylostoma*, for, in the first place, the two are not infrequently associated and, secondly, the mode of infection is somewhat similar in both cases. In other respects, however, the worms are widely divergent for *Strongyloides* exhibits the remarkable phenomenon of heterogenesis which has been so often erroneously ascribed to *Ancylostoma*. Fülleborn’s investigations are full of interest.

The important veterinary matter of horse Strongyles has received a considerable amount of attention in this country at the hands of Yorke and Macfie (1918), Boulenger (1916-17) and Leiper (1913). Further references to the pathogenic action of these worms are to be found by Coureur (1915) and Parodi and Widakowich (1918). The latter deal with the mechanical action of the worms in the intestine of their host, while the former is concerned with the general cachetic state which may result. Bang (1913) deals with the subject of infection in young horses, while Leiper records a new Cylicostome from London horses. Horse Oxyurids have received attention from Railliet, Bedel and Donnat (1918) who form the opinion that the presence of the worms is associated with colic and emaciation.

The interesting hypothesis of worms as a cause of appendicitis has received a large amount of attention during the past ten years chiefly on the part of Rheindorf (1920) and of Innes and Campbell (1914). Additional cases are recorded by Sénéchal and Engel (1912), Borini (1914), Glines (1916) and Hueck (1913). If the view that *Oxyuris* and other intestinal worms are a direct or indirect cause of appendicitis can be substantiated it is evident that appendicitis, at least of a certain type, must be regarded as a disease of infection and must therefore be amenable to public health measures for its prevention, a view which Rheindorf strenuously maintains.

The occurrence of “pneumonia” during the course of helminth infection has been brought into much prominence by recent work on *Ascaris* infection. Ever since Looss’s memorable discovery of the lung route of the infecting larvae of *Ancylostoma* the occurrence of pneumonia symptoms associated with intestinal helminth infection has been fraught with significance. In 1914 I personally recorded the occurrence of fatal pneumonia in dogs undergoing experimental infection with *Ancylostoma caninum*. At that time I was inclined to ascribe the fatal issue to a superimposed microbial infection with a species of *Pasteurella* which was isolated from the lungs of the dead dogs by Dr J. A. Arkwright. Both Ransom (1919) and Stewart (1920), however, have definitely concluded that pneumatic symptoms are a regular concomitant
of *Ascaris* infection and indeed Stewart indicates that he regarded the onset of such symptoms in his experimental animals as definite evidence that *Ascaris* infection had taken place. Ransom, moreover, associates the pneumatic disease, known as “thumps” in young pigs, with *Ascaris* infection and by analogy suggests that many cases of “pneumonitis” in human beings, particularly children, are possibly the result of invasion of the lungs by infecting *Ascaris* larvae. In view of these observations it is obvious that the indifferent attitude displayed by some people in regard to *Ascaris* infection must be abandoned.

In addition to the pneumonia caused by migrating larvae, however, pulmonary symptoms may be caused by worms which have their final habitat in the lungs. The best known instance of this in human parasitology is, of course, the lung fluke *Paragonimus*, but instances in domesticated and other animals are by no means uncommon. Of late years chief attention has been called to the “worm pneumonia” of sheep due, mostly, to the lung-nematode *Dictyocaulus filaria*. This matter has been dealt with by, amongst others, Blum (1911), Knuth (1912) and Romanovitch and Slavin (1915). It is evidently of no small economic importance.

The toxic effects of parasitic worms is a matter which has called forth a considerable amount of investigation, and with which the name of Weinberg has for some time been associated. Since 1912 his principal contribution to the subject is that, in collaboration with Seguin, on eosinophilia (1914). A certain amount of work, apparently negating Weinberg’s conclusions, has been published but his views have received positive support from the work of Bedson (1913), Paulian (1918), Rachmanov (1914), Pomella (1921), Simonin (1922) and others. The frequent, though not invariable, occurrence of high degrees of eosinophilia associated with helminthic infection is a phenomenon of some import and it appears almost certainly to be correlated in some measure with toxic action. Weinberg and Seguin (1914) and Guerrini (1914) have published some interesting work on this subject.

The association of *Strongyloides* with dysentery has been dealt with by Noc (1915) in Cochin China. He came to the conclusion that the worm is not absolutely definitely pathogenic, but that it is frequently associated with bacillary and amoebic dysentery and may possibly accentuate the symptoms of these diseases.

A matter of considerable interest and perhaps of some importance is the reported occurrence of Nematodes of the genus *Trichostrongylus* as parasites of man. Instances have been recorded in India, Egypt and Japan. Species of this genus are not uncommon parasites in ruminants and other mammals, but the most familiar species in this country is that associated with grouse disease (*T. pergracilis* Cobbold). The first discovery of such worms as human parasites was apparently made by Ogata in 1889 and they were identified by Ijima (1895) as *Strongylus subtilis*. In 1914, however, Jimbo decided that these worms which are apparently very common parasites of man, and of man only,
in Japan and Korea represents a distinct species which he named *Trichostrongyulus orientalis*. Other species of this genus which have been reported as occasional parasites in man are *T. vitrinus* Looss 1905 in Egypt, *T. probolurus* (Railliet 1896) also in Egypt and *T. colubriformis* (Giles, 1892) (which is usually referred to as *Strongylus subtilis* or *T. instabilis*). It would thus appear that four distinct species of this genus may be occasional or even common parasites of man. Their small size and inconspicuous appearance render their detection a matter involving more than usual care. A somewhat similar, though larger, cattle Nematode, *Haemonchus contortus*, was reported as a human parasite in Brazil by Magalhaes in 1908. These worms must be taken into account in human pathology as potential causes of anaemia and enteritis.

Another worm of considerable economic importance is the kidney worm of swine (*Stephanurus dentatus*). This is yet another case in which it has been demonstrated that infection may take place through the skin (Bernard and Bauche, 1914).

Allied to the rat tumour *Spiroptera*, to which reference has already been made, is a worm of greater economic importance, namely, *Spiroptera (Spirura) sanguinolenta*, a not uncommon parasite of dogs in certain parts of the world. Following the work of Grassi in 1888 it was tentatively accepted that the larval stage of this worm occurs in the common cockroach (*Blatta orientalis*). Seurat (1912), however, maintains that this larva is that of *Spirura talpae*, a similar parasite of the mole, and that the larvae of *S. sanguinolenta* occur encysted in fowls, hedgehogs and lizards, a somewhat extraordinary variety of intermediate hosts.

The last matter of Public Health interest which need be dealt with here is that of house-flies as carriers of parasitic infection. Since 1911, when I published some experimental observations on this subject, Shircore (1916) and Wenyon and O’Connor (1917) have confirmed these results by observations under natural conditions. Shircore found eggs of *Trichocephalus dispar*, *Taenia saginata*, *Ancylostoma duodenale*, and *Ascaris lumbricoides* in or on flies from hospital wards in British East Africa. Apparently 10–12 per cent. of these flies were functioning as natural “carriers.” Wenyon and O’Connor examined the faeces of “wild” flies under natural conditions and found eggs of *Taenia saginata*, *Ancylostoma duodenale*, *Trichocephalus*, *Heterophyes* and lated-spined eggs of *Bilharzia*. These observations in conjunction with the classic experiments of Grassi and Calandruccio leave little doubt that house-flies play an actual part in the dissemination of worm infection, particularly of such forms as *Trichocephalus* and *Ascaris*. Buxton (1920) also recorded the natural occurrence in flies of eggs of *Hymenolepis nana*, *Strongyloides*, etc.

With regard to the medicinal treatment of parasitic worm infections a few notes only are necessary. The most extensive work on this subject is that of Hall (1919–1920) who has experimentally tested the value of various anthelmintics. Perhaps the most important new treatment is the use of carbon tetrachloride in ancylostomiasis. Results appear to show that this is the most
efficient drug which has hitherto been employed in the treatment of hookworms, with oil of chenopodium as probably the next best. The use of salvarsan and of antimony tartrate in the treatment of Bilharzia disease is also worthy of note. Railliet (1915) has dealt at some length with the medicinal treatment of diseases caused by Nematodes, and Hall (1917) has called attention to the neglect of medicinal treatment in dealing with veterinary helminthic infections.

I have endeavoured to discuss these various matters as briefly as possible and to allocate to each of them a space approximately proportionate to the amount and importance of the work done. The same plan has been applied to the subjoined list of references which, while omitting a considerable number of important works, will nevertheless, it is hoped, be found to include all the most useful publications up to the end of 1921 together with a few of more recent date.

REFERENCES.


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W. NICOLL 397


— (1912). Notes on some Entozoa. Ibid. XXIV. 43-91.

— (1918). Notes on certain Entozoa of Rats and Mice. Together with a catalogue of the internal parasites recorded as occurring in Rodents in Australia. Proc. R. Soc. Queensland, XXX. 53-78.


KEMNITZ, G. VON (1912). Die Morphologie des Stoffwechsels bei Ascaris lumbricoides. Ibid. VII. 463-663.


— (1917). Ancylostoma duodenale as a parasite of Felis tigris. Ibid. v. 210-216.


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NARABAYASHI (1914). On the migratory course of Schistosoma japonicum in the body of the final host. Kyoto Igaku Zasshi, xii. No. 1.


— (1915). Le parasite de la dermite granuleuse des Equidés. Ibid. viii. 695-704.


Parasitic Worms


NOTES ON THE COLLECTION AND PRESERVATION OF PARASITIC WORMS.

By H. A. BAYLIS, M.A., D.Sc.

(British Museum (Natural History)).

At the request of Prof. G. H. F. Nuttall, F.R.S., the writer has attempted to put together a few notes on methods found useful in obtaining well-preserved specimens for helminthological purposes. Of the large number of methods that have been recommended for the various groups by different authorities, only a limited selection can be given, and preference is given, where possible, to those which are comparatively simple and can be employed in the field or under the limited conditions of a field laboratory, while at the same time experience has shown them to give sufficiently good results for ordinary purposes.

Some few years ago the writer drew up a pamphlet on "worms" for the series of Instructions for Collectors issued by the British Museum (Natural History). In this were given in short form some selected methods for the treatment of the parasitic groups, which had been specially recommended by well-known authorities. These require some little modification and expansion, and are given here in greater detail, together with a few others taken from various sources. The writer has had the benefit of some advice from helminthologists whose work on special groups is well known, and has made use of the published writings of others. Thanks are especially due to Dr C. L. Boulenger and Dr F. J. Meggitt, of the University of Birmingham, and to Capt. R. Daubney, of the Ministry of Agriculture and Fisheries, for valuable suggestions and ungrudging help.

Collecting.

The majority of the parasitic worms of vertebrate animals are, of course, found in the alimentary canal. It must not be forgotten, however, that many inhabit other organs and tissues of the body, and when searching for intestinal worms an examination of the rest of the body of the host should by no means be omitted. As examples of the situations, other than the alimentary canal, in which worms, either adult or larval, may be found, may be mentioned the thoracic and abdominal cavities and their lining membranes; the liver and bile ducts, and the gall-bladder; the nasal cavities and orbits; the mouth; the trachea and bronchi, and the lungs generally; the heart and larger blood-vessels (especially the portal system); the kidneys, ureters, bladder and urethra;
the subcutaneous connective tissue and muscles; in fishes and in certain amphibia and reptiles, the outer surface of the skin; and in fishes the swim-bladder and gill-cavities.

It must also be borne in mind that while most of the "intestinal" parasites are found lying freely in the lumen of the stomach or intestines, or merely attached to their walls, some also burrow in the mucous membrane or in and between the muscular coats, and inhabit galleries or tumours formed in these situations. In birds, certain groups of nematodes occur between the coats of the gizzard, which should be stripped apart during the dissection.

To search for intestinal parasites the gut of the host should be slit up with scissors (preferably blunt-ended or enterotome), and after the removal of the gross contents and any obvious parasites, the mucous membrane should be washed with normal salt solution or water, and carefully examined for the less conspicuous forms that may be adhering to it.

In the case of small animals, the whole of the gut may sometimes be opened under water in a dish, or opened and then shaken up in a jar, and the entire contents thus washed out. This last method has the disadvantage that it is sometimes impossible to say from what region of the gut the parasites came.

Many small worms, especially such forms as the nematodes of the Trichostongyle group, are not easily detected unless the contents of the part of the intestine under examination (e.g. the caeca of a bird) are scraped out and shaken up in the washing fluid. A good plan in such cases is to scrape off the matter adhering to the mucous membrane with an instrument such as the back of a knife, and stir and shake it up in a tall jar of the fluid. On allowing this to stand for a few minutes, the worms will sink to the bottom, and most of the dirty fluid may be decanted off. Clean fluid is then added, and the process is repeated until the worms are comparatively free from other matter. The jar is then emptied into a flat dish, and very small specimens may be picked up on a needle, to which they will readily adhere, or if necessary with a pipette, under a lens or dissecting microscope.

"Prospecting" for such worms as Trichostrongyles may sometimes be carried out to advantage by a very simple method. This consists in placing a small quantity of the material scraped from the mucous membrane on a glass slide, and then placing another slide on the top of it, and exerting just enough pressure to spread the material out into a thin layer, in which the worms can easily be seen, if present, either with the unaided eye or under a low magnification.

In collecting cestodes gentle treatment is necessary, as they are often very fragile, and some care must be taken to avoid leaving their "heads" (scolices) adhering to the mucous membrane. In the case of small specimens the membrane should be gently scraped with the back of a scalpel, or some similar instrument. If the heads of comparatively large specimens are very firmly attached, gentle persuasion with a scalpel or needles in a dish of salt
solution may induce the worms to loosen their hold. The same remarks apply to Acanthocephala, but these must sometimes be literally dissected out.

In dealing with nematodes in capsules, cysts or tumours, or buried in tissue of any kind, it should be remembered that it is usually easier to remove and clean them in the fresh condition than after the surrounding tissue has been hardened with fixatives. Even when fresh, they are often very difficult to extract whole and undamaged. They should be dissected out as carefully as possible. Sometimes, if the enclosing tissue be cut out and placed for a short time in salt solution, the worms will emerge of their own accord.

Washing.

All intestinal worms should be washed, after collecting and before killing, to free them from adherent mucus and other matter. This, in most cases, is best accomplished by shaking up in a tube or jar of normal salt solution or tap-water. In the case of nematodes possessing a mouth-capsule, the shaking should be vigorous, in order to remove epithelium and other matter from the mouth. No damage will result to nematodes and trematodes from a fairly severe shaking, but with cestodes care must be exercised. The specimens, obtained by scraping or otherwise, should be gently shaken in a jar with plenty of fluid, or merely washed in a dish. When they are long and tend to become tangled, they should be kept as far apart as possible in a shallow dish, or rinsed separately and spread out on a glass plate with a minimum of fluid. For washing cestodes, Dr Meggitt recommends plain lukewarm water in preference to salt solution, unless it is important to preserve the cuticle in perfect condition. In this case cold salt solution should be used.

Washing should in no case be too prolonged. In the case of small nematodes, immersion in a watery solution for more than a few minutes is apt to cause local or general swelling of the cuticle, and sometimes the specimens will burst. It is therefore desirable, as a rule, to kill the worms as soon as possible after they have been collected. In the case of certain elongated and active nematodes, such as the lung-worms of sheep and pigs, it is necessary to keep them apart as far as possible during the washing process, and to kill them quickly, in order to prevent them from becoming a tangled mass.

Should it be desired to keep worms alive for a time, it is better, as a general rule, to leave them in their natural element than to remove them to water or salt solution.

Fixation and Preservation.

It is important to use methods of killing which will fix the tissues in a condition as nearly as possible resembling that of life, and leave the specimens in a suitable condition for subsequent microscopical examination, without

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1 A 1 per cent. solution of salt is sufficiently accurate for practical purposes. In the opinion of some authorities, tap-water is better for intestinal nematodes than salt solution or distilled water. For nematodes from the alimentary canal of fresh-water fishes, Prof. H. B. Ward recommends a 3 per cent. salt solution.
causing undue shrinkage. Since this cannot be done equally well for all the groups by any one method, the various groups must be dealt with separately.

N.B.—Corrosive sublimate (perchloride of mercury) forms the basis of many of the methods in general use. It should be remembered that when this substance is used the specimens should not be handled, at any time during the process, with metal instruments. They may be moved about by means of a splinter of wood, a quill, or a glass implement.

Nematodes.

Owing to the peculiarly resistant cuticle, rapid penetration is not easily effected by cold solutions, and hot fixatives are essential if good results are to be obtained. Elaborate cytological fixatives possess no advantage for ordinary purposes. No method has yet been devised which gives better results than the simple one introduced by Looss—viz., immersion in hot 70 per cent. alcohol. The spirit should be heated until it is steaming, but not boiling. A temperature of 50°–60° C. has been recommended, but Dr Boulenger considers this is not quite sufficient, and prefers 70° C. When of fair size the worms should be picked up separately with forceps (without using pressure), or with a needle, and dropped into the previously heated spirit. The dish in which the spirit is contained should be of sufficient width at the bottom to enable the worms to straighten out, which they usually do almost at once. The fluid is allowed to cool, and the specimens then bottled in fresh 70 per cent. alcohol.

When the specimens are small and numerous, it is not necessary to remove them entirely from the food-remains and other débris with which they are frequently mixed. In such cases it is easier, after draining off as much as possible of the washing fluid, to pour the hot alcohol over the material. When cool, the whole may be poured into a tube or bottle, and allowed to settle. The used fluid may then be replaced by fresh.

In the case of small specimens, it is sometimes an advantage to use a 5 per cent. solution of pure glycerine in 70 per cent. alcohol, instead of the plain alcohol. The method of procedure is the same.

These methods almost entirely avoid shrinkage or collapse of the specimens, if they be alive immediately before fixation, and usually cause them to die in a straight and extended condition.

For storage the worms may usually be kept in good condition in pure 70 per cent. alcohol, but in hot climates or when some time is likely to elapse before the tubes or bottles are unpacked, it is preferable to add glycerine in the proportion of 5 per cent. to the 70 per cent. spirit. This prevents the drying-up of the material, and keeps it in good condition for examination, while, when small specimens are to be examined in glycerine, the alcohol and water may simply be allowed to evaporate slowly, leaving nearly pure glycerine.

1 The temperature originally recommended by Looss was 80°—90° C.
2 For travelling purposes, enamelled iron dishes, which can be heated over a spirit lamp, will be found useful.
Formalin should not, as a rule, be used for preserving nematodes if its use can be avoided. It is frequently the cause of serious damage to the specimens by bursting or collapse, either at once or when they are subsequently transferred to other fluids prior to examination. Sometimes, however, alcohol is not available, and in such cases a possible method is to kill the worms in hot water (about 70° C.), and then transfer to 3 per cent. formalin. As has been mentioned, heat is necessary for the best results, but, in the writer's opinion, if alcohol is available but heating impossible, it is better simply to place the worms in cold 70 per cent. alcohol than to use formalin.

In cases where it is desired to preserve the histological details of nematodes for minute study, Prof. H. B. Ward recommends killing the worms in a mixture of equal parts of (1) acetic acid; (2) absolute alcohol; and (3) saturated watery solution of corrosive sublimate with 0.25 per cent. osmic acid.

**Acanthocephala.**

These worms swell up if left long in salt solution or water. They may be cleaned rapidly in the washing solution with a small brush, and then subjected to gentle pressure between two slips of glass until the proboscis is protruded. They may then be killed by running in strong alcohol between the slips, an even pressure being maintained (Braun and Lühe, *Handbook of Practical Parasitology*). After killing, they should be stored in 70 per cent. to 90 per cent. alcohol.

Acanthocephala can also be treated by the hot alcohol method employed for nematodes, but it is important to fix them with the proboscis extruded if possible.

When Acanthocephala are to be stained for examination (this is not always necessary for identification), Van Cleave recommends fixation in saturated corrosive sublimate solution, with 1 per cent. acetic acid, for about 15 minutes. The worms are then washed in water, and brought through 35 and 50 per cent. alcohol to 70 per cent. alcohol, and stored in the last.

**Trematodes.**

The following simple procedure gives satisfactory results for general purposes. After the preliminary shaking-up in salt solution, the dirty liquid is poured off, and a small quantity of fresh salt solution introduced. The worms are again shaken vigorously, and an equal volume of saturated corrosive sublimate solution (with, preferably, a few drops of acetic acid) quickly added. The shaking is then continued for a few minutes. The vigorous shaking not only cleans the specimens, but prevents muscular contraction, and so causes

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1 In certain special cases, such as Mermithidae and many of the Filariidæ, formalin seems to give better results than alcohol. After very rapid cleaning, these worms may be killed in a 5 per cent. solution of formalin. They may either be kept in this, or transferred to 70 per cent. alcohol by very gradual stages through 30 and 50 per cent. alcohol. It is better not to wash them in water after formalin.
them to die in an expanded condition. The worms can be left in the sublimate mixture for a few days if desired, or may be transferred to water after a few minutes. In either case they must be washed in running water for 12–24 hours, or transferred to 70 per cent. alcohol in which enough iodine has been dissolved to give it the colour of sherry. This should be changed repeatedly until the colour of the iodine ceases to disappear. Finally the specimens should be stored in 70 per cent. alcohol.

The following alternative methods are recommended for trematodes by Braun and Lühe.

(1) Wash in normal saline, brush off mucus, etc., and stretch on a slide. Put a drop of the fixing fluid on a coverglass, and apply it with slight, but not excessive, pressure, using paper supports or small weights as required, according to the size of the worms. [For large, fleshy forms, pressure between two slides, by means of rubber bands or thread, may be employed.] Add more fluid at one edge of the coverglass, drawing it off with blotting-paper at the opposite edge.

The fixative recommended by these authors is Hofer's (saturated watery solution of picric acid, 50 parts; water, 48 parts; glacial acetic acid, 2 parts). When the worms are rigid, wash off the slide into a dish of the fluid, and allow this to act until the entire worm is opaque. Then wash in water for a few seconds, and bring up through 45–50 per cent. alcohol to 70 per cent. alcohol, and change this frequently to remove the yellow colour of the picric acid.

(2) Another fixative, which is especially useful for demonstrating yolk-glands, and may be used in the same way as the last, is Müller's fluid (potassium bichromate, 2·5 parts; distilled water, 100 parts; sodium sulphate, 1 part). The worms should be soaked in this until the yolk-cells have become brown, then washed in running water and dehydrated with increasing strengths of alcohol as before.

If other fixatives are not at hand, hot or cold alcohol may be used; or formalin (10 per cent. solution) may be substituted for the corrosive sublimate mixture in the first method, and the worms afterwards stored in 3 per cent. formalin.

Cestodes.

After washing the worms, the main difficulty is to keep them straight and extended, and to avoid tangling. This may be accomplished either by laying the specimens out, slightly stretched, on a plate of glass or porcelain, and pouring the fixative over them, or, better, by picking up each worm by the end remote from the scolex and allowing it to hang down, when its own weight will usually cause it to stretch sufficiently. It may then be dipped quickly several times into a jar of the fixing fluid. Small forms, whose weight is not sufficient to stretch them, may be drawn along the side of the vessel after each dipping, so as to exert a slight longitudinal pull on the strobila. Very long specimens may be wound spirally round a wide glass tube or bottle before fixation, and the whole immersed in the fixative. When the worms cease to show any sign
of contraction, they are left in a dish of the solution for a longer or shorter time, according to size.

Dr Meggitt is of the opinion that fixatives for cestodes should be used cold, as heat tends to make the specimens brittle. The fixative most strongly recommended by Dr Meggitt is Zenker’s fluid (corrosive sublimate, 5 grms.; glacial acetic acid, 5 c.c.; potassium bichromate, 2 grms.; distilled water, 100 c.c.). After dipping three or four times, as recommended above, the worms are placed in a dish of the fixative for 4 to 24 hours, after which they are washed in running water for 24 hours, or in iodised 70 per cent. alcohol, as described for trematodes.

Bouin’s fluid (saturated aqueous solution of picric acid, 75 parts; formol, 25 parts; acetic acid, 5 parts) may also be used, and is better than Zenker’s for cytological purposes, but does not give such straight and extended specimens. It has the advantage that the material may be kept in it as long as desired without deterioration. Before examination the specimens should be washed in as many changes as possible of 70 per cent. alcohol.

A simple fixative which, in the writer’s experience, gives fairly satisfactory results, and is easily made up as often as required, consists of roughly equal parts of a saturated watery solution of corrosive sublimate and 70 per cent. alcohol, to which, preferably, a few drops of glacial acetic acid should be added. (This is roughly Schaudinn’s fluid.) Specimens should remain in the fixing bath for ten minutes to half-an-hour, according to size, and are then washed in running water for several hours (e.g., overnight), or in iodised 70 per cent. alcohol. They are finally stored in 70–90 per cent. alcohol.

A modification of this method, useful for rapid treatment of large masses of material, is simply to pour over the washed specimens a mixture of 90 volumes of saturated aqueous sublimate and 10 volumes of glacial acetic acid. As a fixative for minute cytological purposes this is not to be recommended, but it is sufficiently good for the identification of the material.

Another method which has been recommended¹, where minute details are not required, is as follows. After being washed in tap-water until they are “completely relaxed and dead,” the worms are dropped into a mixture of equal parts of pure glycerine, 70 per cent. alcohol and distilled water. The mixture is changed as often as it becomes turbid. This method “tends to keep the worms soft, and such specimens, after the excess of glycerine has been washed out by distilled water, are found to stain very well with Ehrlich’s acid haematoxylin.”

In the absence of other reagents, hot or cold alcohol will preserve cestodes sufficiently well for identification. Formalin may be used in case of necessity, but is not satisfactory, as specimens fixed in it are often difficult to stain.

For immediate examination of the hooks or eggs, Dr Meggitt uses hot absolute alcohol as a fixative, clearing the specimens in xylol as soon as the alcohol has become cool.

L'Abbate Spallanzani.

1729—1799.

(Portrait-plate XVIII.)

BY WM BULLOCH, F.R.S.

Lazzaro Spallanzani, one of the great natural philosophers of the eighteenth century, was born in 1729 at Scandiano, and was educated in Reggio and in Bologna where he came under the influence of his cousin Laura Bassi who was professor of mathematics and physics there. He was intended for the law but at the age of 28, having taken holy orders, he became professor of Greek, logic and mathematics in the College of Reggio. It was here that he began to interest himself in biological questions and by correspondence he became associated with Charles Bonnet and Haller in Switzerland. In 1760 he accepted a chair in the University of Modena where he remained eight years. This was one of the most fertile periods of his life for in addition to dissertations on Greek inscriptions, on problems in mathematics and physics he produced his epoch-making works on spontaneous generation, on the regeneration of lost parts and on the circulation of the blood. By the end of the Modena period he was elected F.R.S. and was known as one of the foremost scientists of his time. In 1768 he was appointed by the Empress Maria Teresa to be the Conservator of the Museum of natural history in Pavia and he held this post till his death in 1799. In 1776 he published his great Opuscoli di fisica animale e vegetabile and in 1780 Dissertazioni di fisica animale e vegetabile, works which must compel the most captious critic to range the Italian abbate among the greatest experimental philosophers of all time. In these works he dealt exhaustively with the problems of spontaneous generation, and the origin of the animalcules of infusions, the nature and origin of spermatozoa, the effect of stagnant air on animals and vegetables, the death and resurrection of animals, the nature of moulds, digestion and generation. In addition to his research and teaching
work in Pavia he undertook journeys for the purpose of collecting objects, biological and mineralogical, to enrich his museum, and in general to elucidate Nature. Thus he visited the Gulf of Genoa, the quarries of Carrara, the Apennine and Euganean hills, the island of Elba and Lake Comacchio. In 1785 he undertook a long and hazardous journey to the east, visiting the Aegean islands and Constantinople, returning by land through Bulgaria, Roumelia, Transylvania, Hungary and Austria. The results of his travels in the volcanic districts of Italy and Sicily led to the publication of his famous *Viaggi alle due Sicilie* (1792–1797) in six volumes, a work which according to the most modern writers laid the foundations of the science of volcanology. He ascended Vesuvius, Etna and Stromboli and in 1788 descended into the active crater of Vulcano, in the Lipari, at a temperature which burned his feet and set fire to his staff.

In addition to these researches the Abbate was an early student of the pneumatic chemistry of Black, Priestley and Lavoisier and carried out a vast number of experiments on the composition of the air under the most diverse conditions and at his death left the manuscript records of nearly 12,000 experiments dealing with the respiration of animals and plants of every kind. This work, published posthumously, remains a classic and established the fundamental doctrine of the respiration of the tissues. His amazing industry and resource are not to be measured by the works referred to for in addition he left completed manuscripts on regenerations, the natural history of the sea, journeys to Switzerland, Turkey and Hungary and a work on the natural history of bats. He wrote papers on corals, sponges, nocturnal lights at sea, waterspouts and on the electrical organ of the torpedo, the divining rod, swallows, owls and eels.

All who knew him relate that he was instantly able to grasp a problem in all its bearings. Until, however, he had put it to experimental tests in an infinite series of variations he was most guarded in the expression of his opinions. His early training in logic, his incomparable experimental skill and the range of his mental vision guided him almost invariably to an unerring judgment. There is no research which he carried out a hundred and more years ago that would not be regarded as meriting distinction if it were done to-day.

Spallanzani's chief claim for mention in Parasitology must rest on his admirable experimental and microscopic researches on spontaneous generation. These were first published in 1765 under the title *Saggio di osservazioni microscopiche concernenti il sistema della generazione di Signori di Needham e Buffon*. In this work he adversely criticized the view of Needham and Buffon that living animalcules are developed in heated organic infusions from the so-called "organical parts" by a vegetative force. Spallanzani gave a minute account of the forms and movements of the organisms of infusions which, he held, showed all the attributes of "animality." By studying infusions, boiled and unboiled, he saw multitudes of "infusoria" appear even when the flasks were plugged with wool, paper, cork, or wood. Even after the vessels were her-
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metrically sealed, the animalcules still made their presence known by a cloudi-
ness which suffused the previously clear infusion. Animalcules still appeared
in organic extracts deprived of air. In other experiments he first heated his
flasks and having introduced his clear infusions and hermetically sealed the
vessels heat was again applied for an hour. At last no infusoria could be found
in the fluid even examined with the magnifier. If perchance a crack occurred
in the vessel animalcules appeared sooner or later. He thus concluded that
infusions sufficiently heated and kept from contact with unheated air ex-
hibited no evidence of spontaneous generation. These experiments were con-
tinued on a large scale the results being published in the *Opuscoli* (1776). He
confirmed the observation of de Saussure (1769) that most of the animalcules
of infusions reproduce by a process of division and by ingenious experiments
he followed *Volvox globator* through thirteen generations. "It must be noted," he
says, "that there are appointed times for these minute animalcules to
originate and be destroyed as with other creatures that multiply to excess.
Nature has with the wisest provision destined that when one species begins
to be excessively numerous it is reduced either from the greater number of
the animals perishing from disease or a violent death by the voracity of other
animalcules for it is a perpetual and inviolable law with numberless animals
that one lives upon another and mutual destruction preserves each species."

Our portrait of Spallanzani is a copy from the original which was drawn
and engraved by C. Rampoldi and appears in the edition of his collected
works. It has been redrawn and lithographed several times and appears
with an added coat and stock in Pavesi's book. There is also a picture of
Spallanzani in clerical dress in Salimbeni's book. His bust in terra cotta,
by Barberini, graces one of the rooms in the Physiological Institute in
Modena.

**BIOGRAPHICAL NOTICES OF SPALLANZANI.**


Pavesi, P. L'Abbate Spallanzani a Pavia. Milano, 1901.


Senebier, J. Notice historique sur la vie et les écrits de L. Spallanzani, in *Mémoires sur
la respiration par Lazare Spallanzani.* Genève, An. xi (1803).


Tourdes, J. Notices sur la vie littéraire de Spallanzani in *Tourdes' French translation of
Dei fenomeni della Circolazione.* Par., An. viii also English transl. Lond., 1801.

**SPALLANZANI'S PRINCIPAL WORKS.**

1. *Saggio di osservazioni microscopiche concernenti il sistema della generazione di Signori di
Needham e Buffon* 1765. Reprinted in *Nuova raccolta d'opuscoli etc.* Venezia, 1767,
xx, 208–323.


4. *Contemplazione della natura del Sig. Carlo Bonnet tradotta in Italiano e corredata di note e di curiose osservazioni.* Modena, 1769.

5. *De' fenomeni della circolazione osservata nel giro universale de' vasi etc.* Modena, 1773. 343 pp.


LAZZARO SPALLANZANI

1729—1799

Separate copies may be obtained from the University Press, Cambridge
T. R. Lewis.
1841—1886.

(Portrait-plate XIX.)

BY CLIFFORD DOBELL, F.R.S.

Timothy Richards Lewis was, as his name reveals, a Welshman. He was son of William Lewis, and was born on October 31, 1841, at Llanboidy, Carmarthenshire. He received his early education at a private school kept by a clergyman in Narberth, a small town in Pembrokeshire where his family resided. On leaving school, at the age of 15, he was apprenticed to a local apothecary; but after spending a few years in this employment he came to London to study medicine—going first to a dispensary in Streatham, and then, when 19 years of age, to the German Hospital at Dalston. He also attended lectures at University College from 1863 to 1866, but finally qualified at Aberdeen, where he took the degrees of M.B. and C.M., “with honourable distinction,” in 1867. Already as a student Lewis was distinguished. His contemporaries spoke very highly of his skill in the laboratory, and that he was no less accomplished in the ward is shown by his having won the Fellowes Medal for clinical medicine at University College in 1866.

In 1868 Lewis entered the Army Medical Service, receiving his commission as “Assistant-Surgeon in Her Majesty’s Army.” After a brief but remarkably successful period at Netley—he was first in both the examinations for entering and leaving the School—he was sent out to India with D. D. Cunningham to investigate cholera: but previous to this these two friends—whose names are now inseparably joined in the history of tropical medicine—were sent to the Continent together, to familiarize themselves with the mycological and bacteriological work of De Bary, Hallier, Pettenkofer, and others. They reached Calcutta in January, 1869, and were shortly afterwards attached as “Special Assistants” to the Sanitary Commissioner with the Government of India. Together they studied cholera and other Indian diseases for about a dozen years, publishing during this time a number of important reports on their researches. Lewis was promoted to the rank of Surgeon in 1873, and Surgeon-Major in 1880. He married in 1879, and returned to England for good in 1883.

1 In all the published biographies of Lewis it is stated that he was born at Crinow, Narberth. Col. W. Johnstone, however, in his Roll of Graduates of the University of Aberdeen, 1860–1900 (Aberdeen, 1906), gives Llanboidy as Lewis’s birthplace; and that this is correct is attested by Lewis himself, for there is, at Aberdeen, a schedule filled up in his own hand in 1866, when he entered the University, and in this he gives his birthplace as above. For this information I am indebted to Dr W. Bulloch, F.R.S., and also to Mr P. J. Anderson, Librarian in the University, who kindly examined this document at his request. I offer my best thanks to both for enabling me to make this important correction.

Parasitology xiv
Notes on Portrait-plates

on his appointment to the post of Assistant-Professor of Pathology at the Army Medical School, Netley. Lewis held this position until his unexpected and untimely death on May 7, 1886. He died from pneumonia at his house at Woolston, Southampton, after only a few days’ illness; and from entries in his note-books it was inferred that his death was caused by his accidentally inoculating himself with micro-organisms in the performance of some experiments.

Most of Lewis’s work was published in the form of “Appendixes” to the Annual Reports of the Sanitary Commissioner with the Government of India (Calcutta, 1869–1881). Some of these papers were subsequently issued separately (sometimes with additions and corrections), and after his death his chief works were carefully reprinted in a memorial volume by his friends (Physiological and Pathological Researches, arranged and edited by W. Aitkin, G. E. Dobson, and A. E. Brown, 4°, London, 1888). Some of Lewis’s reports were also printed, more or less completely, in contemporary volumes of the Quarterly Journal of Microscopical Science. The manner of their publication has doubtless been partly responsible for the unmerited neglect which these classical memoirs have suffered in recent years. Their contents—in so far as they are of parasitological interest—may now be briefly indicated.

The first report describes “The microscopic objects found in cholera evacuations, etc.” (1870), and contains the first authentic account of amoebae from the human intestine. In 1872 and 1874 appeared two important supplementary memoirs (jointly with D. D. Cunningham) recording “Microscopical and physiological researches into the nature of the agent or agents producing cholera.” In 1872 we also find—by Lewis alone—researches on “The bladder-worms found in beef and pork,” and the famous paper “On a haematozoan inhabiting human blood: its relation to chyluria,” containing the first account of “Filaria sanguinis hominis”—Lewis’s name for the worms now known as *F. bancrofti* (i.e., microfilariae). Hardly less important is the next contribution, on “The pathological significance of nematode haematozoa” (1874), containing a description of filariais in dogs and discussing the relation of the filarial parasites to chyluria and elephantiasis in man. With Cunningham he then (1875) exhaustively investigated “The soil in relation to disease,” and inquired into “The fungus-disease of India” (i.e., Madura Foot, etc.). In the following year (1876) the same workers studied and described “The Oriental Sore as observed in India”—though without discovering the specific parasites (*Leishmania*); and in the next year treated of “Leprosy in India” (1877). This was followed by the celebrated memoir—by Lewis alone—on “The microscopic organisms found in the blood of man and animals, and their relation to disease” (1878), which contains important contributions to bacteriology, protozoology, and helminthology—especially noteworthy being an

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1 The dates here given are those of actual publication. The Reports are for the preceding year in every case. For example, the paper here cited as 1870 will be found in the Sanitary Commissioner’s Report for 1869: and so on. The difference in dates has led to some confusion.
admirable account of the spirochaetes of relapsing fever, and the first description of the rat-trypanosome which now bears his name (Trypanosoma lewisi S. Kent). Further contributions to the study of cholera were made in the Reports for 1878 and 1882; and finally, a supplementary note on T. lewisi was published in the Quarterly Journal of Microscopical Science in 1884.

The foregoing rough list of works gives but a poor idea of Lewis's manifold contributions to Parasitology. To be appreciated properly the originals must be read, and read in the light of our present knowledge—always remembering, of course, the ignorance which prevailed in his day. They are written in a simple—almost homely—style, but their candour, good sense, and obvious veracity lend them a singular charm.

Lewis's place in the history of tropical medicine and medical parasitology is not difficult to define. He was, like Manson, a pioneer. He was born three years earlier, but did not take his medical degree until two years after Manson took his at the same university. Manson was already at work in Formosa when Lewis sailed for India; but it was Lewis, nevertheless, who made the first fundamental discoveries in connexion with filariasis. Moreover, Manson continued to live and labour for thirty-six years after Lewis's premature death—to the no small benefit of Science; so that it is now difficult to realize that they once belonged to the same generation. But if Manson is the "Father of Tropical Medicine" then assuredly Lewis is at least its Godfather: for it will not be forgotten that, in addition to his personal contributions to science, he founded, both in India and at Netley, a school and a tradition whose fruits are now visible to all the world in the researches and discoveries of the officers of the Indian Medical Service and the Royal Army Medical Corps.

Shortly before his death Lewis was recommended by the Council of the Royal Society for election to the Fellowship—an honour which he did not live to see consummated. (He died before the election took place, and Adam Sedgwick was elected in his stead.) In his lifetime he received no other signal distinction from science or state. The Lancet, at the time of his death, remarks: "It is not, we think, very creditable to the Government of this country or that of India that Lewis received no State recognition of his important services." Those who value this kind of recognition will now agree that he was treated hardly; but those who do not will feel small sorrow that his name has become great without the embellishment of such ephemeral trappings.

Lewis was an honest man and an honest worker. Honesty, common sense, and sound judgement, are the outstanding characters of his work. After reading his scientific papers we hardly need to be told that in his private life he was the same. His colleagues all spoke of him in similar terms. "Lewis was a man of the most amiable and estimable character. Kindly in disposition, true as steel, brave, honest and faithful in all relations of life. He was an indefatigable worker, and as conscientious and careful in the observation of facts, as he was cautious and clear in their interpretation." Could any man desire a nobler epitaph? This was the saying of a friend at the time of his
death, and it might therefore be regarded as a pardonable exaggeration. But probably it was not, for we could say the same to-day from a study of his printed work alone; and it is safe to prophesy that, in years to come, others will see that it is true, and will say the same. And this surely is, for the man of science no less than for the poet, that "monumentum aere perennius, regalique situ pyramidum altius" which all desire, many claim, but only the very few ever win.

Lewis's chief works have already been mentioned. Biographical notices will be found in the memorial volume of his researches (cited above)—which also contains the portrait here reproduced—and in the following journals: Indian Medical Gazette (1886), xxi. 179, 249; Lancet (1886), i. 993; British Medical Journal (1886), i. 1242; Nature (1886), xxxiv. 76. No obituary notice was published in the Proceedings of the Royal Society, and by a strange irony of fate Lewis has not yet been found worthy of a place in the Dictionary of National Biography.
THE LIBRARY
OF THE
UNIVERSITY OF ILLINOIS
Thomas Spencer Cobbold
1828—1886.

(Portrait-plate XX.)

BY GEORGE H. F. NUTTALL, F.R.S.

Thomas Spencer Cobbold was born at Ipswich, Suffolk, on 28 May, 1828, and died 20 March, 1886, at Maida Hill, London; he lies buried in Paddington Cemetery, Willesden, London, N.W.

After being apprenticed to Cross, the Surgeon of Norwich, he went to Edinburgh, where he worked under John Goodsir and Edward Forbes and took his M.D. in 1851, being subsequently appointed Curator of the Anatomical Museum, where he worked on comparative anatomy. In 1858 he removed to London and became Lecturer in Botany, Zoology and Comparative Anatomy at Middlesex Hospital Medical School, where he for a time gave instruction on parasitic diseases, devoting himself thereafter chiefly to the study of helminths. In 1865 he began to practise medicine and continued to do so for a period of about ten years whilst keeping his hold on science. He lectured on helminthology at the Royal Veterinary College until within some months of his death. In 1868 he became Swiney Professor of Geology at the British Museum and held the post for five years. He was a hard worker, a good writer and teacher, sociable and musically gifted, having a remarkable voice. He was elected Fellow of the Royal Society in 1864 and honorary member of various learned societies abroad, in recognition of his excellent original work.

Cobbold published about five hundred memoirs and papers on a variety of subjects. His work on helminthology, both systematic and experimental, was important and survives as a monument to his originality and industry. From the classified list of 108 papers given by Brumpt, it is seen that 41 relate to helminthology in general, 29 to Nematodes, 22 to Trematodes and 16 to Cestodes. The bibliography of Cobbold’s publications given by Stiles and Hassall (vide infra), starts with a paper published in 1855, wherein Cobbold describes Fasciola gigantica. His well-known book, Entozoa, an Introduction to the Study of Helminthology, with Reference more particularly to the Internal Parasites of Man (xxvi + 480 pp., 82 figs., 21 pls., 8°), appeared in 1864. His Entozoa: being a Supplement, etc. (to the foregoing), appeared as a quarto in 1869, and his treatise, Parasites, was published in 1879. This work contains a most noteworthy introduction and it sums up Cobbold’s knowledge of parasitology at that date. His last publication appeared in 1886 (Trans. Linnean Soc.).

The appreciations and biographical notices of Cobbold which appeared during his life and after his death bear testimony to the high esteem in which he was held by his contemporaries both for his personality and as a man of science.

Our portrait is reproduced from a photograph, taken in 1884, for which his son and daughters have expressed a preference.

In conclusion, I have much pleasure in acknowledging the kind help I have received from the late Prof. Cobbold’s son and daughters, namely Dr C. Spencer Cobbold of Bath and the Misses Cobbold of Worthing, who have generously presented to our Parasitological Library in Cambridge three bound volumes of Cobbold’s printed papers and MSS (gift of Dr Cobbold), original drawings, copies of most of the biographies above cited, and prints of the three portraits to which reference has been made under sources of information Nos. 1–9 (gift of the Misses Cobbold). The bound volumes were Cobbold’s private copies and they contain notes by him. These mementoes of the great helminthologist have found a fitting place in the library of the first Institute of Parasitology to be established in England, and nowhere could they be more appreciated.
THOMAS SPENCER COBBOLD
1828—1886
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PARASITOLOGY

EDITED BY

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