THE GENUS *PRISTIRHYNCHOMYIA*,
BRUNETTI, 1910

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The genus *Pristirhynchomyia* was created by Brunetti to include a single species, *lineata* (Brunetti, 1910), which he separated from the genus *Philaematomyia*, Austen, on account of certain differences in the proboscis. The following is a portion of his original description of the genus:

'With the exception of an important modification of the proboscis, identical with *Philaematomyia*, Aust., the general characters, the venation and chaetotaxy agreeing exactly.

'The two parts of the proboscis, however, are structurally reversed, the wide basal part being fleshy and flexible, the second part (of about equal length) being sub-cylindrical, black and distinctly chitinized, possibly retractile to the extent of its withdrawal partly or wholly within the fleshy basal portion. At the end of the chitinous portion is a soft fleshy tip, the terminal orifice being of the shape of a triangle with a rounded base (the edges being thickened somewhat by a rim bearing the teeth). At the apex of the triangle is a single black tooth, whilst arranged around the orifice above are three pairs of similar black teeth.

'Under high microscopic power the apparent "rim" of the orifice is seen to be the base of each tooth extended considerably on each side, so that the "rim" is not continuous.

'The new genus is intermediate between *Philaematomyia* and *Musca*, but the presence of the teeth suggests that it can hardly be other than a "biting fly."
We have examined several preparations of the mouth parts of this fly, including one made from a co-type, identified by Brunetti, for which we are indebted to Dr. Annandale, Superintendent of the Indian Museum, and have found that the description quoted above, and the figures which accompany it, are inaccurate and misleading, and that such deviations as there are from the type of Philaematomyia are differences in degree and not in kind. The proboscis is not 'structurally reversed' but consists of a proximal portion, the rostrum, containing the fulcrum, and a distal portion, the haustellum, which bears the oral lobes; that is to say, the proboscis is of the ordinary muscid type, and is as retractile as that of Musca domestica, Lin. There are five pairs of teeth (one pair of which is rudimentary), and not three as stated by Brunetti. The 'rim' is not formed by the bases of the teeth, but corresponds to the discal sclerite of non-blood-sucking muscids. It is obvious, from the terms used in the description, that the writer of it is not familiar with the structure of the proboscis of a typical Musca.

We infer from Brunetti's paper that the description was written after examination of pinned specimens and proboscides mounted in Canada balsam without special manipulation. Under such conditions it is extremely difficult, in fact impossible, to make out the finer details of the parts. To study the chitinous structures satisfactorily it is essential to clear the preparations in potash, and to mount in balsam in varying positions. In the case of this fly, one can, with a little care, dissect off the chitinous ring to which the teeth are attached, and mount it flat.

The proboscis of this fly closely resembles that of Philaematomyia insignis, Austen, which will shortly be described in detail by one of us (F.W.C.). All the structures found in the latter fly are represented in lineata, and it will only be necessary here to indicate the points of difference between the two. The proximal portion, or rostrum, is relatively somewhat larger than in insignis; the distal part, or haustellum is, contrary to what one would infer from the original description, considerably less densely chitinized, and therefore less rigid. The theca is shallower, and the thickening of its lateral margins not nearly so well marked. The middle portion of the membrane which stretches between the two
lateral borders of the theca is not chitinized into a definite 'labial gutter' such as one finds in Philaematomyia insignis and Stomoxys; in place of this there are two rod-like thickenings, between which the membrane is only slightly chitinized, the whole forming a trough to accommodate the labrum-epipharynx and hypopharynx.

The labellar rods, which are the lateral arms of the discal sclerite, are articulated, as in insignis, on the ends of the labial rods. The main portion of each is conical, the thickest part lying in front of the end of the labial rod, the sharp internal angles projecting inwards towards one another at the level of the tip of the labrum. The upper ends of the rods are pointed, and diverge widely from one another. The lower and outer angle of each of these wedge-shaped rods is produced downwards and inwards, and directly downwards at the tip, where it projects beyond the axial apophysis. This downward prolongation gives attachment to the inner ends of the teeth.

The axial apophysis is V-shaped, its pointed apex forming the apex of a triangle, from the sides of which the teeth appear to arise. It is situated, however, posterior to the downward prolongation of the labellar rods, and is not directly connected with the teeth. The proximal ends of its arms are attached to the labellar rods on their posterior surface.

The teeth resemble those of insignis, but are considerably more slender and pointed. They arise from the membrane between the pseudo-tracheae by expanded bases, the inner ends of which are elongated and attached to the downward prolongation of the labellar rods. The second, third and fourth teeth on each side are approximately equal in size. The fifth is smaller, but similar, while the first pair, which lie on either side of the tip of the axial apophysis, are about a quarter the size of the others, and project very little from the surface of the membrane. The 'serrated blades' of Philaematomyia insignis are represented by four pairs of spine-bearing chitinous strands. These arise from the membrane between the base of the teeth, a little away from the distal portion of the labellar rods. Each runs outwards parallel to the teeth, and bifurcates in U-shaped manner at the level of the most distal portion of the attachment of the teeth to the membrane. At the point of bifurcation the lateral arms split up into three or four filaments,
which lie to a certain extent super-imposed on one another, and are somewhat difficult to see.

The *Pseudo-tracheal membrane* presents no peculiarities, being identical with that of *insignis*, except that the channels are a little wider. The fourth to the seventh channels, counting from the front, terminate between the lateral arms of the spine-bearing strands, the filaments arising from the strand lying parallel to the horseshoe-shaped chitinous rings of the pseudo-tracheal channels.

From the foregoing it will be seen that this fly corresponds in all essential particulars to Austen's description of the genus *Philaematomyia*, and we are of opinion that it should be placed in that genus, since we think that it is unjustifiable to create new genera on minor details of structure which cannot be made out without dissection.

One of us (W.S.P.) has bred *Philaematomyia lineata* from the egg. Its breeding habits are identical with those of *insignis*, shortly to be described by us. From thirty to forty eggs are laid in cow dung, all in one place. The eggs are slightly smaller than those of *insignis*, but are otherwise similar. The larvae behave in the same way when about to pupate, and have the same lemon yellow colour. The puparium is similar but smaller.

Brunetti states that Dr. Annandale has frequently seen this fly distended with blood, while feeding on cattle. We have not ourselves observed this, although this fly is fairly common here during the colder months. The fact that it has been taken distended with blood is, of course, no proof that it can obtain blood independently; it may, like *Musca pattoni*, Austen, and *Musca convexifrons*, Thomson, suck up the blood which exudes from the wounds made by other biting flies.

The male fly, which Brunetti does not appear to have seen, is much like the female, but has a distinctly lighter abdomen. It will be described fully on another occasion.

**NOTE**

Since writing the above, one of us (W.S.P.) has found a new species of *Philaematomyia*, the habits of which are identical with those of *insignis*. It is distinguished by its large size (0.7-0.8 cm).
long) and its coloration. There are the usual four admedian dark stripes on the thorax, and a narrow median dark line on the dorsal surface of the abdomen; the lateral halves of each segment are a light olive green colour. The proboscis resembles that of *insignis*, but has five large teeth and two rudimentary ones on each side. We propose naming this fly *Philaematomyia gurnei*, sp. nov., after Mrs. Patton, who was one of the first to see it. A detailed description will be published later.

REFERENCES


CRAGG, F. W. (1911). The Structure of the Proboscis of *Philaematomyia insignis*, Austen. (Will be published in the 'Scientific Memoirs Series.')
EXPLANATION OF PLATE XXV

Fig. I.—The proboscis, seen in profile, drawn from a potash preparation.

f. Fulcrum.
M. Membraneous wall of the rostrum.
sl.d. Salivary duct (enclosed within the membrane).
l.ap. Labral apodeme.
\( p \). Palp.
l.ep. Labrum-epipharynx.
hy. Hypopharynx.
lb.r. Labial rod.
l.r. Labellar rod.
fu. Furca.

Fig. II.—The teeth and connected structures, seen from the front, when extended. Drawn from a potash preparation.

pt. Pseudo-tracheal channel.
s. Spine-bearing chitinous strand, representing the serrated blades of *Philaeatomyia insignis*.
l.r. Labellar rod.
lb.r. Labial rod.
l.ep. Labrum-epipharynx.
1. Teeth.
THE LIFE HISTORY OF PHILAE-MATOMYIA INSIGNIS, Austen

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It is not a little remarkable that, although only two years have elapsed since Mr. Austen described *Philaeatomyia insignis* as a new genus and new species, the fly has already been found to be widely distributed throughout the East. It has been recorded from most parts of India, from Ceylon, from Cyprus and also from Central Africa and Socotra. Moreover, a new species, *lineata*, has been described by Brunetti (placed, erroneously, as we believe, in a new genus *Pristirhynchomyia*), and we ourselves will shortly describe another species from Madras, under the name of *Philaeatomyia gurnei*. It is extremely probable that the genus is a large and widely distributed one, which has escaped the attention of entomologists on account of its close resemblance to non-blood-sucking muscids. One of us (F.W.C.) will shortly describe the biting apparatus of *insignis*, and it will then be shown that, although the teeth are quite formidable weapons, they are so concealed by the pseudo-tracheal membrane that even in potash preparations a certain amount of dissection is required to expose them. In pinned specimens it is only exceptionally that one can see them.

These flies are of very considerable interest, on account of the well-defined position they occupy in the muscid group. Structurally, they are intermediate between *Stomoxys* and *Musca*, while as regards their habits, they are intermediate between the non-blood-sucking *Musca (M. domestica, Lin., and M. nebulo, Fabr.)* and
such flies as *Musca pattoni*, Austen, and *Musca convexifrons*. Thomson, which have no piercing apparatus, and yet feed entirely on blood, sucking up that which exudes from the bites of *Tabanus, Chrysops, Haematopota* and *Philanematomyia*.

The breeding habits of this fly resemble in general those of non-blood-sucking muscids. The eggs, fifty to sixty in number, are laid in cow dung, the fly appearing to prefer small patches, freshly dropped, rather than larger collections of dried dung. On alighting, the female crawls over the surface until its finds a small crack or crevice; the ovipositor, which is similar to that of *Musca domestica*, is now thrust into the dung, the abdomen being depressed, and all the eggs are deposited in a heap, from $\frac{1}{8}$ to $\frac{1}{4}$ of an inch below the surface. The process takes from six to ten minutes.

When there are a large number of flies about, one often sees half a dozen or more all depositing their eggs in the same spot, their ovipositors being close to one another, while their heads are turned outwards. When the flies have finished laying their eggs an irregular heap of several hundreds will be found just beneath the surface. The eggs are laid from early morning until noon, rarely later.

![Fig. 1—Egg.](image)

The egg (Fig. 1) measures from 2 to 2.2 mm. long by 0.4 mm. broad. It is of the usual muscid shape, an elongated ovoid, gently convex along one margin and concave on the other; one end is slightly more pointed than the other, but it has no spine. It is a yellowish white colour and densely opaque. On the concave margin there is a shallow groove, difficult to distinguish at the pointed end, but widening out towards the broader end of the egg.

The larvae hatch out, through the groove, in from eight to nine hours, that is, on the evening of the day on which the eggs were laid. When mature, they measure about 1.25 cm., their greatest
breadth being about one-seventh the length. They are cylindrical, pointed at the oral end, and are composed of twelve segments, of which the posterior seven are of approximately equal size. They are bright lemon yellow in colour, and on this account are readily distinguished from other muscid larvae. All the larvae remain together up to the evening of the second day, and then migrate, still in a company, from the dung, passing out from its under surface, and burrow in the ground, under leaves, etc., to pupate. This habit of migrating together is somewhat remarkable, and has not been observed in any other of the Muscids we have studied here.

The puparium resembles that of Musca. It measures, on the average, 0.5 cm. long by 0.18 broad, though there is a considerable amount of variation in this respect. It is of a light mahogany colour, and eleven segments can be distinguished. At the posterior end there are two conspicuous kidney-shaped spiracles, raised somewhat above the surface; these have characteristic markings, as indicated in the figure, which are conspicuous on account of their orange colour, and which are distinctive of the species.
Breeding takes place in Madras throughout the year. The total time occupied is from six to seven days, varying a little according to the temperature. The large size of the egg and the short time in which it hatches suggest that the eggs undergo some development before they are laid.

About eight hours after hatching the fly is ready to feed. Both sexes suck blood, which forms their main if not their only food, though we have seen them rubbing their proboscides on the surface of cow dung when about to lay their eggs, in a manner which strongly suggests that they suck up the juices from the surface, possibly using their teeth to penetrate the crust.

![Fig. 4.—Posterior Spiracles.](image)

They feed almost exclusively on cattle and, as far as we have observed, they only occasionally bite human beings. They do not ordinarily exhibit any preference for any particular part of the skin of the host, though we have found them especially attached to the abdomen of calves which have been shaved for vaccination. When feeding, the fly lies closely pressed against the skin of the host, its body being parallel with the surface. They remain until fully gorged, and are not easily disturbed; they can, in fact, be easily picked off with the fingers. Like most blood-suckers, they pass out a clear watery fluid, and later apparently unaltered blood, from the anus as the abdomen distends.

From the somewhat lethargic habit of the fly, and from the fact that it breeds rapidly and (in Madras) throughout the year, one would expect to find that it has natural enemies, which keep down its numbers. The chief of these is a small Hymenopteron (not yet identified). The habit of this wasp is to settle on the dung and to
watch for a fly laying its eggs. Having marked a victim, it crawls up to within an inch or two of it and then makes a short rapid flight, settles on the fly, and after stinging it through the head carries it away, holding it by means of its sting and its hind legs. We have seen as many as five of these wasps on the same patch of dung. The fly, busily engaged in laying its eggs, usually falls a ready victim to its extremely active enemy; should it escape the first assault, and fly away, the wasp will follow it and either catch it as it settles on a blade of grass, or later when it returns to the dung. The wasp also frequently attacks flies while feeding on cattle. Unfortunately, the wasp is so small and flies so rapidly that we have been unable to follow it to its nest.

Several small species of spider also prey on Philaematomyia, catching them while laying their eggs. There is also a small Asilid which has the same habit, and can often be seen to swoop down on a fly and carry it off, grasped in its forelegs, to a neighbouring twig, where it sucks out its juices.

Lastly, we have for several years observed a small Tachinid, which rests on a blade of grass close to a piece of dung in which Philaematomyia is laying its eggs. Its behaviour is very remarkable and suggestive. It sits with its head directed towards the fly, and every now and then darts towards it, in a very direct and business-like manner, and at once returns to its perch. It certainly does not catch, or attempt to catch, the fly. We have dissected specimens of this Tachinid caught in the act, and have found that its ovaries contain well-developed larvae, enclosed in thin transparent membranes, through which one can see the larva making active butting movements, as if in the endeavour to free itself. The fly, like most of the members of that family, deposits larvae and not eggs, and we suspect that the presence of this Tachinid is associated with that of the Hymenopteron mentioned above, and that the Tachinid deposits its larva on the Philaematomyia with the intention that it shall be carried to the nest of the wasp, where it would find ample food. We hope in time to settle this interesting biological problem.

The simplest way of breeding Philaematomyia insignis is to watch for a number of flies laying their eggs, and then to scoop up the whole patch of dung and place it in a long tin tray about
two to four inches deep. The dung should be placed at one end of the tray and a quantity of sand at the other. The larvae, when about to pupate, will migrate to the sand, from which the pupae can be removed to a closed vessel.

REFERENCES

A NEW SPECIES OF PHILAEMATOMYIA, WITH SOME REMARKS ON THE GENUS.

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The first known species of this genus, insignis, was described by Mr. Austen in 1909, from several localities in India. Since that time it has been found to be distributed throughout the Oriental and Ethiopian regions, being recorded from Ceylon, Borneo, Sokotra, Senegal and the Congo Free State. It appears to be a common fly in the tropics, and in Madras at any rate it is the commonest biting fly found on cattle. There can be little doubt that it had for long been taken for a species of Musca, and indeed such a mistake might well be excused, for there is, as a rule, nothing to indicate that it is a biting fly if only pinned specimens are examined, and if the observer is unacquainted with it in the field.

In the next year Brunetti described a second species, lineata, erecting for it, however, a new genus, Pristirhynchomyia. We have given in a recent paper our reasons for retaining this fly in Mr. Austen’s genus, and at the same time we recorded the discovery of a third species,

* Professor Bezzi informs us that he has come to the conclusion that Philaematomyia insignis is identical with Musca crassirostris, Stein, described from Egypt in 1903. If this is the case the name crassirostris will have precedence. We have not had the opportunity of examining this species, and therefore express no opinion.
for which we proposed the name qurnei, but were compelled to defer a detailed description for want of material.

In this paper we propose to give this description, more material having come to hand after a long delay, and at the same time to describe the male of lineata, which was not included in Brunetti’s account. We take the opportunity to offer some remarks on the genus, considering that the time has come when it should be more closely defined, and to emphasise the points by which it is distinguished from Musca. This is all the more necessary in that it is usually not possible to recognise a fly of this genus by the ordinary methods in vogue among dipterologists.

Philæmatomyia lineata, Brunetti, 1910. (Plate VI.)

**Male.**—Length of eight specimens examined 3·8 to 4·5 mm.; width of head 1·8 to 2 mm.; length of wing 3·8 to 4·5 mm.

**Head.**—Width of front at centre one-sixteenth total width of head; parafrontals and parafacials grey; frontal stripe black with a silvery pubescence. Seven moderately strong fronto-orbital bristles. Antennae, basal joints black, third joint smoke grey. Arista with seven to nine bristles on its upper surface, and four to five on its lower. Palpi dark grey, like those of Musca.

**Proboscis** typically Musca-like in external appearance, and completely retractile; the haustellum is, however, proportionately longer and more distinctly chitinised than that of Musca, and appears of a distinctly darker tint in dried specimens. The labellae are not so conspicuous, and bear fewer hairs. The mouth parts are as described in our last paper, there being no difference between the sexes in this respect.

**Thorax.**—Ground colour varies from silvery grey to dark grey, and in parts yellowish grey. Thoracic chaetotaxy as in *insignis*, except that there are usually 5 to 6 dorso-central bristles.

**Abdomen.**—Ground colour ochraceous; first segment dark brown, with a distinct central longitudinal stripe, sides dull yellow. Second segment, central portion dark brown forming a distinct longitudinal stripe, sides shimmering yellow, with indistinct lateral brown bands. Third segment with a central brown stripe, and brown patches at the sides, remainder yellowish. Fourth segment yellow at the apex, and brown at the sides.

Wing venation as in the type species, *insignis*.

**Life History.**—Philæmatomyia lineata lays its eggs in the same
way as *insignis*, but unlike that species it will readily oviposit in heaps of dung, apparently selecting these more frequently than isolated patches. The egg is an elongated ovoid of the usual Muscid type, and measures from 1.2 to 1.5 mm. in length. It is of a dirty white colour, and closely resembles that of *insignis*. The larvae hatch in about twelve hours, and become mature in about three days. They are of a bright lemon yellow colour, and measure about 8 mm. when full grown. The puparium is of a darker mahogany colour than that of *insignis*, and is from 4 to 4.5 mm. in length. The larvae always leave the dung when about to pupate.

This species occurs not uncommonly in the grounds of this Institute during the cold weather, but is never seen in the same large numbers as *insignis*. We have also found it in the hot weather in Kodaikanal, in the Pulney Hills, South India, at an elevation of 6,000 feet. We have studied its habits carefully whenever opportunity occurred, and can now say with certainty that it is not able to make a wound for itself, but that, like certain species of *Musca* dealt with in another paper, it relies on other flies to puncture the skin for it, as one might expect from the structure of its mouth parts. At the same time it is certainly a true blood-sucking fly, and we have frequently seen it distended with blood, thus confirming the observation of Dr. Annandale. We have seen it feeding on fresh cowdung.

*Philomatomyia gurnei*, Patton and Cragg, 1912. (Plate VII.)

**Male.**—Length of seven specimens examined 8.5 to 9 mm.; width of head 2.5 to 3.4 mm.; length of wing 7.5 to 8 mm.

**Head.**—Front narrow, width at centre from one-sixth to one-eighth total width of head. Ground colour of front silvery to blackish grey with a moderately broad dark stripe. Ten large fronto-orbital bristles and external to these several vertical bristles, as follows: One small bristle external to the first orbital, another just external to the space between the second and third bristles, a pair external to the sixth, another pair near the eighth, and the last pair opposite the ninth. Antennae, basal joint dull black, third joint mouse grey. Arista with ten bristles on the upper surface and five on the lower. Palpi dark grey and similar to those of *insignis*.

**Proboscis.**—This has already been described in detail by one of the present writers (F. W. C.), but for the sake of completeness in a formal description of the fly the essential points may be recapitulated
here: externally Musca-like, but with the haustellum elongate and more chitinised, and therefore more conspicuous, than in lineata. Mentum elongate and trough-like, deepest about its middle, and bearing few and short macrochætæ. Labial gutter thick and pigmented, with a keel in its upper third. The hypopharynx is completely separated from the gutter. Furca a horseshoe-shaped arch. Discal sclerite separated into labellar rods, which are flattened laterally and wedge-shaped, and axial apophysis, which is a stout pointed wedge lying between and behind the rods. Eight peg-shaped prestomal teeth on each side, the external ones of the row being rudimentary. The interdental armature consists of a simple pair of leaf-like blades between each two teeth. Pseudotra-chal membrane present and Musca-like, but reduced in extent.

We may add that the original observations, made from one specimen have been confirmed by the examination of others.

Thorax.—Ground colour greyish black, with four clove brown to black broad longitudinal stripes, the outer pair of which is interrupted at the suture; shoulder silvery grey. Thoracic chaetotaxy as in insignis, except that there are usually five dorso-central bristles.

Abdomen.—Ground colour silvery to yellowish grey; first segment, sides and centre dark brown, median longitudinal stripe incomplete, leaving a narrow grey area which is continuous with the lateral grey bands; a narrow dark band along the lower border. Second segment with a broad black median stripe, and a dark brown somewhat rectangular stripe on each side continuous with the similar area on the first segment; the intermediate spaces form rectangular greyish to yellowish patches, the sides of the segment being greyish. Third segment with a somewhat diffuse dark central longitudinal band and a narrow rectangular yellowish grey patch on each side; external to these a dark brown lateral stripe joining the similar one on the segment above. Fourth segment with an indistinct narrow dark central longitudinal band and on each side a small rectangular silvery patch; a narrow dark brown lateral stripe converging toward the apex, the sides of the segment having a small triangular grey patch. Legs black, greyish in some lights. Wings pale grey; veins yellowish grey.

Female.—Length of four specimens examined 8 to 8·5 mm.; total width of head 3·5 to 3·8 mm.; length of wing 7 to 7·5 mm.

Head.—Front broad, widest at vertex, one-fifth to one-sixth total width of head; front yellowish grey; parafacials and parafrontals silvery to yellowish grey. Eight strong fronto-orbital bristles, and two
PLATE VII.

PHILEMATOMYIA GURNEL.

Male.

Female.

Keith M. Patton, ad. nat. det.
rows smaller vertical bristles. Antenna, arista and palp the same as in the male.

Thorax.—Ground colour silvery to yellowish grey, in parts smoke grey to isabeline grey, with four light brown longitudinal stripes narrower than in the male; otherwise similar to the male.

Abdomen.—Ground colour yellowish grey in the centre and silvery at the sides. First segment dark brown with a central stripe, and sides with clove brown bands bounding yellowish rectangular patches. Second segment with a light grey centre, and a dark brown central longitudinal stripe, narrower than in the male; a lateral clove brown stripe, also narrower than in the male; sides silvery grey. Third segment markings similar to those of the second, except that the central stripe is narrow. Fourth segment without any distinct central longitudinal band, but with a diffuse central brown patch; two lateral converging brown stripes. The types are in our collections. Professor Bezzi of Torino has co-types.

A single female was taken in 1911 feeding on a cow in the grounds of the Institute, but since that occasion we have never seen it in the plains. As we have since found it in Kodaikanal, it appears to be a hill species, and we suppose that the original specimen reached the Institute with calves imported for vaccination from Salem, which is at the foot of the Sheveroy Hills. Even in Kodaikanal it was a rare fly, and was confined to certain parts, for although many flies were taken at all parts of the station, this species was only found in one field on a tame pony. The closest search failed to reveal its breeding habits. It seems certain that it does not breed in dung. It was shown to be oviparous by dissection.

When feeding it is invariably found on the legs of the animal, not on the back, which is the favourite place of *insignis*. It is very like *Musca bezzii* in size and colouration, and is best distinguished from it by the manner in which it settles down to feed, thrusting its proboscis between the hairs, and not searching for another biting fly to make a wound for it.

The genus *Philatomyia*, Austen.

As Austen and Brunetti have pointed out, these flies are very closely allied to *Musca*, and were it not for the remarkable modifications found in the proboscis, would hardly be distinguishable from it structurally. Even in their habits, one species approaches very closely to the haematophagous species of *Musca*, together with which they both breed and
feed. On the other hand, a close examination of the anatomy of the proboscis from the comparative point of view has shown that the specialisation for the biting habit, which is found in them, is directed in the same way as that in the Stomoxinae, in the phylogenetic history of which there have undoubtedly been forms closely resembling the modern Philæmatomyia, as far as the structure of the mouth parts, and the feeding habit to which they are adapted, are concerned.

It is a striking fact, that whereas two of the species so far known are rare flies, not numerous in the places where they occur, the third species, insignis, is not only widely distributed, but is very abundant. It is just those two species which are the least well adapted for the blood-sucking habit which are the least often found, and one is tempted to regard the predominence of the one which is the best equipped as an instance of the survival of the fittest. P. lineata, although it is a blood-sucker, has not yet arrived at the point at which it can make a wound for itself, while it is encumbered with relatively heavy prestomal teeth, probably only sufficiently powerful to scrape the dried serum in order to allow the fly to suck up the moisture underneath. The next species has teeth with which it can make a wound, and obtains blood for itself, though not so readily as insignis. It would hardly be too much to say that there we have a genus in the making: as time goes on, the less efficient will cease to maintain their position, and will be replaced by others more perfectly adapted. But insignis itself, voracious biter as it is, can hardly be regarded as other than a transitional form, at present halfway between its Musca-like ancestor and the form with an elongate piercing proboscis which it will become. When local races, varieties arising in connection with a change of host, and so on, assert their individuality and reproduce their kind, the predominant species may be expected to split up into numerous forms each exhibiting a different degree and kind of adaptation to the habit on which they depend for food.

It is considerations such as these which invest the genus with a peculiar interest, and one may confidently hope that as these flies, and the hematophagous species of Musca which are so nearly allied to them both by descent and by habit, come to receive more attention, some of the intermediate forms will be brought to light. We will venture to indicate the three main considerations which must guide our observations on this matter as follows: Firstly, flies with a proboscis apparently identical with that of Musca may be in reality armed with formidable cutting teeth; secondly, flies seen distended with blood and in the act
of feeding on animals are not necessarily biting flies; and lastly, and arising out of these, a microscopical examination of the proboscis is an essential part of the study of such forms. With regard to the last perhaps it will be well to state the technique employed for the benefit of those not familiar with laboratory technique of this nature.

The proboscis is to be cleared for examination by the solution of the soft parts. This is accomplished by macerating it for two or more days in a two per cent. solution of caustic potash. To allow of free penetration of the fluid, the head of the fly should be removed from the body. The specimen is then washed in water, dehydrated in absolute alcohol, and placed in clove oil or xylol. It is then mounted on a slide in canada balsam. Two specimens at least are necessary for good results, one mounted on the side and one showing an anterior view, and in the case of this genus it is best to slit the oral surface in halves in the middle line for the specimen which is to be mounted front upwards, as this gives a better view of the parts. For finer work, of course, elaborate dissections and sections are necessary, but the above is enough for routine examinations.

With these considerations in mind, we can proceed to the definition of the genus. The prepostoral teeth are evidently the salient feature, by which it is distinguished from Musca, and on the development of which the other modifications are directly dependent. In a genus such as this, which is so markedly atypical,—using the term in the sense in which it is used by Townsend—we may well expect the teeth to differ, and the other structural features to differ with them, but even a considerable variation would in such a case be no ground for splitting up the genus till many more of the species which doubtless exist have been studied. We, therefore, suggest the following amendment of Mr. Austen’s definition, to make it include the three species so far known, and such others of those which come to light as show a corresponding degree of adaptation to the biting habit.

“Proboscis Musca-like, but showing a tendency to an increase in the length of the haustellum as compared with the rostrum; retractile, and concealed in the position of rest. The mentum more or less strongly reinforced with chitin, trough-shaped, having its lateral borders produced forwards so as to diminish the membraneous part of the wall of the haustellum. The labial gutter thick, with a posteriorly directed flange or ‘keel’ in the upper portion in most cases. The hypopharynx free from the labial gutter, not partially fused with it as in Musca. The
disctal sclerite thickened, variously altered in shape, and split up into two lateral portions, the labellar rods, and a median posterior portion, the axial apophysis, to a varying degree. The posterior joint between the labellar wall and the fork of the mentum more consolidated than that of *Musca*, but similar in principle. The prestomal teeth not very numerous, arranged in one row on each side and thickened and elongated to various degrees. The pseudotracheal membrane present and functional, but reduced in extent. Haematophagous, usually capable of piercing the skin. Immature stages like those of *Musca* generally."

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ON CERTAIN HÆMATOPHAGOUS SPECIES OF
THE GENUS MUSCA, WITH DESCRIPTIONS
OF TWO NEW SPECIES.

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The true biting and blood-sucking flies of the family Muscidae are found in three groups, viz., the genus Philematomyia, Austen, the Stomoxydinae,* and the genus Glossina, Wied. In each of these groups the structure of the proboscis is adapted to the method of feeding of the fly in a characteristic manner, showing different degrees of differentiation from the prevalent type of the family as seen in Musca domestica. In Philematomyia, the three species of which have been described and depicted by us in another paper, the Musca facies is retained, but the delicate pre-stomal teeth of Musca are replaced by strong cutting weapons; in the Stomoxydinae the proboscis is a definite piercing organ, but in some of the species traces of the pseudotracheal membrane still persist; even in Glossina, which is much more advanced in specialisation, the structures

* In the Stomoxydinae are included the following genera:—Stomoxys, Geoffrey; Stygeromyia, Austen; Haematobia, Andinet-Serville; Bidididurynx, Austen; Haematomboeca, Bezzi; and Lyperosia, Rondani. In the latter genus we include Glossinella, Grünberg, as Professor Bezzi informs us, in a private letter, that in all essential features it agrees with Lyperosia. These genera are very closely related to one another, and if the distinctions between them, and their grouping as a sub-family, are to be retained, then the genera Philematomyia and Glossina should be raised to equal rank as the Philematomyinae and Glossininae, as they represent equally distinct groups.
which go to make up the piercing stylet can be definitely homologised with those of *Musca*.

In view of the close anatomical relationships, as regards the structure of the mouth parts, it is of peculiar interest to find that in the genus *Musca*, in which the proboscis is of the type from which the Muscid biting flies have evolved, there are certain flies which habitually feed on blood. Though they are confirmed blood-suckers, and have no other food, the proboscis is not adapted for piercing, and presents no notable deviations from the type of the genus. In fact, the only feature which might be supposed to indicate a special relationship between them and *Philometomyia lineata*, the least specialised of the flies included in the above list, is that the number of pre-stomal teeth is reduced, only one row being present instead of three as in *Musca domestica*; this is of little importance, as there are other flies in the same group with different habits which have the same peculiarity.

The great interest and importance of the group of flies which forms the subject of this paper lie in the remarkable manner in which they obtain their food. Totally unable to penetrate the skin of the host themselves, they rely on other and better equipped flies to do it for them, and feed on the blood and serum which exudes from their bites. For this purpose they associate themselves with the biting Muscids, such as *Stomoxys*, *Bdeilloarynx*, *Philometomyia*, and *Lyperosia*, and with the *Tabanidae*. An observer unacquainted with this group would remark on the large number of what appear to be ordinary house flies on the cattle and horses in places where any of the above are numerous. Closer examination, however, reveals the fact that extremely few, if any, of the species present are really those which are habitually found in houses, but are flies which are seldom found away from cattle. We have frequently confirmed this by the examination of large numbers of flies brought from the neighbouring bazaar, and by the capture of flies from cattle.

The behaviour of these flies is extremely interesting to watch. On approaching a biting fly in the act of feeding, the *Musca* will endeavour to thrust its proboscis into the wound, and to oust the rightful occupant; often several will beset the same biter, and when they succeed in dislodging it, or when it has completed its meal, will thrust down their proboscides to suck up the blood which exudes from the wound. When a single individual succeeds in placing itself in position over the wound its attitude is exactly that assumed by the true biting Muscids, such
as *Philamatomyia* and *Stomoxys*, for it crouches down and remains motionless while its abdomen can be seen to distend. Like the true biting flies also, they will gorge themselves with blood from one feed, and fly away to rest afterwards; more frequently, however, the conditions are not sufficiently favourable, or the competition for food too great among themselves, to permit of this, and they will feed again on another animal after having exhausted or been dislodged from one source of supply. We have very frequently seen as many as six individuals of *Musca Pattoni*, a large fly, assembled around a single small *Philamatomyia insignis*, waiting for it to withdraw its proboscis. The larger Tabanids, such as *Tabanus albimedius* and *T. striatus*, which make a deep and bleeding wound, provide a frequent supply of food.

These flies are not entirely dependent on other biting flies for their food, for they will readily suck up the moisture which exudes from sores on the skin. That their food is blood and serum can be readily shown by dissection. Their food and the manner in which they obtain it raises the interesting question, discussed elsewhere by one of the present writers, as to whether we have in this group of flies a first step in the evolution of the blood-sucking and biting forms.

The practical importance of the group lies in their probable capacity to transmit disease from one animal to another, and we cannot but think that they have been somewhat overlooked in this respect, particularly as regards what is termed "accidental infection." They are necessarily to a large extent intermittent feeders, passing from place to place on the same animal, and from one animal to another, in the search for food, and always inserting their proboscis, perhaps bearing on its surface the infective matter, into or near a wound or broken surface. True biting flies, on the other hand, habitually take a full meal from one wound, and allow an interval of from one to three days to elapse before feeding again, in order to digest the meal. It is only when they happen to be disturbed while feeding that they will fly off to another animal, and in the majority of cases we must assume, if "accidental infection" is to occur in a state of nature, that the infective organism is capable of living for at least twenty-four hours exposed to the external air. In cases where such a method of infection is suspected, the non-biting but hæmatophagous flies deserve at least as much attention as the true biters.

An excellent case in point is provided by the experience of Darling in the Panama Canal Zone. In recording his observations on an epidemic of equine trypanosomiasis (murrina), Darling states that having failed
to find any evidence incriminating any of the biting flies, he turned his attention to the non-biters, noting that swarms of flies of the general *Musca, Compsonyia*, and *Sarcophaga*, were attracted to the excoriated patches which formed on the necks, heads, and other parts of the diseased animals a few days before death. Coming to the conclusion that the parasite was being transmitted mechanically by these flies, he had all the wounds and raw surfaces protected from the flies by creolin and other disinfectants, with the result that the epidemic was stopped. The particular species of *Musca* which were present are not mentioned, but it is quite possible that some of them are blood feeders such as are described in this paper. It would be well to bear in mind that surra in this country may be transmitted in the same way. The further possibility should not be forgotten, that since these flies, like many others, defaecate while feeding, infective organisms might be passed on to the skin of the host in the neighbourhood of the wound or on to an abraded surface.

We will now describe* the four species we have studied in South India. They all resemble the common house fly superficially, and identification is not always easy. We need hardly say that we will be glad at any time to assist in the identification of species from this or any other part of India.

We have pleasure in expressing our thanks to Mrs. Patton for the admirable and accurate manner in which she has drawn the flies.

*Musca gibsoni, sp. nov. (Plate I.)*

**MALE** (Plate I, Fig. 1).—Length of 30 specimens examined 5·5 to 7 mm.; width of head 2 to 3 mm.; width of front at centre 1·5 to 2·2 mm.; length of wing 5·8 to 6·2 mm.

*Head.*—Front very narrow with central dark stripe; parafacials and parafrontals silvery to yellowish grey. Eyes reddish brown and bare; occiput and ocellar triangle black. Frontal bristles 12 to 14 in number, with 7 to 8 vertical bristles, extending in a row to the back of the head. Ocelli light amber with four pairs of bristles; a long stout bristle on each side of the posterior border of the vertex. First and second antennal joints black, second joint with one stout and two smaller bristles; third joint elongate, mouse grey to silvery grey. Arista dark

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* In the descriptions and drawings of these flies the insect is held with its head towards the observer, so that the light falls on its body from the front.
grey with nine to ten bristles on its upper surface and five on its lower. Palpi dark grey to black with many long bristles. Proboscis as in the type species, *domestica*, Linné.

**Thorax.**—Ground colour slate grey to dark grey with a yellowish tinge in some lights. Four broad black longitudinal stripes on the dorsum of the thorax, the median pair meeting in front and not interrupted at the transverse suture of the thorax, behind which they soon fade away. Outer pair broad and directed inwards in front of the suture, behind it slightly narrower, and reaching to the posterior border of the thorax. In most of the specimens the black bands are not easily seen owing to the dark colour of the thorax. Thoracic chaetotaxy as in *domestica*. Scutellum slate grey to dark grey, with lighter sides; bristles as in *domestica*.

**Abdomen** reddish brown with shimmering white bands. First segment black except for two yellowish brown horizontal bands at lower border. Second segment with a broad black central longitudinal stripe broader at the upper end of the segment, and narrowing towards the lower end; on each side a narrow shimmering yellowish grey band; remaining part of segment reddish brown, with a white patch at the lateral edge. Third segment with a narrow black central longitudinal stripe, and a larger white patch at the lateral edge, the markings otherwise similar to those of the second segment. In almost all the specimens the lower border of the third segment is dark brown, forming two conspicuous spots at the sides. The fourth segment is dark grey to olive grey in the centre, with the faintest indication of a stripe, and a narrow white line on each side as in the other segments; the lateral clove brown stripes are well marked and the extreme edge of the segment is silvery. Legs black and with bristles as in *Musca domestica*.

Wings grey with a darker tinge near the base; venation as in *domestica*. Squamae large, greyish white; halteres, stalk grey, and knob yellowish.

**Female.**—(Plate I, Fig. 2).—Length of 40 specimens examined 6 to 8 mm.; width of head 2 to 3 mm.; width of front at vertex about 1 mm.; length of wing 5 to 7 mm.

**Head.**—Front wide with a broad central stripe and yellowish grey sides; parafrontals and parafacials silvery grey to yellowish grey. Eyes reddish brown, bare; occiput and ocellar triangle black with three amber-coloured ocelli. Ten frontal bristles, and two rows of from 8 to 12.
10 vertical bristles, the inner row with four stout ones extending to sides of ocellar triangle; a pair of stout bristles at each side of the posterior border of vertex. Three pairs of ocellar bristles. Antennae and palpi as in male. Arista with nine to ten bristles on its upper surface and five to six on its lower.

Thorax yellowish grey with markings as in the male; scutellum yellowish grey with an indistinct dark central band.

Abdomen reddish yellow. First segment as in male, except that the lower border is dark, forming a transverse band, and the lighter areas are reddish brown. Second segment with a central dark longitudinal stripe narrower than in the male, a yellowish grey broad shimmering patch on each side and a lateral ochraceous band joining the lower border of the first segment, which is dark brown, to black lower border with a transverse band; extreme edge of segment grey. Third segment markings similar to those of second segment, the longitudinal stripe narrower as is also the transverse band at the lower border. Fourth segment with two lateral clove brown narrow stripes at the sides, centre olive grey, sides silvery grey to reddish brown. In all other respects the female is like the male.

Egg (Plate I, Fig. 3) white with greyish tinge, and with a long spine expanded at its extremity. Length without spine 2 mm., length of spine 1 mm.

Larva (Plate I, Fig. 4) closely resembling that of domestica; length 10 mm.; anterior spiracles with seven papille. Puparium (Plate I, Fig. 5), greyish brown, reddish brown in some lights; length 5.5 to 6 mm.

This fly was found by one of us (W. S. P.) at Kodaikanal, in the Pulney Hills, South India, at a height of 6,000 feet. We have never seen it in the plains. It was extremely common during the month of September, so much so that on one occasion over two hundred specimens were taken in two hours, on a horse. It appeared to cause more annoyance to cattle and horses than did the true biting flies. The species on which it was dependent were mainly Stomoxys calcitrans and Bdello- larynx sanguinolentus, which it provokes in a remarkably persistent manner, as many as a dozen being frequently seen around a single biter, endeavouring to make it dislodge its proboscis. The biting fly appeared to resent these attacks, and to endeavour to protect itself by raising its body and vibrating its wings, but it was frequently seen to be turned round by these attacks, on the fixed point provided by its proboscis. Both male and female suck blood.
The eggs are laid in cowdung as it lies in the field, and not in places in which it has been collected. About fifty are laid at one time. On alighting on the dung the female crawls about until a sufficiently moist place is found, and then extrudes its ovipositor, depressing the body at the same time. When one egg is laid down the ovipositor is rapidly withdrawn from the dung, but kept still extended until the next egg has passed down and is ready to be laid. The separate eggs are laid close together but not in the same hole. When all the eggs have been laid, the surface of the patch of dung presents a peculiar appearance, for with a low power lens all the spines can be seen projecting upwards.

We proposed the name *gibsoni* for this species, in acknowledgment of the valuable help received from Miss Gibson in the hunt for its breeding place.

**Musca pattoni**, Austen, 1910. (Plate II.)

This fly has been fully described by Mr. Austen, so that we need only mention some of the main points by which it may be distinguished from *gibsoni*, which it closely resembles. Both sexes are distinctly larger and stouter than *gibsoni*. The thorax of the male *pattoni* is lighter and of a yellowish grey colour, and the longitudinal stripes are clove brown instead of black. The abdomen of the male *pattoni* is much lighter than that of the male *gibsoni*, and the central longitudinal stripe is distinctly narrower and, unlike that of the latter, is only present on the first three segments. The first segment of the male *pattoni* is almost entirely yellowish, while the second and third segments have the same markings as those of *gibsoni*, except that the silvery patches on each side of the central longitudinal band are broader and slightly interrupted at the lower borders of the segments, not forming a continuous silvery stripe down each side of the central band. The lateral brown bands are much lighter and narrower. The fourth segment has the same markings as the third except that the median longitudinal stripe is wanting. The female *pattoni* also resembles the female *gibsoni*, but is much yellower in colour. The first segment is much lighter, and has only a very narrow and indistinct black longitudinal stripe, while the corresponding segment of *gibsoni* is almost black, and the central stripe is broader. The lateral brown bands in the female are broader and lighter, and the transverse black bands at the lower borders of the segments 1, 2 and 3 are very much less in evidence.
Length of male (Plate II, Fig. 1) 5.5 to 8.5 mm.; female 6 to 7.5 mm.; width of head in male 2 to 3 mm.; in female (Plate II, Fig. 2) about 2.5 mm.; width of front of female at vertex 8 to 1 mm.; length of wing of male 5.5 to 7.5 mm.; of female 6.5 to 7 mm.

The egg of *pa-toni* (Plate II, Fig. 3) is similar in shape and size to that of *gibsoni*, except that the spine is more delicate and less expanded at its apex. The larva (Plate II, Fig. 4) is also very similar, but the anterior spiracles have only five papillae. The purpurium (Plate II, Fig. 5) is of a characteristic dirty greyish white colour, and measures from 7 to 7.5 mm. in length.

This species of *Musca* is abundant in Madras throughout the year and regularly breeds in the heap of cowdung kept in the Sewage Farm of this Institute. It never lays its eggs, at any rate at Guindy, in isolated patches of cowdung. Each egg is laid singly and never more than one in the same tunnel, but as many as eight flies may be found ovipositing close together. The larvae, unlike those of *gibsoni*, never migrate from the dung but pupate in it. The flies, for both sexes behave in the same way, are only seen on cattle and horses and they worry *Philometomyia insignis* and *Stomoxys calcitrans* in much the same way as does *gibsoni*.

*Musca convexifrons*, Thomson 1868. (Plate III.)

This fly was described from China in 1868 by Thomson, but as his paper contains only a short description, and is exceedingly rare, it is very necessary to redescribe it.

**Male** (Plate III, Fig. 1).—Length of 50 specimens examined 5 to 5.5 mm.; width of head 2.5 to 2.8 mm.; length of wing 4.8 to 5 mm.

**Head.**—Front very narrow; fronto-orbital bristles 10 to 12 in number and mostly moderately long. Arista with 11 to 12 long bristles on its upper surface, and 6 to 7 on its lower. Palpi and proboscis as in *domestica*.

**Thorax.**—Ground colour slate grey to dark grey, and in some lights bronze black. Four broad black longitudinal bands on thorax, the outer pair slightly curved and directed inwards in front of the suture, at which they are interrupted. Thoracic chaetotaxy as in *domestica*; scutellum slate grey.

**Abdomen** yellowish brown, first segment with a broad black transverse stripe at its upper border and a broad central black longitudinal band. Second segment with a broad black central longitudinal band, and a silvery patch on each side, the remainder of the segment yellowish and the edges white. Third segment with markings similar to those of the second
except that the central band is narrower. Fourth segment olive grey in the centre, and brown at the sides, sometimes with two narrow lateral brown bands.

Female (Plate III, Fig. 2).—Length of 60 specimens examined 5 to 6.5 mm.; width of head 2 to 3 mm.; width of front at vertex 5 to 8 mm.; length of wing 5 to 5.2 mm.

Head.—Frontal stripe moderately broad; fronto-orbital bristles 10 in number; vertical bristles two rows of 7 to 8. Arista with 9 to 10 bristles on its upper surface, and 6 to 7 on its lower.

Thorax grey to greyish black with the usual four black longitudinal stripes, somewhat narrower than in the male.

Abdomen reddish brown, first segment almost entirely yellow with a narrow to broad central longitudinal band. Second segment with a narrow median longitudinal band, and silvery patches at its sides, and in some lights a reddish brown lateral band. Third segment with similar markings except that the central longitudinal band is much narrower. Fourth segment dark at the centre and apex and brown at the sides.

Egg (Plate III, Fig. 3) with a slender spine similar to that of pattoni; length 2.5 mm.

Larva (Plate III, Fig. 4).—Length 8 to 9 mm.; dirty grey with an orange tinge on its dorsal surface. Puparium (Plate III, Fig. 5), length 5 mm.; almost pure white, in some lights greyish white.

This fly is common in Madras throughout the year, but has not been found in any of the hill stations. It lays its eggs in isolated patches of cowdung in exactly the same way as does gibsoni. Both sexes feed on cattle.

Musca bezzii, sp. nov. (Plate IV.)

Male (Plate IV, Fig. 1).—Length of ten specimens 7.5 to 8.5 mm.; width of head 3 to 3.5 mm.; width of front at centre 5 mm.; length of wing 8 to 8.5.

Head.—Front very narrow with a dark black stripe filling up the entire interocular space; parafrontals silvery grey; eyes reddish brown, and bare; occiput and ocellar triangle black. Normally 26 fronto-orbital bristles, the lower 12 stout, the remainder moderately strong; no distinct vertical bristles. Antennae; first and second segments almost black, second segment with three strong short bristles; third joint mouse grey to shimmering grey. Arista dark grey to black with 14 to 15 bristles on its upper surface and 9 on its lower; there are many minute
hairs between the long bristles on the upper surface. Palpi dark grey to black; proboscis as in domestica, labella with many long black hairs.

Thorax.—Dark grey to black or bluish grey, in parts yellowish grey with four broad black longitudinal stripes; the median pair broad at their anterior ends, narrowing slightly near the suture, at which they are not interrupted, but broader behind the suture, fading away just in front of the posterior border of the thorax. Outer pair narrower and directed inwards, slightly interrupted at the suture, behind which they broaden out and extend almost to the posterior border of the thorax. In most of the specimens examined the outer stripes almost fuse with the median pair, thus giving the dorsum of the thorax a general bronze black appearance. Chaetotaxy as in the type species. Scutellum with a large black triangular black patch at its centre, with yellowish grey sides and apex; bristles as in domestica.

Abdomen.—Ochraceous buff with shimmering yellowish grey patches. First segment with a black transverse band, not extending to the outer and lower borders; in some specimens the band is smaller. Second segment buff with a central broad longitudinal stripe, extending in front to the sides of the segment; a small light grey shimmering patch on each side of the central band. Third segment with a narrower longitudinal stripe, and an adjacent very distinct rectangular yellowish grey shimmering band, and external to these a brown somewhat rectangular stripe; sides of the segment silvery and extending round to the ventral surface. Fourth segment with a narrow black longitudinal stripe, and a light grey rectangular band on each side, lateral reddish brown band, and silvery sides. With the light falling on the abdomen the central black band is seen to be bordered by a grey band, and this again by a reddish brown patch. Legs black with bristles as in domestica. Wing grey with a yellowish tinge near the base and along the anterior border; venation as in domestica. Squamae large grey; halteres grey with canary yellow tips.

Female (Plate IV, Fig. 2).—Length of 100 specimens examined 8 to 8.5 mm.; width of head 3 to 3.5 mm.; width of front at vertex 1.2 to 1.5 mm.; length of wing 7 to 7.5 mm.

Head.—Front wide with central black stripe, and yellowish sides; parafrontals and parafacials silvery grey. Ten fronto-orbital bristles; three pairs of irregularly arranged vertical bristles more or less in rows; arista with 13 to 14 bristles on its upper surface and 8 on its lower; a few minute hairs between the upper bristles.
Thorax silvery grey to dark grey, not as dark as that of the male. Four longitudinal stripes narrower than those of the male. Scutellum dark grey with a central almost black patch.

Abdomen dark grey and in parts yellowish grey. First segment almost entirely black, sides usually lighter. Second segment with a broad black central longitudinal stripe, with a rectangular yellowish grey patch on each side; a clove brown to black lateral band, narrow and pointed at its upper end, broad and transversely elongated at its lower end, thus forming a transverse band to the lower border of the segment. At the extreme edge of the segment there is an oval greyish to yellowish patch extending round to the ventral surface. Third segment markings the same as those of the second, except that the central longitudinal band is narrower. Fourth segment with a brown central longitudinal band, a yellowish patch on each side, and a broad clove brown lateral stripe converging slightly towards the apex of the segment; yellowish grey patches at the sides of the segment. In many of the specimens the two lateral brown stripes are interrupted or only present at the lower end of the segment. Legs, wings and other parts as in the male.

Larva (mature) (Plate V, Fig. 10).—Length of six specimens examined 10.5 to 12.5 mm.; width 2.5 to 3.5 mm. Yellowish white, in parts greyish white with a lemon yellow tinge on the dorsum towards the anterior end. Anterior spiracles with ten papillae. Ventral surface with eight well marked spined ridges.

Puparium (Plate V, Fig. 10).—Length of six specimens examined 6 to 6.5 mm.; width 3 to 3.6 mm. Dirty grey and in some lights with a yellowish tinge; in general colouration and appearance closely simulating the puparium of Musca pattoni.

The male of this handsome fly was first found at Kodaikanal in 1910 by Mrs. Patton. Two years later the junior writer found it in Kotagiri, in the Nilgiri Hills, and was able to follow its life-history. It appears to be only present in large numbers during the middle of the year; it was only rarely seen in Kodaikanal in September. We have never seen it in the plains.

When the second batch of flies was first examined, this species was taken for our Philomatomicya gurnei, to which it bears a very close resemblance, and it was only after an examination of the mouth parts in a cleared preparation that we recognised that we were dealing with a new species. As at the time we had only one headless and somewhat dilapidated specimen of gurnei, the mistake was perhaps excusable.
We mention it, however, to emphasise the difficulty of distinguishing flies of the genus Philomatomyia from those of Musca, without dissection of the proboscis. There is reason to believe that many specimens of Philomatomyia insignis, which has a most efficient biting apparatus, were present in collections as species of Musca, until Mr. Austen described it and erected the genus. When the proboscis is retracted, as is usually the case in pinned specimens, there is nothing to indicate the difference. In this case the observer was led astray in the other direction by seeing the fly gorged with blood.

This species is of special interest in that it is larviparous, having a reproductive habit similar to that of the Musca corvina* Fabr., (ovipara, Portsch.) of Europe, which, by the way, is also a hæmatophage. Its reproductive organs are correspondingly modified. Its habit is totally different to that of the larviparous Tachinids, and to that of Glossina, for only one young larva is produced at a birth, and this passes through the next two phases exactly like the larva of gibsoni or pattoni.

Oviposition under natural conditions was not observed, but the fly was induced to lay without any difficulty in captivity. A large number of females was caught and placed in large test tubes, which were inverted over fresh cowdung, it being supposed that the larvae would have the same habitat as those of the species of similar mode of feeding. Out of each batch a few of the flies would settle on the dung after a few minutes, and proceed at once to deliver themselves. The ovipositor is extended exactly as in the egg-laying species, and bent downwards and

* Through the kindness of Professor Bezzi, Baron Surcouf and Dr. Schenkeling we have had the opportunity of examining a long series of specimens of Musca corvina, Fabr. (ovipara, Portsch.), and Musca corvina, Fabr. (vivipara, Portsch.), as well as the puparia of the former. According to Portschinsky the variety ovipara lays a spined egg in Northern Europe, while in the Crimea in the spring and summer it deposits larvae. From the oviparous form white puparia are developed while from the larviparous variety brown puparia. On examining the two varieties we find that ovipara is much smaller (5-6 mm.) than vivipara (7-8 mm.). In the male vivipara the first abdominal segment is not entirely dark as in the male ovipara. In addition it has a wide marked silvery band on the second and third segments on each side of the median line and pollinose lateral bands. The female vivipara is also distinctly different from the female ovipara, but the markings are very similar. From our knowledge of the oviparous convexifrons (spined eggs and white puparium), pattoni (spined eggs and dirty grey puparium) and the larviparous bezii, it appears to us that Portschinsky has confused two distinct species of Musca, one entirely oviparous and the other entirely larviparous. Otherwise it seems difficult to understand how a species of Musca can lay spined eggs at one time and larva at another. It is to be hoped that those who have the opportunity of studying the life-histories of these two interesting species will settle this point.
The reproductive organs of *Musca bezzi*, the uterus being empty; ov. 1, ov. 2, ov. 3, ov. 4, the ova in order of succession; ov. d, oviduct; s. ov., common oviduct; sp., spermatheca; a.g., accessory glands; ut., uterus.

Figure 2.—The distal end of one of the accessory glands.

Figure 3.—A spermatheca.

Figure 4.—The reproductive organs of *Musca bezzi*, the uterus containing a larva about to be born. Reference letters as in Figure 1.

Figure 5.—Muscle fibres from the wall of the uterus, showing the regular arrangement. A single layer of circular and one of longitudinal fibres are present.

Figure 6.—Puparium of *Musca bezzi*.

Figure 7.—A larva immediately after birth.

Figure 8.—The posterior end of the puparium.

Figure 9.—The egg.

Figure 10.—The larva when full grown.

The scale on the left of Figure 1 applies to Figures 1 and 3. That under Figure 3 to Figures 2, 3 and 5.
a little forwards over the surface of the dung. Then, after a few explosive efforts, a small white larva appeared at the tip, and passed out at the next contraction of the abdominal muscles. The young larva is exceedingly active from the moment of birth, and at once crawls away from the mother and burrows into the dung, being rapidly lost to sight. The female does not introduce the ovipositor below the surface but merely drops the larva on to the dung.

On two occasions a large egg was laid instead of the larva. Probably this is quite abnormal, and in the nature of an abortion, due to the excitement of capture and close confinement. One of the eggs was preserved and kept in moist dung, but it did not hatch.

Young larvæ obtained in this way were kept, and supplied with fresh cowdung daily. They rapidly increased in size, assuming the ordinary appearance of Muscid larvæ. After about fifteen days, when they were full fed, they left the dung and crawled into moist earth provided for them, and after two or three days pupated. The imago hatched in three weeks in the climate of the hills, but some pupae sent down to Madras by post hatched in a fortnight.

The reproductive organs (Plate V) are of the type of the Pupipara, except as regards the spermathecae, which resemble those of Musca. There are two ovarian tubes (as in Glossina, while there are four in Melophagus), the ova in which are never equally developed. Usually four ova can be distinguished in each side, the one nearest the open end of the tube being always the most nearly ripe of its side, and very much bigger than the next. When the most mature ovum of one side is nearly ready to pass down, it distends the tube in both directions, extending downwards to near the junction of the two ovarian tubes which form the common oviduct, and also upwards into the abdomen to such an extent that the tube above it, containing much less mature eggs, is bent on itself, the terminal portion containing the undifferentiated cells becoming adherent to the side of the tube near its middle, so that the whole tube comes to have a retroflexed appearance. The two oviducts fuse to form a common chamber, their length depending on the degree to which the lower portion of each is distended by the growing egg. The tube distal to this junction, which we may regard as the uterus, is a very strong and muscular organ, the shape of which is entirely dependent on the stage at which the reproductive process happens to be. When empty it is elongate and spindle shaped, as shown in figure 1, and is only distinguished from the corresponding part in Musca by its
greater length, but when it contains a growing larva, it becomes bent and distended in its upper part to accommodate its increased contents. The internal end, as in the uterus of *Melophagus*, is pressed forwards into the abdomen below the ovaries, and forms a conspicuous projection, at the upper end of which is situated the head of the larva.

There are two small tubular accessory glands, corresponding to the milk glands of *Glossina*, arising about the middle of the common chamber. They are simple and unbranched, and have a wall consisting of small round cells with large nuclei; they terminate in attenuated ends, as shown in figure 3. Their small size would appear to indicate that the sojourn of the larva in the uterus is not a long one, as is also shown by its size when born. The spermathecae are small oval bodies with a densely chitinised wall. They are attached to the uterus by rather long ducts, which are twisted in a spiral manner. The walls of the ducts are composed of an inner layer of chitin and an external coat of muscular fibres. Two of the ducts open into the uterus by a common opening.

It will be seen that there is no special apparatus developed for nourishing the larva, the accessory glands being similar to and not much larger than those of *Musca*. The eggs, on the other hand, are very much larger than those of *Musca*, or even of *Philematomyia insignis*, when they leave the ovary. Unfortunately apparatus for making good preparations of the organs was not available in the hills, nor could it be obtained in time, so that a complete study of the development of this interesting fly could not be made. The preparations from which the organs have been drawn were made from flies which were brought down to the laboratory by train, and which had been dead some hours before they could be dissected. The appearances seem to suggest that development proceeds to an advanced stage before the egg is passed down to the uterus.
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THE INDIAN JOURNAL OF MEDICAL RESEARCH,

A PRELIMINARY NOTE ON FERTILIZATION IN CIMEX.

BY

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The phenomena connected with fertilization in the bed bug, extraordinary though they are, have remained almost uninvestigated since their atypical nature was first pointed out by Berlese in 1898. Berlese’s papers appeared in a journal in which one would not expect to find detailed studies in insect morphology, and probably few entomologists were aware of their existence until the publication of his book in 1909. For the rest, no one looked for the new and marvellous in so common an insect, and its nocturnal habits, and the circumstances under which it is commonly met with, assisted in concealing from observation the salient feature of the reproductive function, namely, the peculiar method of copulation. It is nevertheless remarkable that the presence of so large an organ as that described by Berlese—always visible to the naked eye after dissection, and under certain circumstances visible even in the intact insect—should have escaped notice. Landois, whose account of the anatomy of the bed bug is otherwise very minute and precise, made no mention of it.

The anatomical features peculiar to the bed bug are briefly as follows: In the right lateral half of the ventral plate of the fourth segment there is a longitudinal slit, extending through about half the width of the chitinised portion of the sternite. Lying upon the plate internally, and immediately above this incision, there is a spherical organ, termed the organ of Berlese; this structure is a solid mass of cells, with no lumen, and no duct to establish connection with any of the internal organs. In sections of this organ taken from caught specimens one practically always
finds it to contain very numerous spermatozoa, either collected together in a central mass occupying from a half to a quarter of the total area of the section, or in strands, fine or coarse, infiltrated among the cells. The cells themselves present very varied appearances, and may be simple and regularly nucleated, or containing numerous granules, or they may be vacuolated to any degree. Even more striking than the presence of this unilateral organ and the connected incision in the integument, is the almost constant presence, in caught specimens, of great numbers of spermatozoa free in the hematocele of the abdomen. These spermatozoa are collected into large masses which might easily be mistaken for fat body, from which, however, they are to be distinguished by their dense white colour and greater opacity. In addition to the free masses of sperms, there are usually other masses attached to the oviducts at their point of junction to form a common trunk. The ovaries, which are composed of seven ovarian tubes on each side, are of the usual type and the common oviduct terminates at the posterior end of the body in the usual manner between modified ventral plates so arranged as to permit of expansion of the genital orifice at the moment of oviposition. The evident peculiarities of the female are, therefore, the unilateral organ containing sperms and the presence of masses of sperms in the hematocele. It is to be noted that there is no connection, by duct or otherwise, between the organ of Berlese and the female organs proper.

Berlese, who had very evidently never seen, or at least never recognized, the act of copulation, founded upon those facts and other connected observations a most elaborate and, it must be admitted, a very attractive theory. According to his account the spermatozoa from the male, passing into the oviduct and spermathecae, pierce the walls of these, become free in the abdominal cavity and enter the special organ, where by a special process they become broken up and absorbed, to be utilized as food material during the sexual life of the female; other sperms pass upwards in the oviduct, and it is stated definitely (indeed the statement is printed in italics) that the epithelial cells of the oviduct and ootheca contain spermatozoa in large numbers. To this process he gives the name hyperganesis.

Whatever truth there may be in the theory of hyperganesis, it is certain that the process described by Berlese—which, it may be noted, was very strongly criticised by Carazzi, who was also ignorant of the essential fact—is directly contrary to the actual course of events. The
spermatozoa do not enter the posterior genital orifice at the time of copulation; they pass directly into the organ of Berlese, through the incision in the integument already noted, and from thence into the hæmatocœle. The genital orifice at the posterior end of the abdomen functions only in oviposition; the copulatory orifice is the incision in the fourth sternite.

These phenomena were briefly discussed in a recent publication by Major Patton and the present writer. At the time of writing, however, the original papers dealing with the question were not available, and time did not permit of any systematic investigation at first hand. Since then sufficient progress has been made to warrant the presentation of a preliminary account.

The previous workers appear to have limited themselves to the examination, by dissection and by the preparation of sections, of female bugs taken at random. Berlese’s theory, in fact, represents an attempt to fit together the conditions found by such observations as component parts of a connected process, though he does not hesitate to express it as if it were definitely ascertained fact. The chances of error in such a method are obviously very great. To obtain any sure foundation it is essential to start from the only condition which can be regarded as standard, namely, that of the newly hatched and unfed virgin female, and to make observations at definite intervals after copulation has occurred. This method has been adopted, and a large number of experiments have been carried out with bred specimens of which the entire history was known. A corresponding number of observations on caught females taken at random have been made, to serve as controls. In the majority of cases the specimens have been examined in serial sections of the abdomen, prepared by the clove oil-celloidin-paraffin method.

The main fact, that of the entry of the spermatozoa directly into the organ of Berlese during the act of copulation, has been abundantly confirmed. In no case have spermatozoa been found elsewhere than in this organ immediately after copulation, of the kind described, has been seen to occur in previously virgin females. The description of the act, however, must be somewhat modified. The bugs on which the first observations were made were kept in tubes containing a small number of both sexes, and had thus unlimited opportunities for copulation. Under such circumstances the very rapid performance of the act is frequently observed, and it is usual to find that after it has been seen to occur the organ is distended with sperms, but under more
favourable conditions the act is much more deliberate and prolonged. If a male and a female, two or more days old and unfed since the last moult, be placed together in a small tube the mouth of which is applied to the skin of the forearm, they will usually feed readily and at once. After they are gorged they wander away from the skin and remain quiet for a few minutes, ejecting black excreta from the rectum at short intervals. After some time the male moves toward the female and places himself obliquely across her body in such a way that the penis is applied to the opening in the ventral surface of the female, that is, his body points forwards and to the left. The male remains attached in this position for a considerable time, retaining his hold by grasping the thorax of the female with his legs. The posterior end of the abdomen of the female can be seen to be quite free from all contact with the male, and the actual entry of the penis into the opening in the fourth segment has frequently been seen with the aid of a binocular microscope. Usually the pair remain at rest in this position for two or three minutes; at times, however, the female runs about the tube and endeavours, generally without success, to dislodge the male. If the act is interrupted it is often repeated after a short interval. Occasionally the pair will remain attached for quite long periods, even up to half an hour.

It is probable that fertilization only occurs when such deliberate congress of the sexes takes place. The very rapid grasping of the female by the male, which was at first thought to be the normal performance of the act, is only seen when several individuals of each sex are kept and fed together in one tube, and has not been observed when a single pair have been under observation, except occasionally when they have been fed in a bright light, a condition which they would ordinarily avoid in a state of nature. Nevertheless, when momentary attachment has been observed one almost always finds that the organ of Berlese, dissected out immediately afterwards, is filled with sperms, and is in much the same condition as after the deliberate performance of the act. Probably the female in such a case has only recently been fully impregnated, and the act is abortive partly on that account.

Contrary to what is usually the case in insects, bed bugs copulate very frequently during their sexual life. Individuals which have been kept isolated from the opposite sex, will usually become attached at once when given the opportunity, if they are shielded from bright light and allowed to remain undisturbed. The act is not necessarily associated with feeding, though it only occurs in well-nourished individuals,
and does not, so far as present observations go, occur before the first feed in the adult stage.

The course of events subsequent to the entry of the sperms into the organ of Berlese has been followed by dissection and by the examination of serial sections of the whole abdomen, made at stated intervals after copulation had been seen to occur. It was soon found, however, that the elucidation of the problem was by no means so easy as might have been expected, on account of the very great irregularity with which the function of reproduction is carried on. In the first place, the amount of spermatozoa injected by the male at one act varies within wide limits, and the period which elapses before the organ of Berlese, which receives the spermatozoa, is again free from them varies in a corresponding manner. Quite frequently, also, the female is found to have remained unfertilized even when the male has been seen to take up the position indicated above and to remain attached for the usual time. The irregularity as regards oviposition is very striking; usually two or three eggs are laid on the third or fourth days after the first feed and the first copulation, but oviposition may be delayed for a week or more or may not occur at all. In several experiments in which no eggs have been laid by a fertilized female after the requisite time, numerous spermatozoa within the oviduct, and partially mature ova, have been found in the sections, thus proving that fertilization had really occurred, although it was not followed by oviposition. The number of eggs produced by different females, of the same age, after one meal of blood and one impregnation, may range from three to nine in the first four days; with an ample supply of food and unlimited access to the other sex the number of eggs produced may vary from an average of one every second day, or less, to one per day or even more. These wide variations in a normal process have made frequent repetitions of the experiments necessary.

Sections of the organ of Berlese, fixed within a few minutes after copulation, show a dense felted mass of filamentous sperms in the centre of the organ, occupying from a quarter to a half of the total area. The mass is definitely bounded, though there is no structure separating it from the cells of the organ. Such masses of sperms are very well shown in Berlese’s plates, and in one of the figures in his book, though he did not know, of course, that this stage represented in reality the beginning of the process of which he has given so elaborate a description. The mass is so dense that no detailed structure, except numerous more
CRAGG—A preliminary note on Fertilization in Cimex.
Fig. 1. A section of the organ of Berlese, eight hours after copulation has occurred. The spermatozoa are on their way from the central mass to the periphery, and at one point, on the right of the figure, a mass has passed into the haematocoele. x 80.

Fig. 2. The ovaries and oviducts, twelve hours after copulation. Some development of the ova has already occurred. The mass between the oviducts is a clump of spermatozoa; several similar masses, which are only very slightly adherent, were detached in dissection. The spermathecae are distended with sperms. x 25.
darkly stained particles, which are apparently the heads of the spermatozoa, can be made out. If such organs are dissected in saline in the fresh condition the sperms are seen to be actively motile. The cells of the organ surrounding the central mass are shrunken, and are separated by many fissures and clear spaces, while those towards the periphery are not different to those in the virgin condition. No spermatozoa are to be found outside the organ, either in the body cavity or in the oviducts or so-called spermathecae.

During the subsequent few hours the central mass becomes dissociated into strands, which are directed towards the periphery of the organ. The edge of the mass first becomes wavy and fringe-like, and the strands as they break off thrust themselves between the cells of the organ, in some places enclosing small islands of vacuolated cells. By the end of four hours the mass is usually entirely broken up, and the bundles of sperms are concentrated at the periphery, and after eight hours some of the bundles have passed out of the organ altogether, and are free in the body cavity, lying between the organ of Berlese and the right oviduct. By the end of twelve hours the streaming out of sperms into the body cavity is well marked, and large masses of them are found in the neighbourhood of the oviducts. Many have already pierced the wall and are lying inside the duct, and inside the spermathecae, though as yet the number outside is much greater than that inside. The ovaries show considerable development, some of the follicles being nearly half their full size. In twenty-four hours after copulation the number of sperms in the organ is very small, while there is a large mass around the oviducts and in the angle between them, and the spermathecae are now greatly distended.

During subsequent days there is a gradual transference of the spermatozoa from the organ of Berlese to the body cavity, and from thence to the interior of the spermathecae. Under normal conditions, with free access to the male, the body cavity is never without numerous spermatozoa from this stage onwards, since copulation again occurs and the flow from the organ of Berlese continues. If females are kept isolated there is a gradual reduction in the number of sperms, but they are still present in small numbers at the end of three weeks.

The early development of the embryo is a noteworthy feature of reproduction in Cimex. In the majority of insects the actual fertilization of the ovum by the male element is believed to take place immediately before oviposition, the spermatozoa being stored in small chambers,
the spermathecae, which are connected to the lower part of the common oviduct by means of a narrow chitinized duct. In the bed bug it is quite certain that the ovum is fertilised—in a considerable number of cases if not always—while still contained in the follicle, for embryos at a late stage of development, showing pigmented eyes, are frequently seen in the body cavity in dissections, both in caught specimens and in those which have been kept under experimental conditions, whilst in sections of bugs which have laid a number of eggs one almost always finds at least one ovum which shows a developing embryo. The ovarian tubes in ovipositing females are always very unequally developed. At the most only one tube on each side—the most internal in position—contains an egg ready to be laid, the remaining tubules being in various stages, and some of them apparently as in the virgin condition. These facts suggest very strongly that the bed bug is not far removed from the larvivorous habit, and call to mind the common occurrence of this method of reproduction among insects which feed solely on blood.

The amount of spermatozoa received by the female is not only altogether out of proportion to that necessary for the fertilization of the ova, but is exceptionally large in relation to the size of the insect. The testes of the male are of unusual size, and the long spindle-shaped vasa leading from them are usually packed with sperms; copulation is repeated every few days under favourable conditions, a second batch of spermatozoa being received while the first is still surrounding the oviduct. It is not uncommon to find at least a half of the area of a section through the posterior part of the abdomen to be occupied by masses of sperms. The reason for this enormous excess, and the ultimate fate of the spermatozoa, are as yet unexplained.

A word with regard to the spermathecae. In the publication referred to above it was stated that they are absent in Cimex. Further examination, however, has shown that at the posterior end of the common oviduct there is on each side a small diverticulum, which becomes distended with spermatozoa at the same time as the lumen of the oviduct. The structure of the wall of these diverticula is similar to that of the oviduct itself, and the lumen is separated from that of the median oviduct only by a slight constriction, not by a distinct elongate duct as depicted by Berlese. These chambers can only be seen clearly in dissections of virgin females or of females which have been kept apart from the other sex for a long period, as under normal conditions they are hidden by the masses of sperms accumulated in this region. They
are difficult to make out in sections of fertilized bugs on account of the
great distension and consequent thinning of the wall. It is doubtul
if they should be termed spermatheca, since neither in structure nor
in function do they correspond to the chamber so named in other insects.

A discussion of the statements and conclusions of previous observers
is postponed to a later paper. It will suffice to state here that Berlese’s
account of a cycle of changes, commencing with the penetration of the
individual cells of Berlese’s organ by spermatozoa, and the fusion of
the spermatozoon with the nucleus of the cells, as male and female
nuclei, has not been confirmed. Spermatozoa have not been found
in the cells of the ovaries, where, however, the spireme stage of mitotic
division is frequently seen.

The complex armature of teeth and spines surrounding the open-
ing into the organ of Berlese, which was taken for a stridulating organ
by Ribaga, and supposed, from its occurrence only in adult females,
to be used to summon the male, is evidently a device to enable the penis
to be kept in position during copulation, and corresponds in function
to the claspers so common in other insects.

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THE ALIMENTARY TRACT OF CIMEX.

BY

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The only available accounts of the alimentary tract of the bed bug appear to be those of Landois, who wrote in 1868, and of Patton and Cragg. Landois' paper is very incomplete and is inaccurate on many points, while the latter account was not offered as the result of a detailed study. A large number of sections of the insect, including each instar and specimens at all stages of digestion, having been prepared in the course of an investigation into the phenomena of fertilization, material has been available for a more complete investigation, the results of which are given here. As will be seen, there are many interesting anatomical features in the alimentary tract, while the process of digestion and absorption of food differs considerably from that observed in blood-sucking Diptera, resembling rather the course of events in the ticks.

The Pharynx.

The pharynx (Plate LXXXVIII, fig. 1) is a sucking pump, by means of which blood is drawn from the host. It is a flask-shaped chamber, broadest about the junction of its middle and posterior thirds, and flattened in the dorso-ventral direction; it lies anterior to the brains and passes obliquely backwards from the region of the clypeus, to which it is attached, to the ventral wall of the head. In transverse sections its lumen appears as a transverse slit, while in longitudinal sections it curves evenly in the direction indicated. Anteriorly its lumen becomes directly continuous with the food channel formed by the converging stylets of the mouth apparatus; posteriorly its abruptly narrowed end merges into the oesophagus without the intervention of any valve or sphincter muscle to control the inflow of food.
FIG. 1. The pharynx in situ. f. ch., food channel formed by the converging stylets. a., the chitinous arch which connects the lateral borders of the pharynx and the lateral borders of the clypeus. an., antenna. st., salivary pump. st., the mouth stylets. ph., the pharynx.

Fig. 2. The salivary pump and hypopharynx. hy., hypopharynx. s., opening of salivary duct. p., the piston of the salivary pump.

Fig. 3. The anterior toothed plate in the dorsal wall of the pharynx.

Fig. 4. A transverse section of the head of Cimex, in front of the eyes. d. ph., dilator muscles of the pharynx. m., retractor muscle of the maxilla. st., the basal portions of the stylets. s. d., the salivary ducts, two on each side at this level. ph., the pharynx.

Fig. 5. A transverse section of the head in front of the antennae. a., the arch connecting the lateral borders of the pharynx with the clypeus. lb., the labium. lr., the labrum, on the ventral aspect of the base of the proboscis. ph., the cavity of the pharynx. s. r., the salivary pump. s. d., the salivary ducts, one on each side at this level.

Fig. 6. A portion of the wall of the lower part of the posterior intestine. The black mass is the remains of food in the lumen. Note the faint but definite striated border of the cells. x 312.

Fig. 7. A portion of the wall of the lower part of the stomach, not fully stretched out. s. a., striated area. m. c., circular muscle fibres. m. l., longitudinal muscle fibres. x 840.

Fig. 8. A longitudinal section through the junction of the mid-gut and hind-gut, a little to one side of the middle line. m. g., wall of mid-gut (posterior intestine), enclosing a mass of black food residue. m. t., Malpighian tubes, the one on the right cut longitudinally as it enters the gut. m. g. o., the orifice of the mid-gut. r. p., the rectal plug. nl., wall of the rectum. x 160.
PLATE LXXXVIII.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Craw—The Alimentary Tract of Cimex.
The dorsal wall, which is more strongly chitinised than the ventral, is marked in its posterior two-thirds by a series of minute interlacing ridges, which divide up the area into a large number of very small fields, producing a mosaic pattern. The majority of these fields are regularly hexagonal, but near the lateral borders the ridges become thicker and the fields smaller and more elongate. The anterior and narrow third of the dorsal wall is composed of thin, even, and only slightly pigmented chitin, and is probably of the nature of a flexible membrane rather than a rigid plate. The salivary pump is situated at the apex of this region; immediately behind it there is a series of five delicate chitinous plates, four or five times longer in the transverse diameter than in the longitudinal, with gently curved anterior borders and straight posterior ones. The first of these plates is much the largest and best defined, and bears upon its straight posterior border a row (Plate LXXXVIII, fig. 3) of very minute pigmented chitinous teeth, which are directed towards the lumen of the pharynx, and appear in transverse sections as a sort of comb guarding the entrance to the chamber. The succeeding plates are ill-defined, and do not bear teeth.

The ventral wall is smooth and without markings. The lateral portions of both plates are turned sharply in the dorsal direction, so as to form lateral pockets at right angles to the main portion of the lumen, giving the whole the shape of a broad U in transverse sections (Plate LXXXVIII, fig. 4). Near the anterior end of the organ the space between the two walls of these pockets becomes obliterated, and the lateral prolongations from the dorsal and ventral plates become fused to form a strong ridge on each side; these ridges pass upwards and outwards, and fuse with the epicranium at the sides of the clypeus (Plate LXXXVIII, fig. 5). The entire pharynx is thus held firmly in position by means of these lateral supports, while at the same time the dorsal wall—the chitinous portion of which is posterior to these ridges—is free to move away from the ventral wall.

In serial sections of the head the dorsal wall of the pharynx can be traced forward as an uninterrupted and stout lamina which eventually becomes continuous with the ventral surface of the labrum. The ventral wall, on the other hand, becomes much thinner as it is traced forwards, and is eventually lost in front of the salivary reservoir, where the mouth styles converge to form the food channel. In successful cleared pre-

* On the ventral aspect but visible through the only slightly pigmented chitin.
parations of this part one can observe that while the main portion of the ventral wall ceases at the level of the anterior end of the salivary reservoir, there is an exceedingly delicate sword-shaped slip which extends forwards in the middle line in the angle formed by the converging stylets (Plate LXXXVIII, fig. 2). On the lateral margins of this prolongation there are several short and stout tubercles, similar to those so commonly found in the hypopharynx of insects; from the presence of these tubercles, and from the position of the structure, one may justifiably regard it as representing the hypopharynx.

The dilator muscles of the pharynx pass between the internal aspect of the dorsal wall of the head and the dorsal wall of the pharynx (Plate LXXXVIII, fig. 4). They fill up almost the whole of the anterior part of the head, and appear to be of considerably greater bulk than one finds in most blood-sucking insects, a circumstance which may perhaps be explained by the length and extreme narrowness of the food channel in the probosces in contrast to the relatively enormous capacity of the chamber which has to be filled with blood at each meal. The general direction of the fibres is vertical, the anterior ones, which are inserted into the softer anterior portion of the dorsal wall, being much shorter than the rest. The dilator muscles extend right to the anterior end of the pharynx, and are present even where, on account of the fusion of the lateral portions to form the supporting ridges, the two plates cannot be pulled apart to any considerable extent.

The mechanism of the sucking pump of Cimex is evidently of a comparatively simple type. The absence of any evident valvular arrangement and of controlling sphincter muscles is rather remarkable. The inflow of blood is brought about by alternating waves of contraction and relaxation of the muscles, passing from before backwards; the strong fibres inserted into the chitinised portion of the dorsal plate draw it away from the ventral plate, and open out the lateral pockets, the membranous nature of which permits of a considerable degree of separation. The general pressure of fluid in the hematocele will assist in the approximation of the two plates during the relaxation of the dilator muscles. The pulsation of the pharynx during the act of feeding can be watched in the young and transparent larva; its rate is about seventy per minute.

The Oesophagus.

The oesophagus commences at the abruptly narrowed end of the pharynx, and passes at once between the supra and infra-oesophageal ganglia, and thence directly through the thorax, to join the mid-gut
about the level of the meta-thorax. It is a simple thin walled tube, lined by cubical epithelium with a well marked chitinous intima, and surrounded by a thin layer of circular muscle fibres, which are nowhere accumulated to form a definite sphincter. In sections the tube usually shows a radiate appearance in its lower part, due to the compression of the cellular lining by the surrounding bands of muscle. The portion which lies between the large ganglia is exceedingly thin and is lined by only a few flattened cells and has no muscular layer.

The Mid-Gut.

The mid-gut occupies by far the greatest portion of the alimentary tract, and shows some interesting features which are related to the mode of life of the insect. In addition to its function as a digestive chamber, it serves as a reservoir for the storage of food, by means of which the insect is enable to live apart from a host for a considerable time. It commences abruptly in the meta-thorax, without the intervention of any sphincter or proventriculus between it and the œsophagus, and terminates at the usual point, namely, where the Malpighian tubes enter the alimentary tract. As the hind-gut is very short the apparently low insertion of these tubes is a conspicuous feature of the alimentary tract.

The mid-gut consists of two parts, which are sharply distinguished both by their shape and intimate structure. The first of these, which may be termed the stomach (the "magen" of Landois) is an oval or spindle-shaped chamber, which occupies almost the whole of the abdominal cavity in the majority of specimens. In bugs which have fed fully and recently this chamber is very greatly distended, and its walls perfectly smooth and even in contour; when digestion and absorption have proceeded for some time the surface is indented here and there at irregular intervals by circular furrows, which are due to the contraction of the circular muscle fibres in the...
Alimentary Tract of Cimex.

wall. After a period of starvation the anterior and posterior ends of the chamber are more contracted than the middle, the whole assuming an irregular spindle shape.

In fresh dissections the wall has the same appearance throughout, but when the organ is placed in a fixative it is seen that the anterior portion at once becomes opaque and white, indicating that the wall is thicker and more cellular at this point.

At about the level of the fifth or sixth segment, according to the state of distension of the gut, the lumen becomes suddenly constricted, and the remainder, which may be called the posterior intestine, is tubular. This portion, when stretched out, is longer than the stomach, and is about as long as the whole abdomen. It is therefore convoluted to accommodate itself to the cavity of the posterior end of the abdomen. The disposition of the coils and their relative size vary according to the stage of digestion, but the general course is always much the same. The posterior intestine leaves the stomach a little above its lower end and on the right side, and passes backwards to the right of the middle line and in front of the right oviduct; after a short course it bends suddenly upwards, and passes up to the level of the organ of Berlese, in the right lateral angle of the abdomen. It then passes directly inwards to the middle line, and dips downwards and again upwards, forming a loop on the dorsal aspect. After forming this loop it runs transversely to the left, dips downwards to below the level of the rectum, and rises to join the latter slightly on its left side.

![Image](image_url)
PLATE LXXXIX.

Fig. 1. A portion of the wall of the upper end of the mid-gut, in longitudinal section. m. l., longitudinal muscle fibres. m. c., circular muscle fibres. v., vacuoles at the inner end of the cells. v., vacuoles at the basal end. n., nuclei. \( \times \) 840.

Fig. 2. A portion of the wall of the upper end of the posterior intestine. st., striated border, beneath which is a denser area, and beneath this an area of vacuoles. m. l., m. c., longitudinal and circular muscles. \( \times \) 840.

Fig. 3. A portion of the wall of the lower part of the stomach. b. m., basement membrane. \( \times \) 840.

Fig. 4. A portion of the wall of the rectum. o., cells. m. l., m. c., longitudinal and circular muscle fibres. m. s., sheath of the muscle fibre. \( \times \) 840.
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

ORAGG—The Alimentary Tract of Cimex.
The transverse portions, including the descending dorsal loop, are those which are usually the most distended. The distension may be uniform, but more commonly the lumen is alternately dilated and contracted to form a series of rounded chambers. The changing contour is due to course to irregular contractions of the circular muscle fibres. The upper and lower parts of the tube, joining the stomach and rectum respectively, are usually contracted and empty.

The structure of the mid-gut indicates that the functions of digestion and absorption of food are not carried out equally by all parts. The lumen is lined throughout by a single layer of epithelium, which is supported by the usual two layers of muscle fibres, an inner circular layer and an outer longitudinal one; the cells of this epithelium, however, differ remarkably in different areas.

The cells lining the upper fourth of the chamber (Plate LXXXIX, fig. 1) are high and cylindrical, and are very evidently goblet cells, such as are believed to elaborate and to discharge into the lumen a digestive ferment, the action of which upon the contained food is to convert it into an assimilable substance. The free ends of these cells are very greatly expanded and are irregular in outline; the protoplasm is almost entirely replaced by vacuoles, the substance contained in which does not take on either acid or basic stains. The largest of these vacuoles are at the free border of the cell, and often occupy its whole extent. The membrane enclosing the vacuoles is of considerable thickness when the cell is still intact, but is ill-defined and fragmentary in those cells which have discharged their contents. Smaller vacuoles lie beneath the larger ones, the whole occupying about a third to a half of the cell. The middle part of the cell is composed of delicately reticulated protoplasm containing few vacuoles.

The nuclei are situated about the middle of the cells, or rather nearer the attached border, and show a considerable variation in shape. Many are spherical or oval, while in others the end directed outwards is drawn out to a point and slightly bent, giving the impression of a nucleus which has recently divided and has failed to round itself off. In many cells there is a second nucleus near the base of the cell; these are always smaller than the principal nuclei, and are irregularly shaped, usually having the part opposite the free border drawn out to a point or to a narrow neck, which, in some cases, is directed towards the corresponding pointed end of the larger nucleus.
The chromatin of the nuclei is for the most part distributed about the periphery in coarse grains, though in some cases, and especially in those nuclei which have pointed ends, the grains are very fine. A large nucleolus, having the shape of a short rounded rod, is present in almost all the nuclei. Although the nuclei evidently divide freely, no mitotic figures are present.

The outer ends of the cells of this epithelium show an unusual condition. Instead of resting on a basement membrane they are expanded and project freely towards the haematocele and beyond the fibres of the muscular coat, thus coming to have an appearance not unlike that of the free inner ends. The expanded portion, which makes up about a quarter of the whole cell, is almost entirely occupied by clear vacuoles which do not take on any stain. The vacuoles are regular and spherical, more or less equal in size, and are never seen in the act of discharging their contents into the haematocele, the free border being always entire. The secondary nuclei are situated at the inner limit of this vacuolated area.

The individual cells vary considerably in shape, according to the stage at which the process of formation and discharge of secretion happens to be. Many are regular and cylindrical, while others are strongly compressed in the middle and expanded widely at the free vacuolated ends; others are compressed throughout, having discharged their contents and not yet elaborated a fresh supply of the ferment. The amount of vacuolation is generally in inverse proportion at the two ends of the cell. When the inner end is greatly swollen and evidently about to discharge its contents, as in the cell on the right of the figure, the succeeding part is compressed to a narrow neck, and there are few or no vacuoles at the base. When the inner end contains few vacuoles, as in the sixth cell from the left of the figure, the middle portion is bulky, and the basal end crammed with vacuoles.

The arrangement of the muscular layer in this region is altered by the peculiar nature of the outer ends of the cells. The layer is very scanty and forms a loose network, through the meshes of which the cells can project freely into the body cavity. In a longitudinal section the circular fibres are found wedged in between the vacuolated ends of the cells, some little distance from the free borders, and here and there one finds, in thin sections, small portions of longitudinal fibres, which appear to be upon or within a cell, but which are, in reality, between two adjacent ones. The arrangement must allow of a very free com-
munication, through the cells, between the fluids in the lumen of the gut and in the hæmatocæle.

The layer of goblet cells only extends over about the upper fourth or fifth of the stomach. Below this level the height of the cells rapidly diminishes, and the remaining portion of the chamber is lined by a layer of flattened non-secreting cells (Plate LXXXIX, fig. 2). These conform closely to the type of cell met with in the gut of blood-sucking insects when distended with recently ingested blood. The cells are flattened, and have oval nuclei, the long axis of which is always directed tangentially to the contour of the gut; they never show definite nucleoli, but have the chromatin evenly distributed in coarse grains; the inner surface is covered by a fine intima, while externally there is a stout basement membrane. There is nothing, in fact, to suggest that these cells take any part in the functions of digestion or absorption of nutriment. In a few cases, and especially when the gut has not been greatly distended with blood, one finds a portion—never the whole—of the lower part of the stomach lined by cells such as those shown in Plate LXXXVIII, fig. 7. Here the outline of the cell layer is most irregular and wavy; in some places it is much flattened, and here the cytoplasm is finely granular and free from vacuoles and is bounded internally by a narrow and only faintly striated intima. Between these narrow portions there are pointed or hatchet-shaped elevations which are covered by a delicate and distinctly striated intima; the nuclei are situated in these broader portion and are usually inclined obliquely inwards. In the largest elevated portions the nuclei show clear nucleoli, and there may be a few cle.-r vacuoles in the cytoplasm. The basement membrane is distinct but is not so thick as when all the cells are flattened; the muscle layers, on the contrary, are more evident. It is only rarely that one can distinguish any limiting membrane between adjacent cells. The whole appearance of such portions of the epithelium suggests that the cells originally cubical, with a striated border and a vacuolated cytoplasm, have been partially stretched.

The epithelium of the posterior intestine presents a variety of appearances in different portions of the same specimen, and in the same portion in different specimens, being in fact always in an active condition. Its function appears to be mainly that of absorption of food material from the lumen of the gut. All stages between a high cylindrical epithelium and a flattened pavement of cells can be seen in the same series of sections. In the upper part, close to the opening from the stomach, the
cells are usually as shown in Plate LXXXIX, fig. 2. These are cylindrical in shape, but irregular, some cells being much higher than others in the same area; the main portion of the cytoplasm is finely striated in the long axis, and is without vacuoles. The inner border is marked by a well defined striation, immediately below which the cytoplasm is denser and retains basic stains more firmly than elsewhere. The inner portion of the cell is filled with regular spherical vacuoles, the largest of which are nearest the striated border. The basal ends of the cells are rounded, and project through the meshes of the muscular layer in the same manner as at the anterior end of the stomach. The nuclei are rounded or oval, and usually show a distinct round or rod-shaped nucleolus, the rest of the chromatin being arranged around the periphery in coarse grains. Some cells have two small nuclei, which are without nucleoli, and it may be noted that such cells are usually smaller than the rest and contain only a few vacuoles.

The inner surfaces of these cells are in contact with the black mass, derived from the ingested blood, which always fills the lumen of this part of the gut. When the adjacent portion of the gut is moderately distended the cells are cylindrical, as those shown in fig. 2. When, however, digestion has proceeded further, and part of the assimilable substance of the mass has been absorbed, the cells become elongated and thrust out their free ends towards and into the mass, becoming club-shaped. The swollen inner ends are filled with vacuoles, and are connected to the rest of the cell by narrow necks. The striated border is stretched out and can no longer be clearly distinguished over the vacuolated area. The appearance of sections gives the impression that the cells are penetrating the food mass, and absorbing from it its fluid constituents, which are passed from the vacuoles into the substance of the cell, and thence into the haematocoele.

Where the posterior intestine is greatly distended, and especially at its transverse loop, the cells are cubical or flattened, as shown in Plate LXXXVIII, fig. 6. In such cells the striated border can be clearly made out, but there are no vacuoles in the cytoplasm.

The Rectum.

The rectum comprises the whole of the hind-gut. When distended with food residue it is typically pear-shaped (see text-figure 1, page 709), and is of relatively large size. It lies in the middle line, but its com-
munication with the posterior intestine is situated a little to the left. Its wall is remarkable for the strength of the muscular coats and the scanty nature of the lining epithelium. The latter consists (Plate LXXXIX, fig. 4) of a single layer of very small irregularly cubical or cylindrical cells, which do not form a compact row, but are usually only attached to the basement membrane and not to one another. Many are slightly swollen at their free ends, while others are a little flattened and triangular. The cytoplasm is very finely granular and without vacuoles. The nuclei are of an unusual type. Each consists of a number of coarse granules of chromatin, which retain the stain very intensely in the iron-hæmatoxylin method, and are not united to one another or contained within a nuclear membrane; or, if such a membrane is present, it does not stain by any of the ordinary basic stains. These granules are usually arranged in a more or less compact mass but they may be scattered about the free end of the cell. The number and size of these chromatin particles vary very greatly.

The muscular coat consists of very coarse circular fibres, arranged at intervals from one another throughout the length of the organ; in life the contraction of these fibres produces a series of transverse furrows and ridges. The longitudinal fibres are finer and more widely separated from one another. Owing to the coarseness of these fibres and to the manner in which the two layers are arranged in relation to one another, the sarcolemma is exceedingly well shown when the inevitable shrinkage of the contractile substance occurs during fixation.

The most interesting feature of the rectum is the absence of rectal papillæ, and the presence in their stead of a plug of cells, which surround the orifice by which the chamber communicates with the posterior intestine. The orifice is exceedingly small, and is surrounded by a small sphincter muscle; the extremity of the mid-gut dips into the rectum at the point corresponding to the stalk of the pear, but a little to the ventral surface and on the left side, and produces a little papilla on the inner surface, the orifice at the summit of the papilla appearing in sections as a series of radiating crevices. On the rectal aspect of this papilla there is a layer of large cylindrical cells (Plate LXXXVIII, fig. 8), which cover the whole of the broad end of the rectum, and are inclined inwards on the sides of the papilla so as to prolong the narrow channel through which the contents must pass. At the sides this layer of cells terminates abruptly, and is replaced by the scanty layer of small cells already described.
Contents of the Alimentary Tract.

It has already been stated that the mid-gut of Cimex serves both as a digestive chamber and as a reservoir for food. Under natural conditions the tract is almost always well filled, for the bug does not wait until one meal is digested and the food absorbed before taking a second meal, as is the habit of the majority of blood-sucking insects. Digestion and absorption, in fact, go on continuously during the life of the insect, and it is only during abnormal conditions, when food is not available, that the stomach becomes empty. The conditions resemble those met with in ticks.

Bugs kept in captivity under conditions as nearly normal as possible, and at summer heat, feed every day or every second day. The abdomen is always well rounded and the entire alimentary tract always filled with partially digested blood and the non-assimilable residue derived from it. If, however, food is withheld for ten days or more the abdomen becomes flattened, its middle portion becomes lighter in colour and is seen to contain bubbles of gas, which on dissection prove to be within the mid-gut, the wall of which appears as an exceedingly delicate transparent membrane surrounding them. The rectum is the only portion of the tract which contains solid matter; it is distended to a typical pear shape, the outline of which can be plainly seen through the integument.

Such starved bugs feed readily even in a bright light, taking from seven to ten minutes to complete the meal, a period considerably longer than is usual in those which are allowed to feed every second or third day. Usually they attack the host at once, and remain in position till replete, but occasionally they relinquish their hold before they are quite gorged, and return after a minute or so. During the early part of the meal the inflow of blood can be seen distinctly through the integument of the pro-thorax and anterior abdominal segments. As the blood accumulates the mass separates the dorsal and ventral plates until the bug becomes spherical or nearly so in transverse section. In some cases there is also a marked elongation of the abdominal segments, the intersegmental membranes becoming visible as lighter transverse stripes. The reason for this elongation, which occurs only in a minority of cases, is not evident.

Immediately after feeding the bug passes a considerable quantity of thick black fluid—the contents of the rectum, derived from the previous meal. The manner in which this is done is peculiar and characteristic; the bug, moving about in a quick jerky way, suddenly turn
round, moves a short distance backwards, and thrusts the distal end of
the abdomen back with a jerk, expelling the fluid. The fluid is black
when deposited on filter paper, but light brown if smeared out.

A few minutes after the contents of the rectum are passed out a
lighter and more fluid material is ejected, and in many cases this is
followed by a clear colourless fluid in small drops. Probably this second
portion consists of the contents of the Malpighian tubes, at first
tinted by the residue in the rectum. Unaltered blood is never passed out.

After twenty-four hours the bug is still rotund, though not so
obviously distended as before. There is a little black food residue in the
anterior part of the posterior intestine, but the greater part of it is empty.
The rectum is contracted and empty. After two days the stomach is
evidently reduced in size, and now occupies only about half the cavity of
the abdomen; the posterior intestine contains more of the black material,
which is collected into small spherical chambers formed by irregular
contractions of the circular muscle fibres. By the end of the fourth day
the entire posterior intestine is filled with this black residue, some of
which has reached the rectum, while the reduction of the contents of the
stomach proceeds. The rectum becomes distended about a week after
the meal, and by this time the body of the insect has become flattened
with the emptying of the stomach. In ten days the stomach contains
only bubbles of gas and a small amount of brown granular material.

Defecation takes place immediately after feeding, as already de-
scribed, and at frequent intervals during the first two days, though the
amounts passed at each act are successively smaller. During the later
days the food residue accumulates in the rectum, only very small quan-
tities being passed out until the necessary stimulus of a fresh meal is
forthcoming.*

It will be seen that the complete assimilation of one meal of blood
takes at least a week, although many bugs will feed readily within
twenty-four hours of the previous meal, and almost all on the third day.
Under normal conditions, therefore, portions of three or more meals will
be present in the gut at the same time—a condition of affairs very
different to that which is found in other blood-sucking insects.

* A further point may be noted as having perhaps some practical bearing. Bugs
very rarely rest on the skin of the host while feeding. They prefer to remain on the
clothing, or, if fed in tubes as is done in the laboratory, on the filter paper, and reach
the skin from the edge of their resting place. Consequently their excretion usually falls
on to the clothing, not on the skin of the host.
THE PROCESS OF DIGESTION.

In Cimex, as in other insects, the ingested food is digested by an extra-cellular process, that is to say, it is subjected to the action of ferments elaborated by the epithelium of the mid-gut, and thus converted into substances which are subsequently absorbed and carried into the circulation. The blood corpuscles are emptied of their fluid contents, and the non-assimilable residue is passed on to the rectum and subsequently evacuated. During the whole process the substances absorbed remain in solution, and cannot be recognized by any staining reaction. Although the general course of events in the alimentation of the bug appears very similar to that described by Samson for the tick, the granules and intra-cellular deposits found by her are not represented in the bug, in which the transference of the alimentary substances from the lumen of the gut to the haematoccele is brought about by an essentially different process. The absence of any demonstrable series of changes in the cells during the passage of the absorbed substances to the haematoccele renders the subject even more obscure than it is in the Acari.

The most striking fact regarding digestion and absorption in Cimex is that these processes are continuous and regular. Except in the event of starvation for unusually long periods, the different parts of the epithelium are always found in much the same condition, and there is little to indicate, apart from the condition of the contents of the gut, whether the insect has fed recently or is just ready to feed. The large secreting cells at the anterior end of the stomach are always in the condition described above and depicted in the first figure on Plate LXXXIX, and the epithelium of the lower part of the gut is always thin and stretched, showing no evidence of an active function. The epithelium of the posterior intestine always contains vacuoles at one or other part, even after a prolonged period of starvation. This continuity of the process is in sharp distinction to what is found in the blood-sucking Diptera, in which the whole meal is digested and absorbed before another is ingested.

The continuity of the process, and the fact that a fresh meal is ingested long before the previous one has been completely absorbed, render it impossible to obtain much information regarding the various stages, and the parts played by the different areas of secreting cells from a study of the adult alone, and it becomes necessary to have recourse to the young larva. The alimentary tract of the newly hatched and unfed larva differs from that of the adult only in the shorter length and straighter course of the posterior intestine. As the larva feeds
readily on the second or third day after emerging from the egg the condition of the gut at that period may be regarded as one of readiness for the digestion of food.

In the unfed larva the epithelium at the anterior end of the mid-gut is cubical, or in some places cylindrical, and is more regular than that of the adult. It is already in an active condition on the first day, and by the second day the whole layer of cells is secreting vigorously. The free ends of the cells are swollen and project freely into the lumen; they either contain a few large vacuoles which do not take on any stain, or the entire free end of the cell is converted into a single vacuole, which may be entire or may have already partially discharged its contents into the lumen. The upper end of the digestive chamber is entirely filled up with irregular spherules which have been discharged from the vacuolated cells, and which disintegrate into a coarse granular substance as they pass away from the epithelium. The function of these cells is evidently the elaboration of a ferment, since the vacuoles are present and the whole epithelium in an active condition before any food has been ingested. The granules which lie in the lumen are probably produced by the action of the fixative on a coagulable fluid; they are presumably present also in the adult, but cannot be seen on account of the presence of partially digested blood.

The epithelium of the lower part of the stomach in the unfed larva is cubical or slightly flattened, and is not vacuolated. It has a well-defined intima, which is split off from the cells in some preparations in a manner which gives it the appearance of a peritrophic membrane.

The cells lining the short posterior intestine are very different to those in the adult. They are cubical and regular, and are without vacuoles or other signs of functional activity. It is unlikely, therefore, that they take any part in the formation of digestive ferment, and it is reasonable to suppose, from what has been already said regarding the condition of this part of the gut in the adult, that its function is to absorb substances rendered assimilable by the action of the ferments produced at the anterior end of the gut.

The changes which occur in the ingested blood, resulting in the eventual disintegration of the corpuscles and the formation of a pigmented residue, are best followed in the larva. In specimens fixed immediately after feeding the whole of the stomach is found packed with corpuscles, which are not, one may note, in rouleaux. The tubular posterior intestine contains no corpuscles. In two or three hours fine striae of an
eosinophile substance appear among the corpuscles, with here and there a few particles of black pigment, some of which is also found in the upper part of the posterior intestine. The homogenous eosinophile material gradually increases in amount and accumulates about the lower end of the stomach, while a debris of broken down corpuscles and pigment accumulates in the posterior intestine. The corpuscles become compressed and angular, especially at the lower end of the chamber. These initial changes are well marked at the end of eight hours. In twenty-four hours the cells are completely broken up, and the whole of the stomach is filled with a mass of ill-defined light brown granules which are stained only with difficulty by eosin and not at all by hæmatoxylin; intermingled with these one finds granules of black pigment, and here and there faint outlines of red cells.

The contents of the stomach remain in this condition for the next few days, during which there is a gradual increase in the amount of pigmented matter in the posterior intestine. The moult takes place usually on the second day after the first feed and the nymph is ready to feed on the day following. When the next meal is ingested the fresh corpuscles are mingled with the granular debris by means of the peristaltic contractions of the wall of the gut, and become broken up in the same manner. Fresh blood is ingested periodically, while disintegration, digestion, and absorption go on continuously during the life of the insect. Under natural conditions fresh uninjured corpuscles, granular debris, and pigment particles, are always found in the stomach.

REFERENCES.


OBSERVATIONS ON DYSENTERY CASES FROM MESOPOTAMIA.

BY

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During the second half of 1916 and the first two months of the present year the opportunity occurred of examining stools from a considerable number of cases invalided from Mesopotamia on account of dysentery and allied complaints. At the commencement the work was limited to a routine microscopical examination of the stools, the main object of which was the detection of E. histolytica and its cysts. Later on it became possible to supplement this by a bacteriological examination in cases which showed symptoms, and to inquire in detail into the patient's history in cases of special interest.

The condition of the patients on admission varied from time to time. In the early months the majority of the cases were comparatively recent, while later on most of the men were either convalescents sent to India to recruit after a severe or prolonged illness, or had obstinate chronic symptoms.

Six hundred and thirteen cases are dealt with in the period covered by these notes. Twenty-two of these had acute attacks while in this hospital, and in 128 cases mucus, in varying quantities, with or without pus and blood, was present in one or more of the stools sent to the laboratory for examination. These cases showed every degree of severity from a definitely established chronic dysentery to cases in which a small amount of soft mucus, not necessarily indicating the existence of an intestinal ulcer, was found.
Dysentery Cases from Mesopotamia.

Microscopic Examination of the Stools.

1,543 stools, or an average of two and-a-half per case, were examined for protozoa. The majority of these were sent for examination as a routine measure, the plan aimed at being three examinations in each case at weekly intervals; many, however, were sent by the medical officers in charge on account of the presence of symptoms, thus increasing the proportion of abnormal stools. The nature of the stools examined is indicated by the table on page 310, in which a rough classification of three selected series is attempted. It should be noted that a mild saline purge was frequently given to ensure that a stool should be available for examination on the appointed day.

Protozoa were found in the stools of 244 cases, or 40 per cent. of those examined. Eighty-eight of these were found to harbour more than one form. The following table shows the number of cases in which the various protozoa were found. The probable percentage actually infected will be considered later.

<table>
<thead>
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<th>Protozoon</th>
<th>Single Infections</th>
<th>Multiple Infections</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. histolytica</td>
<td>31</td>
<td>33</td>
<td>64</td>
</tr>
<tr>
<td>E. coli</td>
<td>77</td>
<td>64</td>
<td>141</td>
</tr>
<tr>
<td>E. nana</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Lamblia intestinalis</td>
<td>39</td>
<td>39</td>
<td>66</td>
</tr>
<tr>
<td>Trichomonas intestinalis</td>
<td>31</td>
<td>27</td>
<td>58</td>
</tr>
<tr>
<td>Tetramitus mesnili</td>
<td>6</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Isospora</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Undetermined flagellates</td>
<td>3</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>&quot;I cysts&quot;</td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

E. histolytica and Amoebic Dysentery.

The 64 cases in which E. histolytica was found may be summarized as follows:

<table>
<thead>
<tr>
<th>Description</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free form alone</td>
<td>15</td>
</tr>
<tr>
<td>Free form, cysts found subsequently in the same case</td>
<td>2</td>
</tr>
<tr>
<td>Free form, with other protozoa</td>
<td>11</td>
</tr>
<tr>
<td>Cysts alone</td>
<td>14</td>
</tr>
<tr>
<td>Cysts, with other protozoa</td>
<td>22</td>
</tr>
</tbody>
</table>

In all these cases there was a history of one or more attacks of acute dysentery. In eight of the cases in which the free ameba was found the patient was at the time suffering from an acute relapse; in one of these, the most severe and prolonged of the series, the Shiga bacillus was isolated twice, on the first and fourteenth day of the attack. Twelve
cases suffered from chronic dysentery of varying severity, and from two of these, bacilli were isolated, of the Flexner and Y groups respectively. In the remaining eight cases there were no active symptoms at the time of examination, beyond the slight abdominal discomfort and irregularity of the bowels which are so common during convalescence from the disease. Only ten of the cases in which amœbæ were found were examined bacteriologically.

Two of the chronic cases showed interesting features. In one the patient gave a history of a single mild attack of dysentery, but was much emaciated and suffered from a mild but intractable diarrhoea. His stools contained large numbers of Lamblia cysts, and on one occasion the flagellates were found in considerable numbers; he also had heavy infections of Trichomonas and E. coli, and on two occasions a small flagellate, which could not be identified, was found. Out of the nine stools examined, only one contained mucus, and then only in small quantity and showing no signs of blood to the naked eye. Microscopically, however, it was found to contain a large number of very active amœbæ, the majority of which contained blood corpuscles, while no amœbæ, either histolytica or coli, could be found in the faecal matter of the stool. The patient was put on a course of emetine and improved considerably within a short time. Such a case might easily have been regarded as ‘flagellate diarrhoea.’

In another case there was a heavy infection of Isospora and Lamblia in addition to the histolytica. The patient was in a very weak condition on admission, and gave a history of repeated attacks of dysentery for months past, none of the attacks having been severe. The stools were invariably watery, and contained very numerous small flakes of tenacious mucus, with at times a few streaks of blood. The appearance of these stools was remarkably constant, and during the ten days he was under observation he did not at any time pass a motion of the type ordinarily met with in chronic dysentery. On the first two examinations only Isospora, in very large numbers, was found; at the third a single amœba was found in addition. On the next examination, however, there were very numerous amœbæ, many of them containing red cells, although the stool did not show any naked-eye evidence of blood, and only a few corpuscles could be found free. No amœbæ were seen after a prolonged search at the next two examinations, the patient, of course, having been given emetine as soon as the amœbæ were recognized. This patient died within a short time after admission, and, although he
Dysentery Cases from Mesopotamia.

had never shown any signs of dysentery, in the ordinary sense, while in hospital, his stools having been passed without much pain and never having contained appreciable quantities of blood, it was found at the post-mortem that the peritonitis which had been the immediate cause of death was due to the rupture of an old dysenteric ulcer. A considerable part of the large intestine was studded with small ulcers, many of them so deep that the floor was formed by peritoneum only. The appearance of these ulcers suggested bacillary rather than amœbic dysentery.

Of the thirty-six cases in which the cysts of histolytica were found, four were chronic infections, the remainder convalescents with no symptoms. Including the two cases in which free amœbae were also found, the figures show a percentage of 6·2 of the total cases examined. Observations on the persistence of the cyst infection and its response to emetine treatment could not be carried out as, under the arrangements in force at the time the work was done, infected cases were transferred elsewhere at the earliest opportunity.

The Shiga bacillus was recovered from one of the four chronic cases which showed cysts. In the other three neither amœbae nor bacilli were found; the absence of free amœbae from the mucoid stools suggests that the disease from which the men were suffering was not amœbic dysentery in spite of the presence of the cysts.

As regards the diagnosis of E. histolytica, most reliance was placed on the presence of ingested red corpuscles and on the motility, the kind rather than the degree, of the latter being perhaps the most striking point of differentiation between this amœba and E. coli. In the acute cases no difficulty was met with, as amœbae showing the typical characters were usually present in large numbers. In some of the chronic cases, and not always those with the most marked symptoms, very numerous amœbae were found, as easily differentiated as in the acute cases, while in other cases it was necessary to examine a considerable number of individuals before a diagnosis could be arrived at. E. histolytica has not been seen, except in the cyst-carrier case referred to above, in the faecal matter of the stool, but always in the mucus or pus; on several occasions E. coli and its cysts have been found in the faecal matter while the pathogenic amœba has been present in blood-stained mucus in the same stool. In all cases in which the species could not be identified with certainty, a second stool was obtained and examined as soon as possible after it was passed. In the cyst cases little difficulty was met with in determining the presence of an infection, for although in many
individual organisms it was impossible to be certain of the species, further
search, if necessary on subsequent days, resulted in the finding of the
characteristic 4-nucleate form with chromidal rods; no infection has
been diagnosed *histolytica* unless these were present.

The nuclear characters of the amœbæ have been studied in prepa-
rations stained by iron-haematoxylin, and have been found to correspond
to the *histolytica* type as distinguished from the *tetragena*. The character
of the nucleus, as seen in fresh preparations, has been of little service as
a guide in distinguishing between *histolytica* and *coli*.

**ENTAMOEBA COLI.**

This amœba was found in the stools of 141 patients, or 23 per cent
of those whose stools were examined. Of the 452 stools which were
examined from these patients, the amœba, either free or encysted,
was found in 237, or 52 per cent.

The amœba is frequently present only in small numbers, and
may therefore be easily missed in the course of an examination lasting
fifteen minutes or so, so that the actual proportion of the patients
harbouring the parasite must be considerably higher than that stated
above. To ascertain the approximate percentage, the findings in a
series of 87 cases, stools from which were examined on three or
more occasions, have been calculated; three hundred and seventy-six
stools, or an average of 4·3 per case, were examined, and the amœba or
its cysts found one hundred and seventy-five times. The chances of
finding the amœba in an infected case are, therefore, about one in two,
and from this it may be inferred that the actual number of cases infected
is at least double the number in which the amœba was found, i.e., of the
present series of dysentery convalescents probably about 50 per cent
harboured *E. coli*.

Free amœbæ were found alone in 47 per cent of the stools, free
forms and cysts in 19 per cent, and cysts alone in 34 per cent. The
predominance of free forms is to be explained by the nature of the stools,
which, as was to be expected from the nature of the cases examined,
were more frequently soft or liquid than formed. As will be seen from
the table on page 310, normal formed stools were quite exceptional.
The cysts, however, were frequently present in soft or liquid stools, which
suggests that some factor other than a mere change in the density of the
medium in which the amœbæ are living is concerned in bringing about
their encystment. It has frequently been observed that while at one examination only free amoebæ, or free amoebæ and the bi-nucleate cysts, were found, at subsequent examinations, without any change in the nature of the stool, the majority of the amoebæ were encysted and many of them in the final 8-nucleate stage.

The number of amoebæ present in the stools of infected individuals varies from time to time and within very wide limits, as has been noted by several observers. In the majority of cases they are not numerous, only half a dozen or so being found during the course of an ordinary examination of ten or fifteen minutes; frequently there are very marked variations in the numbers present in different parts of the same stool, a fact which makes 'amoeba counts' of doubtful value. In a few of the cases of the present series this amoeba has been found in very large numbers in soft and normal stools; they appeared, in fact, to be more numerous than do the leucocytes in a normal blood film, and must, indeed, have formed a not inconsiderable proportion of the total mass of the stool. The patients in these cases have shown no symptoms which could be referred to the infection. Nine stools from one case were examined during thirty-five days, and at the first eight of these examinations large numbers were invariably present; in cover-slip preparations made on one occasion and stained by iron hæmatoxylin, it has been possible to count over two hundred amoebæ within a space of not more than half a square inch of a thin film. At the ninth examination not a single individual could be found during a prolonged search, nor could any explanation of their disappearance be suggested. In another case (No. 417, vide appendix) large numbers were present on the first four examinations, very few on the fifth, and none at all on the sixth, on which occasion the stool contained a patch of mucus from which the Shiga bacillus was isolated. In this case the recrudescence of the dysentery was coincident with the disappearance of the amoebæ. The slight infection which is found in the great majority of cases, and the occasional occurrence of very heavy infections, suggests an analogy between this parasite and other 'natural' infections, such as Trypanosoma lewisi in the rat, in which the very large numbers which are found when the animal first becomes infected steadily decrease down to a certain level as the parasite becomes established in its new host.

E. coli does not appear to flourish well in acute inflammatory conditions of the intestine, though it is not necessarily killed off. Several cases have come under observation in which it has been found
during the periods when the dysentery was quiescent, and has disappeared on the occurrence of a relapse, to reappear in convalescence. It has never been seen in the stools in cases of acute dysentery, though in several chronic cases it has been found in the faecal matter of the stool. Cases Nos. 471, 510 and 547 (vide appendix) illustrate the occurrence of the amoeba in such cases. One might suggest that the association of this amoeba with normal faecal matter be added as a distinctive feature between it and the pathogenic species.

**ENTAMOEBA NANA.**

In several cases towards the end of the series small amoebae were met with in considerable numbers, together with cysts which could not be identified, but which were certainly not those of *Amoeba limax*. An opportunity occurred later of showing stained preparations of these organisms to Lieutenant-Colonel Wenyon, who diagnosed them as *Entamoeba nana*, the newly published description* of which was not at that time available. Subsequent examination of stained preparations made for the study of other intestinal parasites has shown that this amoeba has been of quite common occurrence, though not recognized in the fresh preparations. In twenty-three of the total cases examined bodies almost certainly protozoal in nature, but not further identified, were present; many of these were possibly degenerated flagellates, but it seems likely, from subsequent experience, that the majority were *E. nana* which had lost its motility.

**INTESTINAL FLAGELLATES AND 'FLAGELLATE DIARRHOEA.'**

*Lamblia* was found to be a common inhabitant of the intestine in the cases examined. The relative frequency of the flagellate and encysted forms, together with an indication of the nature of the stools in which they were found, is shown in the table below. The parasite, however, is of much more common occurrence than the figures would indicate, as on account of the small numbers present in many stools it must frequently have been missed. In a series of 47 cases, all of which were examined three or more times, 95 stools were positive and 98 negative, so that, as in *E. coli* infections, the chances of finding the

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Dysentery Cases from Mesopotamia.

parasite are about one in two. The 69 cases found to be infected out of a total of 613 representing a percentage of 11.25, the actual percentage of cases infected would probably be about 22.

Flagellates are rarely found except in liquid stools, in which they may occur alone or with the cysts. The number varies with the character of the stool. In watery stools containing little or no mucus they are generally scanty, the individuals present being possibly those which were not attached to the mucosa, at the moment the wave of peristalsis carried downwards the contents of the part of the intestine in which they were moving. When mucus is present in considerable amount, and is closely incorporated with the faecal matter, indicating that it has had origin high up in the intestine, the numbers present may be enormous, far greater in fact than is seen in the case of any other intestinal protozoon. In such cases the parasites are often motionless, or show only feeble movements of the flagella, probably on account of the mucus in which they are entangled. It is interesting to note that the flagellates do not appear in the stools during attacks of diarrhoea or after purgation with nearly the same frequency as does Trichomonas under similar conditions. As will be seen from the table, out of 66 liquid stools passed by patients known to be infected, only 21, or 32 per cent, showed the flagellate, as compared with 65 per cent in the case of Trichomonas; the proportion of these stools which contained mucus is more than twice as great in the Lamblia infections as with Trichomonas.

The cysts have been found in stools of every variety, except the stools of blood and mucus of acute dysentery. The number varies very greatly from a fifty or more in one field to only one or two in a whole cover-slip preparation. In several cases they have not been found until the fourth or fifth examination, and in one case, which was examined eight times at approximately weekly intervals, the cysts were only found at the seventh examination, and then in large numbers.

Of the three flagellates commonly found in the human intestine, Lamblia is the one which is the most generally believed to be pathogenic. Statements to this effect are to be found in most text-books on tropical medicine, and in the numerous papers which have been published recently on intestinal parasites the majority of writers have accepted the current belief without remark. The clinical condition for which it is supposed to be responsible is, however, very ill defined, and consists in little more than a sequence of attacks of diarrhoea of a mild type. The mere presence of the parasite in the stools in such a condition is very slender evidence
of its pathogenicity, in view of the very numerous causes to which such a diarrhoea might be ascribed. It was therefore of interest to examine the records of the cases included in the above series, to see if any evidence for or against a pathogenic rôle on the part of this parasite can be found. The series is not ideal for the purpose, but the percentage of infected cases is sufficiently large to make it worth while comparing them with others of the same series in which the parasite was not found; if no differences emerge, the argument against *Lamblia* being able to produce any pathological condition of importance is a strong one.

One would expect, other things being equal, that if *Lamblia* be capable of producing diarrhoea the stools from infected cases would on the whole tend to be looser and more irregular than the stools from non-infected cases. To ascertain if there be any evidence of this, the following table has been constructed from notes regarding the nature of the stools made at the time of the microscopical examination. 1050 stools, of which roughly one-fifth are from cases known to be infected with *Lamblia*, one-fifth from *Trichomonas* cases, and the remainder from cases in which no parasites were found, are classified according to the consistence and the presence or absence of mucus. The notes were made by the same observer and by the same standard throughout, and, though an exact differentiation is hardly possible, the numbers are sufficiently large to bring out any marked differences in the stools of the three classes of patients, if such existed. The cases are all patients with a history of dysentery or diarrhoea, some with symptoms while in hospital and others convalescent; it will be noticed that formed stools are infrequent. The figures show that to ordinary clinical observation patients infected with *Lamblia* are not specially liable to diarrhoea.
Dysentery Cases from Mesopotamia

Table showing the Occurrence of Lamblia and Trichomonas in Various Kinds of Stool.

<table>
<thead>
<tr>
<th></th>
<th>Lamblia.</th>
<th>Trichomonas.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysenteric stools, chronic, showing blood ...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Constipated stools without mucus ...</td>
<td>...</td>
<td>2</td>
</tr>
<tr>
<td>Do. with mucus ...</td>
<td>...</td>
<td>10</td>
</tr>
<tr>
<td>Formed stools without mucus Do. with mucus ...</td>
<td>...</td>
<td>9</td>
</tr>
<tr>
<td>Soft stools without mucus Do. with mucus ...</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Liquid stools without mucus Do. with mucus ...</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Total ...</td>
<td>16</td>
<td>93</td>
</tr>
<tr>
<td>percentage ...</td>
<td>13.3</td>
<td>77.5</td>
</tr>
</tbody>
</table>

Parasitised and Non-Parasitised Cases compared.

(Figures = percentages.)

<table>
<thead>
<tr>
<th></th>
<th>Lamblia:—229 stools from 69 cases.</th>
<th>Trichomonas:—196 stools from 57 cases.</th>
<th>Non-Parasitised:—625 stools from 369 cases.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysenteric stools ...</td>
<td>...</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Constipated stools ...</td>
<td>3.5</td>
<td>26</td>
<td>61</td>
</tr>
<tr>
<td>Formed stools ...</td>
<td>7.9</td>
<td>0.4</td>
<td>8.3</td>
</tr>
<tr>
<td>Soft stools ...</td>
<td>51.5</td>
<td>4.0</td>
<td>55.5</td>
</tr>
<tr>
<td>Liquid stools ...</td>
<td>29.5</td>
<td>8.3</td>
<td>38.8</td>
</tr>
<tr>
<td>Total ...</td>
<td>83.4</td>
<td>16.6</td>
<td>100.0</td>
</tr>
</tbody>
</table>
In the clinical aspect of these cases, and in their progress while in hospital, there was nothing to distinguish a case infected with Lamblia from one which was not. All grades were met with, from men who were practically normal to definitely established cases of chronic dysentery; some of the men improved rapidly with rest and an appropriate diet, while others made little or no progress. The histories of the patients before admission to this hospital similarly showed no indication of the infection. Many of the men had had one severe attack of dysentery with an uninterrupted convalescence, many several attacks, and while some of the patients had a history of repeated attacks of a mild type, or of diarrhoea preceding the dysentery or following it, others had not, and no type could be said to be at all characteristic of a Lamblia infection. In fact, as far as one can tell from this series of cases, the only means of diagnosing 'lambliasis' is by a microscopic examination of the stools, for the condition does not appear to have any distinguishing clinical features whatever. Reports on ten of the cases are included in the appendix to this paper. The cases quoted are chosen as representative of the series, and it may be said that for almost every positive case a parallel one could have been quoted in which Lamblia was not found.

As regards the actual cause of the disease from which the men infected were suffering or had suffered, four of the cases showed free histolytica in the stools, and one showed cysts of the same parasite. In twelve cases the stools were plated out on account of the presence of mucus which appeared to indicate ulceration, and from two of these the Shiga bacillus was recovered. The typhoid bacillus was isolated in two cases, one of which subsequently proved to have enteric fever. In three of the cases Isospora was also present.

**Trichomonas intestinalis.**

This flagellate was not found quite so frequently as Lamblia, the fifty-seven patients found to be infected representing a percentage of 9.3. Ninety-three stools out of the 196 from these men showed the parasite. It was not, however, so frequently seen in the stools of the infected men as was Lamblia, as in a series of 30 cases examined three or more times 63 stools out of 155 were positive, or two out of five. The calculated percentage is therefore 23.

A considerable difference was noted between this parasite and Lamblia as regards the kind of stool in which it was present. As has
been pointed out, *Lamblia* flagellates are rarely found except in liquid stools, and never in large numbers except in stools characterized by the presence of soft mucus which has originated high up in the intestine. *Trichomonas*, on the other hand, is found quite frequently in soft stools and has even been found in normal formed stools, still actively motile when the faecal matter was emulsified in saline solution. Further, though the degree of infection varies a great deal, there appears to be no relation between the kind of stool the patient passes and the number of flagellates present. Very large numbers have been met with in soft stools, normal except in their consistence, and equally heavy infections in watery stools. *Trichomonas* is the only flagellate which has been met with in the stools of blood and mucus in acute dysentery; in two cases it has been found together with the amœba, and in numbers far exceeding those of the latter, and in one acute case it was found in a stool from which Morgan’s bacillus was isolated.

The kind of stool in which it has been found is indicated in the table on page 310. It will be seen that the stools of cases in which *Trichomonas* was present are on the whole distinctly looser than those from non-parasitised cases.

What has been said with reference to the pathogenicity of *Lamblia* applies equally to *Trichomonas*. The most that could be inferred from the nature of the stools passed by infected persons is that this flagellate flourishes better in an abnormal condition of the intestine than in a healthy one. Clinically the cases cannot be differentiated, and the same may be said with regard to the history of the cases from the commencement of symptoms. All types of dysentery, from single acute attacks to cases of long continued irregularity without acute symptoms, have been found to be infected, and so far as can be seen the presence of the parasite has had no effect on the course of the disease.

**Tetramitus Mesnili.**

*Tetramitus mesnili* was probably of much more frequent occurrence than is indicated by the figures, for the cysts have been found in stained films in a considerable number of cases when they were not noticed in the fresh preparation. There was nothing to suggest that the flagellate had any relation to the patient’s condition.

In eleven cases flagellates which could not be identified were found. They were present in small numbers, and not subsequently found in the stools of the same patient. *Prowazekia* was found in one case.
ISOSPORÁ.

In two of the cases in which this parasite was present the men reached this hospital in very bad condition, and died after a lingering illness. One of them has already been referred to on page 303, in connection with the unusual *histolytica* infection which was present. Unfortunately the liver was not examined at the *post-mortem*. Two of the other cases, including one fatal one in which there was an infection with the Shiga bacillus, are recorded in detail in the appendix. The remaining case was a convalescent in whose stools no mucus was seen on the four examinations made; he had also a *Lamblia* infection. The cysts of *Isospora* were only found once in this case, in a liquid bile-stained stool, and the number present was very small. Although there was a very heavy infection in the two fatal cases, in each of them there was other adequate cause for the patient's condition, and no indication of the pathogenicity of the *Coccidium*.

**BACTERIOLOGICAL EXAMINATION OF THE STOOLS.**

The isolation of bacilli of the dysentery group was attempted in 96 cases, of which 60 are included in the last 200 of the series, when the majority of the patients were either convalescents or men with chronic symptoms. A bacteriological examination was only carried out when the stool showed some indication of existing ulceration. MacConkey plates were used, and the selected piece of mucus was washed in several changes of saline to free it as far as possible from faecal matter before it was rubbed on the plates. The colonies selected at the end of 24 hours were put on litmus-mannite-agar, stab and stroke cultures being made in the same tube. Gas-forming organisms being eliminated by the presence of bubbles in the agar, non-motile bacilli were tested against Flexner or Shiga high titre serum, according to whether they produced acid from the mannite or not, and at the same time inoculated in the following media:—broth, litmus milk, lactose, glucose, dulcite, saccharose, mannite, and maltose. In a few cases motile organisms were also investigated, but no attempt was made to follow up the gas-forming organisms. Cultures which gave the reactions of known organisms but which did not agglutinate with the appropriate serum were subcultured repeatedly on broth and tried again.

It was noted that bacilli of the Flexner group form sufficient acid on the mannite-agar tubes to make the medium unfavourable for their
Dysentery Cases from Mesopotamia.

own growth; several cultures on the original tubes were found to be dead at the end of a fortnight.

The results obtained are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Acute cases</th>
<th>Chronic cases</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shiga bacillus</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Flexner bacillus</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Y bacillus</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Morgan's bacillus No. 1</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td><strong>E. histolytica present</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shiga bacillus</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flexner bacillus</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Y bacillus</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Morgan's bacillus No. 1</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Shiga bacillus and Morgan’s No. 1 bacillus</td>
<td>13</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Flexner bacillus</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Y bacillus</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Morgan's bacillus No. 1</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Negative Results—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. histolytica present</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>E. histolytica absent</td>
<td>4</td>
<td>52</td>
<td>56</td>
</tr>
<tr>
<td>Total negative cases</td>
<td>6</td>
<td>57</td>
<td>63</td>
</tr>
<tr>
<td>Total cases examined</td>
<td>14</td>
<td>82</td>
<td>96</td>
</tr>
</tbody>
</table>

Included in the negative results are two cases in which the typhoid bacillus was isolated. The stools in both cases were sent to the laboratory on account of the presence of mucus, there being no suggestion at the time that the patients were suffering from enteric fever.

The Shiga bacillus.—This bacillus was isolated 26 times from the above 21 cases, as follows:

2 platings positive out of 2, in 1 acute case (histolytica present).

1 " " " 1, " 4.
1 " " 3, " 1.
1 " " 1, " 5 chronic cases.
1 " " 2, " 2 " " " .
1 " " 3, " 2 " " " .
1 " " 4, " 1 " " " .
2 " " 3, " 2 " " " .
3 " " 4, " 1 " " " .
1 " " 5, " 1 " " " .
1 " " 7, " 1 " " " (Morgan’s bacillus also present.

In the acute case which was plated out three times with only one positive result, very numerous colonies were obtained on the first occasion, the two negative results being obtained after an appendicostomy had been performed and the intestine irrigated.
The above strains were found to show considerable variations in their agglutinating properties with the high titre serum. Several of them which did not agglutinate at all when first isolated reacted in a normal manner after daily subculture on broth for a week. Most of the cultures were kept in the incubator and subcultured monthly, and at the end of a period of approximately six months they were tested again. The results are as follows:

<table>
<thead>
<tr>
<th>Dilution of serum 1 in</th>
<th>500</th>
<th>1,000</th>
<th>1,500</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 strains, not further tested</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>5 Do. do.</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>3 Do. do.</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>3 strains, on first isolation after monthly subculture</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>1 Do. on first isolation after monthly subculture</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>1 Do. on first isolation after monthly subculture</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>5 Do. on first isolation after subculture on broth, after six months on agar</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td>+ + + +</td>
</tr>
<tr>
<td>4 Do. on first isolation, and after subculture on broth and six months on agar</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Two of the four strains which did not agglutinate even after prolonged subculture were obtained from cases in which typical strains were also isolated; in two of the 21 cases, therefore, the identity of the bacillus was doubtful. It may be added that on two occasions an organism has been isolated which is culturally and morphologically identical with the Shiga bacillus, except that it is actively motile. It is not agglutinated by the Shiga serum in any dilution.

Mannite Fermenting Bacilli.—Of the four organisms which gave the sugar reactions of the Flexner bacillus, only one, that isolated from the acute case, gave a complete agglutination. One strain gave an incomplete reaction (agglutination with the serum at one-fourth the full titre) after subculture on broth for seven days. The other two were entirely negative; one of these was agglutinated by the patient’s serum in a dilution of 1/200, with a trace of clumping at twice that dilution, the other by the patient’s serum in a dilution of 1/50. The two strains which did not ferment maltose gave a complete reaction with Flexner serum, and also a complete reaction with Y serum.
Dysentery Cases from Mesopotamia.

These two strains were agglutinated by the patient's serum in dilutions of 1/200 and 1.50 respectively.

Morgan's Bacillus No. 1.—As has already been indicated, motile bacilli were usually rejected without further investigation. Towards the end of the series, however, a case was met with (No. 524, vide appendix) in which colonies which had the characteristic appearance on the plates, but which were composed of motile bacilli, were met with in considerable numbers at successive platings. Through the kindness of the Director of the Bombay Bacteriological Laboratory several cultures were put through a complete series of tests, and were found to give the reactions of the above bacillus. Unfortunately no high titre serum was available.

In seven of the cases the bacillus was isolated only once each, out of a total of 25 stools examined. In the case quoted above it was found four times out of five, and on one occasion in almost pure culture.

It is probable, of course, that this organism has been quite frequently missed, as most colonies showing motility were not investigated further.

Chronic and Relapsing Dysentery and the Carrier Question.

The recent work of Wenyon and O'Conner has shown that the carrier problem in amoebic dysentery is not nearly so simple as was at first supposed. These workers have found the characteristic cysts in the stools of men who had no history of past dysentery, and further, have demonstrated that, in groups of men who have lived under conditions of active service in countries where dysentery is prevalent, the percentage found to be infected is comparatively little affected by a history of dysentery. In view of such observations, one is compelled to doubt not only whether the 'cyst-carrier' is after all so potentially dangerous, but whether the cysts so frequently described are really related to the amoeba found in acute dysentery.

The above workers, after a very extensive experience, express the opinion that it is not justifiable to detain healthy men in order to detect and treat cyst infections. It will be readily agreed that such detention can only be justified if it be proved that amoebic dysentery is the prevalent form of the disease, and particularly that the chronic and relapsing cases are mainly amoebic.
The cases of dysentery admitted to this hospital from Mesopotamia between the last week of November and the first week of March offered an opportunity for examining the latter class of case. Although a complete examination could not be carried out, circumstances rendered it possible to keep the patients under observation for a longer period than had previously been the case, and to devote more time to the work. In addition to the routine microscopic examination of the stools, a bacteriological examination was made whenever mucus, which from its appearance suggested the existence of a chronic infection, was present. As far as possible the medical history of each case was personally inquired into and recorded, particular attention being paid to the number of attacks from which the patient had suffered, to his condition between the attacks, and to the length of the intervals between them. The patients under observation during this period were either convalescents with no symptoms or chronic cases. Only two developed acute attacks of dysentery, one case being amœbic, the other yielding Morgan’s bacillus. Practically all the men were sufficiently well to be able to walk about the ward within a few days of arrival, and most of them were apparently in fairly good health when transferred elsewhere. There were no deaths.

The results of the microscopic and bacteriological examination of the stools of these cases are shown in the table below. To give a more definite idea of the cases dealt with, they have been grouped under three headings. The first group includes cases in which the naked eye examination of one or more of the stools which were sent to the laboratory left no doubt about the man having chronic dysentery; in the second group are placed the doubtful cases, in which mucus was present, but either in very small quantity or not of the kind which one has learned to associate with a chronic infection. In liquid stools, for instance, it is common to find streaks of mucus, at times tinged with blood, but the mucus is clear to the naked eye, and does not show pus cells or mucosa cells when examined under the microscope. Neither dysentery bacilli nor amœbæ have been found in such mucus. In constipated stools also one frequently finds patches of yellowish tenacious mucus, adherent to and pressed into the faecal matter, which when teased out and examined microscopically shows nothing suggestive of ulceration. The third group is composed of the cases in which no mucus was detected in the stools seen. This group, of course, probably includes many cases which were in reality uncured. The figures relating to the various bacilli and
to the amœbæ indicate the number of cases, not the number of positive examinations.

Table showing the Results of Microscopic and Bacteriological Examination of the Stools of 197 Cases.

GROUP 1. Definite chronic dysentery.

GROUP 2. Doubtful cases.

GROUP 3. Cases showing no mucus in the stools.

<table>
<thead>
<tr>
<th>Groups</th>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>31</td>
<td>32</td>
<td>134</td>
<td>197</td>
</tr>
<tr>
<td>Do. stools examined</td>
<td>219</td>
<td>135</td>
<td>395</td>
<td>749</td>
</tr>
<tr>
<td>Do. stools which suggested dysentery</td>
<td>104</td>
<td>36</td>
<td>140</td>
<td>197</td>
</tr>
<tr>
<td>Do. stools examined bacteriologically</td>
<td>83</td>
<td>36</td>
<td>119</td>
<td>197</td>
</tr>
</tbody>
</table>

| Shiga bacillus | 5 | 2 | 7 |
| Shiga bacillus with Morgan's No. 1 | 2 | 2 |
| Shiga bacillus with E. histolytica cysts | 1 | 1 |
| Flexner bacillus | 2 | 2 |
| Flexner bacillus with E. histolytica | 1 | 1 |
| Y bacillus | 1 | 1 |
| Y bacillus with E. histolytica | 5 | 1 | 6 |
| Morgan's bacillus No. 1 | 2 | 2 |
| E. histolytica, free form | 2 | 2 |
| E. histolytica cysts | 2 | 2 |

19 | 6 | 7 | 32

In interpreting the results of such an investigation, it is necessary to take into account the technical difficulties, or, in other words, to consider the value of a negative result. This is particularly important in the diagnosis between amœbic and bacillary dysentery in chronic cases. Given the requisite experience, E. histolytica, if it be present and be the cause of the patient's condition, should be found without much difficulty in a stool which contains mucus and pus cells. The difficulties in the recognition of this amœbe which have given rise to so much discussion recently relate more to the forms met with when there are few or no symptoms. When there is ulceration it is practically always possible, by examining several preparations from different parts of the stool, and by making sure that the stools examined are fresh, to find amœbes showing the characteristic movement and with ingested blood corpuscles. The isolation of the bacilli is, on the other hand, to a great
extent a matter of chance, unless one is prepared to use a very large number of plates,* for the number present in chronic cases is seldom high; in only one case in the present series was a suspected organism found in numbers at all comparable with what one sees in the acute cases. In comparing the results of microscopic and bacteriological examination, therefore, the number of cases of definite amebic infection may be taken as correct for practical purposes, while the number of bacillary infections recognized must always, in chronic cases at least, be well below the actual number.

Looking at the figures in the above table with these considerations in mind, one can have no doubt that, whatever be the cause of acute dysentery in Mesopotamia, it is the bacillary cases which make up the great majority of those who eventually reach the base on account of dysentery. The argument lies not so much in the actual identification of known organisms and the demonstration of their relation to the patient's disease, as in the absence of the amoebae in the cases which still have symptoms.

With regard to the cases in which the cysts of *E. histolytica* (36 cases of the whole series, or 6 per cent) were present, the work of Wenyon and O'Connor has shown that one cannot regard the cysts as evidence that the patient has suffered from amebic dysentery. It is almost as probable that his disease was bacillary. In one case (No. 516, vide appendix) the Shiga bacillus was twice recovered from the stools of a cyst carrier, and although four out of the six stools which were examined were mucoid, *E. histolytica* in the free and active condition was not found. Such a case shows that it is possible for an amœbic infection to remain quiescent even while the patient is suffering from marked symptoms of dysentery.

It is hardly justifiable, of course, to draw definite conclusions regarding the cause of dysentery in the field from the results of investigations made at the base. The use of emetine in the treatment of the disease appears to have been almost universal, and it is possible that this has controlled the amœbic cases to such an extent that few of them have to be sent down as convalescents or chronic cases. Seasonal prevalence has also to be taken into account. As will be seen by a comparison of the table given above with the statement of the total findings in the microscopical examinations; the proportion of cases in which the amoeba was found was higher in the period between July and November than later.

* Three plates, occasionally more, were used for each stool in the present series.
The predominance of bacillary infections in the cases which are eventually sent to the base, has an important practical bearing on the carrier question. Evidently the number of amœbic cases which relapse and become chronic is not nearly sufficient to justify the detention of large numbers of dysentery convalescents for the purpose of the detection of cyst carriers. On the other hand, the relatively large proportion of the cases which were still excreting the bacilli on their arrival at the base, focuses attention on the chronic and relapsing cases as potential sources of danger.

There is an essential practical difference between the 'cyst carrier' and the man who excretes dysentery bacilli. The existence of amœbæ of the cyst-producing generation in the intestinal tract of the former must be assumed, and it is generally supposed, from the number of cases in which emetine given hypodermically is without effect, that these amœbæ are living in some small unhealed pocket which is cut off from the general circulation. But it is a matter of practical experience that a man may pass large numbers of cysts in his stools for long periods, without at any time suffering from a relapse of dysentery; he may even not have had dysentery at all. So far as our present knowledge goes, it is doubtful whether the seven men in the present series in whose stools the cysts of *E. histolytica* were found are really specially liable to relapses of dysentery, or constitute any special danger to others as carriers of the disease. The case is quite otherwise with those from whose stools bacilli were isolated. In all there were signs of the existence of an inflamed or ulcerated spot in the intestinal mucosa; they were in fact uncured cases, and as such would be specially liable to break down under trying conditions of service, and further, would presumably continue to excrete the bacilli so long as the ulcer remained unhealed. Such men, although in the majority of cases they were in fairly good health and had no symptoms at all except the occasional passage of small quantities of mucus, often without any obvious pus or blood, were not only unfit for active service but constituted a real danger to others as sources of infection.

Whatever policy be adopted with regard to the 'cyst carrier,' the importance of the detection of the chronic cases will hardly be questioned. The detection of chronic dysentery is not, however, an easy matter. Neither the patient's appearance, nor the account which he may give of his condition, is to be relied on, and he may for considerable periods pass normal stools. It is possible for a man to have in his intestine ulcer large or numerous enough to produce mucus and pus, and even blood
in almost every stool he passes, and yet to be well nourished; many of the lesions in chronic dysentery, in fact, appear to cause little disturbance of the general health. Many striking contrasts, between the patient's appearance and the notes relating to his stools, have been met with in the cases of the present series. Cases Nos. 471 and 516 are instances of this. Had these men been ordinary convalescents their appearance would certainly have justified their discharge to a convalescent camp, and neither of them attached much importance to the presence of mucus in the stools. Further, chronic ulcers are frequently quiescent, and it is only occasionally, perhaps on the irritation from unsuitable food, that mucus appears in the stools. Case No. 417 is a rather remarkable example. The only point about the case which was at all suggestive of chronic dysentery was some ill-defined abdominal pain, which might have been otherwise accounted for, yet the sixth stool examined contained a mass of mucus of the kind which is typical of chronic dysentery. Unless one examines a considerable number of stools, the detection of such cases is largely a matter of chance. It will be seen from the table given on page 318 that out of 219 stools examined from cases known to have chronic dysentery only 104 showed signs of the disease.

The carrier problem in bacillary dysentery has received comparatively little attention, and we have no experience, such as has been gained in time of peace in the case of enteric fever, to guide us. Numerous observers have recorded the isolation of dysentery bacilli from the stools of patients convalescent from the disease, and from contacts who have had no definite attack, but these have been mainly organisms of the ill-defined mannite-fermenting group, and not the more definite Shiga bacillus, which is apparently the principal organism associated with dysentery in Mesopotamia. The cases recorded, moreover, are not sufficient to show that bacilli which may cause the disease can live for long periods in the normal healthy intestine, and no case comparable with that of the enteric carrier has been established. If it be unjustifiable to detain men in order to search for the cysts of the pathogenic amoeba, it would certainly be unjustifiable, as well as impracticable, to make a bacteriological examination of the stools of all dysentery convalescents. But cases such as those referred to above are not only 'carriers,' in the sense that, although the men are generally in sufficiently good health to do duty, they are excreting the bacilli intermittently in their stools: they are also suffering from a disability which is likely to render them unfit when
they are subjected to any severe strain. The tendency to relapse is a marked feature of dysentery. If the ulceration of an initial attack be not completely healed before the patient resumes a normal diet and a life of exposure, it does not tend to spontaneous cure; it may remain quiescent for a while, but at any time a chill, or an attack of diarrhoea due to unsuitable food, may light up the inflammation again, and the patient passes through another attack. The histories obtained from these cases illustrate this tendency to relapse very clearly. Single attacks were the exception, while many patients gave a history of three or more definite attacks, at varying intervals. It is not possible to ascertain how many of these were relapses and how many fresh infections, but from the account of intermittent diarrhoea, occasionally associated with the passage of blood, which was so commonly given of the intervals between the attacks, it is extremely likely that the majority of the patients had never properly recovered from the first illness.

The importance of these mild chronic cases cannot be too strongly insisted on, and every possible precaution should be taken to ensure that the men do not return to duty till the disease has been completely got rid of. As has already been pointed out, occasional inspection of the stools will not suffice to detect the infection. Repeated examinations are necessary, and these should be made at a time when the patient is on full diet and taking exercise, not while living under hospital conditions and on a light diet. There would be much to be said for a course of field service rations as the final test as to whether a dysentery convalescent is fit to resume his previous life.

APPENDIX—Illustrative Cases.

LAMBLIA INFECTIONS.


History.—Patient was in Gallipoli for three months and suffered from a mild diarrhoea all the time, but was never sufficiently ill to report sick on that account. He was wounded there, and while in hospital in Cairo his diarrhoea continued, and for a time was more severe than before. He rejoined his unit at Mudros, and while there got rid of the complaint and passed normal formed stools. He spent seven months in Mesopotamia, and for the first three remained quite well and free from bowel complaints. He then began to suffer from alternating constipation and diarrhoea, and has not been well since. On one occasion he was constipated for seven days, and on several occasions for three days. During the last two months he has not been constipated, but has had constant diarrhoea. When admitted to hospital about the end of August he was passing...
about twelve stools per day, without any marked tenesmus; the stools only occasionally contained a little blood, and were never of the usual dysenteric type. He has never been acutely ill.

On admission to this hospital the patient was thin and anaemic, but able to get about. He says he has lost about a stone in weight since leaving England.

Stool Examination.—7th October.—A normal formed stool. A very heavy infection of E. coli, and large numbers of Lambia cysts.

The patient improved rapidly while in hospital, passing two stools per day on the average.


History.—Patient was six weeks in Gallipoli, where he kept fit, and reached Mesopotamia in February. In August, after no previous bowel complaint, he began to suffer from diarrhoea, and after a week or so of this noticed some blood and mucus in his stools. The symptoms were not very severe, and he continued on duty most of the time for six weeks. He was eventually sent to hospital at the end of September, and has not been on duty since, though recovering gradually, with occasional attacks of diarrhoea.

Stool Examinations.—22nd November.—Formed stool, with much adherent mucus. No parasites found. The mucus was plated, and the Shiga bacillus obtained. 6th December.—Liquid stool. Lambia cysts in large numbers. 12th December.—Liquid stool. No parasites found.


History.—After six months in France, where he was wounded, he went to Mesopotamia in January, 1916. He was slightly wounded almost at once, and at the same time had a slight attack of dysentery. Within four weeks he had recovered from both and returned to duty. He remained fit, except for a third wound, till September, when he had a moderately severe attack of dysentery, diarrhoea following which did not subside till the end of October. He now passes two or three stools during the day, and is improving steadily.

Stool Examinations.—23rd November.—Liquid yellow stool. A heavy infection of Trichomonas, and many Lambia cysts. 28th November.—Liquid stool. A few flagellate Lambia, many cysts, and many Trichomonas. 6th December.—Liquid stool, with much soft clear mucus. Heavy infection of flagellate Lambia, its cysts, and Trichomonas.

No. 480, Byrne. Admitted 13th December, 1916.

History.—Patient reached Mesopotamia, direct from home, in January, 1916, and remained fit until August. He then suffered from a mild attack of dysentery, not sufficient to compel him to go to hospital; he continued on duty for six weeks, passing from six to twelve stools per day, with at times a considerable amount of blood and mucus. He was then sent to hospital, where he recovered from his acute symptoms in a few days, without any treatment by serum or emetine. Since the first week in September he has been passing four to six loose stools per day, with occasionally a little blood and mucus.

Stool Examinations.—15th December.—Liquid stool, pale yellow in colour. No parasites. 22nd December.—Liquid slate-coloured stool, containing many Lambia cysts. 27th December.—Soft stool, containing small numbers of Lambia cysts.

The diarrhoea continued, in spite of treatment, while in this hospital.

No. 520, Jones. Admitted 20th December, 1916.

History.—Patient was in Gallipoli from the end of September to the time of the evacuation, and subsequently in Egypt and Mudros for two months, and during this time
had no illness. He reached Mesopotamia in March, and was three weeks in hospital in April with ‘dubility, suspected enteric.’ After this he remained fit till November, when he had a short but acute attack of dysentery. By the first week in December he was passing normal stools, and has since had an uninterrupted convalescence.

**Stool Examinations.**—27th December—Normal soft stool. *Lamblia* cysts and *E. coli* present. 10th January—Soft stool, showing a heavy infection of *E. coli*.

**No. 577, Latchem. Readmitted 21st January, 1917.**

**History.**—Patient reached Mesopotamia direct from home in January, 1916. He had three attacks of dysentery between then and July, and was invalided to Bombay at the end of that month. Between the attacks of dysentery he had intermittent diarrhoea, and appears to have been on duty only about half the time he was in Mesopotamia. Since arrival in Bombay he has been employed as a ward orderly, and has kept in good health except for a little malaria. He frequently has slight attacks of diarrhoea, lasting only two or three days, and consisting in the passage of six or seven loose stools in the day, without blood or mucus. He is well nourished, and has not been off duty since early August.

**Stool Examinations.**—22nd January—Liquid stool, without mucus. No parasites found. 29th January—Liquid stool, without mucus. Large numbers of *Lamblia* flagellates and a few cysts.

**No. 578, Morgan. Admitted 11th January, 1917.**

**History.**—Admitted with a diagnosis of shell-shock. He was six months in Gallipoli and while there had a mild attack of dysentery for which he was a week in hospital. He was in Mesopotamia for eleven months, and except for three weeks in hospital after sunstroke, kept well. He denies having had any dysentery or diarrhoea while there.

**Stool Examinations.**—22nd January—Soft stool, no parasites seen. 29th January—Liquid stool, with much soft mucus. *Trichomonas* present. The mucus was plated, but no dysentery bacilli recovered. 6th February—Liquid stool. *Lamblia* flagellates and cysts, and *Trichomonas*, present.

**No. 582, Pinkenburg. Admitted 22nd January, 1917.**

**History.**—After four months in India, during which he was in good health, he went to Mesopotamia in October, 1916. He remained fit till the third week of December, when he had an attack of diarrhoea; no history of blood in the stools, though there was occasionally some mucus, and the motions did not at any time exceed fifteen in the day. At the end of a fortnight he was almost recovered, though up to the time of admission here his stools were soft and averaged two or three in the day. He was sent to India on account of neuritis. He appears in fair health, though a little thin.

**Stool Examinations.**—25th January—Liquid stool. No parasites. 31st January—Soft stool. Numerous *Lamblia* cysts. 7th February—Constipated stool with a little adherent mucus. No parasites seen. The mucus was plated, but no dysentery bacilli were recovered.

(See also Cases Nos. 303 and 417, on pages 325 and 326.)

**ISOSPORA Infections.**

**No. 276, Lethbridge. Admitted 10th September, 1916.**

**History.**—Patient first reported sick in May, 1916, having had no previous bowel complaints. He then had an acute attack of dysentery, from which he recovered in about a month. He did not, however, get quite rid of his complaint, but had occasional attacks of mild diarrhoea. He was twice readmitted to hospital for such recurrences, none
of which were serious, and states that he has not passed a normal stool since May. At present he passes two or three stools during the day, and one or two during the night. He states that he picks up strength very quickly between these attacks.

Stool Examinations.—12th September.—A soft stool with much incorporated mucus, which shows many pus cells. *Isospora* present in small numbers in the mucus. 16th September.—Liquid stool with much milk curd and a large patch of soft mucus. No parasites found. The mucus was plated, but no bacilli of the dysentery group were recovered. 25th September.—Semi-formed stool, bile-stained, containing much undigested food, and some muco-pus. *Isospora* present but rare, three cysts only seen. 29th September.—A small formed stool with a large patch of mucus and some pus. No parasites found. 1st October.—A soft bile-stained stool with much soft mucus. No parasites found.

The patient's general health improved considerably while in hospital, but there was little change as regards his dysenteric symptoms.


History.—This patient was in Kut during the siege, and was subsequently a prisoner in Baghdad. He suffered much from diarrhoea and colic while in Kut, and was three weeks in hospital. He states that his motions at this time did not contain blood. Since then he has passed no formed stools, but averages six or seven motions per day, evacuation of the bowel being preceded by much pain.

On admission to this hospital he was in a condition of great emaciation, and complained of much gastric pain and dysphagia. Salivation was very marked, and vomiting frequent.

A connected history could not be obtained, but it appeared clear that he had not had an acute attack of dysentery.

Stool Examinations.—19th September.—A strongly bile-stained stool of soft consistence, passed in a sheet, and containing much milk curd and soft mucus. *Isospora* present in considerable numbers. 20th September.—A soft bile-stained stool with much soft mucus. *Isospora* present, but rare. 4th October.—A small stool of bile-stained mucus and faeces. No parasites found. 16th October.—A small stool consisting of bile-stained mucus with a little faecal matter. Part of the mucus was unstained and showed pus cells in large numbers, while the rest was strongly bile-stained and contained few pus cells. In the latter portion *Isospora* was found in large numbers. A few *Lamblia* were found, and another flagellate, spindle-shaped and with flagella at both ends, which could not be identified. The mucus which contained pus cells and was without bile-staining was plated, and the Shiga bacillus recovered.

The patient made no progress while in hospital, and died on October 20th. At the post-mortem examination it was found that the large intestine, from a point nine inches below the valve, was closely studded with small ulcers from a third to half an inch long, many of these being so deep that the floor was formed only by a whitish layer of peritoneum. The mesenteric glands were much enlarged. Nothing unusual was found in the liver. The patient evidently died of inanition, and it was indeed remarkable that he had lived so long, for a large part of his large intestine must have been practically out of action for some time. It is to be noted that no blood was found in the stools at any time, nor were red corpuscles seen on microscopic examination. The extent of the emaciation may be gathered from the fact that the lateral diameter of the thigh at the thickest point was only three and a half inches.
Dysentery Cases from Mesopotamia.

CHRONIC BACILLARY DYSENTERY.


History.—Patient spent a year in France, where he was wounded and gassed, but had no illness. He reached Mesopotamia in January, and six weeks later had a severe attack of dysentery for which he was kept two and a half months in hospital. He returned to duty at the end of April, and kept fit till September, except for occasional attacks of diarrhoea lasting for three or four days at a time. He then had another attack similar to the first but more severe. At the end of six weeks he was passing normal stools, and since then has had no diarrhoea, but is inclined to be constipated. He has now some pain below the right costal arch, especially before and after defaecation, and some abdominal discomfort after meals. He states that he is three stone lighter than he was when he left England.

Stool Examinations.—21st November—Soft stool. E. coli present. 4th December—Soft stool. Heavy infection of E. coli and its cysts. 11th December—Liquid stool, showing a heavy infection of E. coli and Trichomonas. 13th December—Soft stool, showing a considerable number of E. coli and many Lamblia cysts. 15th December—Soft stool, showing a few amebae, and a heavy infection of Trichomonas. 20th December—A large soft stool, with a large patch of soft but tenacious yellow mucus, slightly blood-stained, showing microscopically many pus and mucosa cells. No parasites found. The mucus was plated, and the Shiga bacillus obtained. 23rd December—Soft stool. No parasites found. 30th December—Soft stool, with some undigested food. No parasites found.

The stools of this patient were specially examined on account of the unusually heavy infection of E. coli. It will be noted that the mucus was only found at the sixth examination, and then at least six weeks after the cessation of the diarrhoea which followed his last attack of dysentery.


History.—Patient went to Gallipoli in October, 1915, and had some diarrhoea on the voyage. Shortly after arrival dysentery supervened, and he was in hospital for a month. The maximum number of stools passed was fifteen per day, and he was never acutely ill. After a month in a convalescent camp he returned to duty, but was still passing loose motions. He reached Mesopotamia in February, 1916, and remained well, getting rid of his diarrhoea, till June, when he had a mild attack of dysentery which kept him off duty for a month. He was again on duty for two months, keeping in fair health, but having at times a little blood and mucus in his stools and some abdominal pain and discomfort. These symptoms got gradually worse, and he was admitted to hospital at the end of October, and has not been on duty since. On admission here he appeared in fairly good condition, though rather pale, and states that he has not lost much weight. He passes four to six stools a day.

Stool Examinations.—21st November—Small stool, a mixture of faces and mucus. No blood. 22nd November—A soft stool, with a patch of soft mucus, which shows microscopically many pus and mucosa cells, and some blood corpuscles.

The first stool was plated, and a bacillus obtained which produced acid but no gas in mannite, glucose and maltose; it did not agglutinate with Flexner serum, but was strongly agglutinated by the patient's serum in a dilution of 1/200.
No. 410, PORTAS. Admitted 19th November, 1916.

History.—Reached Mesopotamia direct from home in June, and kept fit and on duty until the end of September, when he had an attack of diarrhoea which was followed by dysentery. The attack was moderately severe, but convalescence was very prolonged, and he has had two relapses since. He now passes two stools per day, morning and evening, and has no complaint of pain on defaecation. General condition good.

Stool Examinations.—21st November—Soft stool with a few shreds of soft mucus. 29th November—Soft stool. The stool of the 21st was plated, and the Y bacillus isolated. This organism was agglutinated by the Flexner serum in a dilution of 1/6000, the full titre, and by the patient’s serum in a dilution of 1/400.

No. 471, WOOD. Admitted 1st December, 1916.

History.—After nine months in France, patient reached Mesopotamia at the end of August. Almost immediately he had an attack of sand-fly fever, and while in the convalescent camp after it he developed dysentery. Apparently a severe but short attack. He recovered from this and was passing normal soft stools, when in the third week of October he had a relapse of four or five days duration. Since then he has had almost constant diarrhoea, never severe, but accompanied by much pain on defaecation. He says he has passed formed stools now and then.

He was apparently in fair health, and well nourished, on admission to hospital, and improved considerably while here.

Stool Examinations.—4th December.—Soft bile-stained stool, with much stiff yellowish mucus. Small numbers of E. coli cysts present. 7th December—Normal stool. Cysts E. coli present. 14th December—Soft stool with much incorporated mucus. Many pus cells and blood corpuscles seen. 18th December—Small stool of blood-stained mucus and facal matter. The mucus contained many pus and mucus cells, and blood corpuscles. 30th December—Soft stool with a large patch of soft mucus. 5th January—Soft stool, with much blood-stained mucus. 13th January—Soft stool, with a large amount of soft mucus, containing many pus cells and blood corpuscles. 19th January—Soft stool, with much soft bile-stained mucus. 24th January—Soft stool, with a large amount of blood-stained mucus. 31st January—Stool of blood-stained mucus only.

In spite of the passage of the kind of stools indicated above the patient continued in fair health and did not lose weight.

Eight of these stools were plated, and from one, that of 19th January, Morgan’s bacillus No. 1 was obtained. Only one colony was found on the three plates.

No. 510, MIDDLETON. Admitted 13th December, 1916.

History.—Patient was three months in Gallipoli, where he had an attack of dysentery, for which he was three months in hospital, and from which, he states, he recovered completely. He reached Mesopotamia early in March, 1916. Except for two mild attacks of malaria, he remained in good health until the middle of November, when he had an attack of diarrhoea; this persisting, he was admitted to hospital about the end of the month. His symptoms were never acute, the maximum number of stools being eight in one day, and there was not at any time much blood, though usually a good deal of mucus. He had much abdominal pain, especially on defaecation. On admission to hospital he was in fair condition, passing one to three stools per day, with a little mucus now and then.

Stool Examinations.—20th December—Soft stool. 28th December—Formed stool, with
a large patch of yellowish mucus, which contained many pus cells. 3rd January—A small patch of blood-stained mucus, with no fecal matter. 11th January—Soft stool, with a large patch of stiff mucus. 18th January—Formed stool, with some adherent soft mucus. 23rd January—Soft stool, with a little soft mucus. A few E. coli present, and numerous E. nana.

The stools were plated on 28th December and on 3rd and 11th January. The Shiga bacillus was obtained on the first occasion.


History.—Patient reached Mesopotamia, direct from home, in October, 1916, and remained fit till the middle of November, when he had a very sharp attack of diarrhea and vomiting. He did not notice any blood or mucus in the stools at the time. A week later he had another attack similar to the first, but this time with dysenteric stools from the start, and he passed through a moderately severe attack of dysentery. At the worst he did not pass more than twelve stools during the twenty-four hours. The acute symptoms subsided in a week, but since then he has frequently noticed a little blood and slime in his stools, and has some pain on defecation. On admission he was rather thin and anaemic, but improved rapidly in this respect while in hospital.

Stool Examinations.—26th December—Normal formed stool with some adherent mucus, and a small patch of soft mucus passed separately. Both pieces of mucus showed abundant pus and mucous cells on microscopic examination. No parasites found. January 2nd, 1917—A small patch of mucus only. No parasites found. 10th January—A hard constipated stool, with much adherent mucus. 17th January—Soft stool, with no mucus. 22nd January—A normal formed stool with a thin coating of soft mucus. No parasites were found in the mucus, but the fecal matter showed many numerous mature cysts of E. histolytica. 29th January—A soft stool, no mucus. E. coli present, with its cysts, and cysts of E. histolytica. Also numerous small amoeba, probably E. histolytica (minuta).

Reports from Busrah accompanying the patient record pus and blood, but no amoeba in the stool on 8th December. The Flexner bacillus was isolated on 12th December.

The stools of 26th December, 2nd and 22nd January were plated out, and on the first two dates the Shiga bacillus was isolated. The first strain was negative to the homologous serum on first isolation, but agglutinated after seven days subculture on broth. The second strain agglutinated normally.

No. 524, Buxton. Admitted 20th December, 1916.

History.—Patient was for three months in Gallipoli, and for two months at Mudros. At the latter place he had a mild attack of dysentery, but having light duty at the time he did not report his condition and received no treatment. He did not pass more than six stools per day. He went to Mesopotamia in February, 1916, and kept fairly well for the first two months. In May he had another attack of dysentery like the first, but rather more severe, and again continued on duty. Throughout the hot weather he had relapses every few weeks, never severe, but he got gradually weaker with each attack and was not able to do full duty. In early November he was sent to hospital with an attack a little more severe than usual. On arrival in Bombay he had obviously been very ill, but his general condition improved remarkably while here, and he put on a good deal of weight. He passed two to four stools a day, and had much trouble with pain on defecation and general abdominal discomfort worse at night. He states that he has not passed a normal formed stool for the past eight months.
Stool Examinations.—27th December—Soft stool, with a small patch of soft mucus. 3rd January—Soft stool, with a patch of soft mucus. 11th January—Soft stool, with much adherent mucus. 17th January—Soft stool, with much incorporated mucus. 22nd January.—Small stool, mainly mucus. 29th January—Normal soft stool, with no mucus. No parasites were at any time found in the mucus or the faecal matter of the stools.

The stools of the first five dates were plated, and on four occasions Morgan's bacillus No. 1 was isolated. The stool of the 11th January gave almost a pure culture of this organism. On the last occasion the Shiga bacillus was also isolated.

No. 547, DEXTER. Admitted 25th December, 1916.

History.—Reached Mesopotamia direct from home in June, 1916. In the following months he had occasional diarrhoea, and at times passed a little blood and mucus, but was able to remain on duty. In early November he suffered from constipation, which aggravated the piles from which he had previously had some trouble. Dysentery supervened, apparently a mild attack, but complicated by the bleeding from the piles. After the acute symptoms had subsided he continued to pass much mucus in his stools, and had a slight relapse shortly before admission here. On admission he was in fairly good condition, passing one or two stools per day, and inclined at times to be constipated.

Stool Examinations.—29th December—Stool of mucus and blood with some hard faecal matter. The mucus showed large numbers of columnar mucosa cells. 30th December—Stool of blood and mucus, showing a heavy infection of Trichomonas. 31st December—Stools of blood-stained mucus with some hard lumps of feces. 3rd January, 1917.—Hard lumps of faecal matter and blood clot, with a little soft mucus. 5th and 11th January—Similar stools, with no mucus and no parasites. 18th January—A soft stool with a large amount of blood, but no mucus. Cysts of E. coli present in small numbers. 23rd January—Formed stool, with some blood, and a patch of muco-pus adherent to it. 30th January—Soft stool, with some soft mucus, showing pus and mucosa cells. 31st January—A normal soft stool, with a heavy infection of E. coli and its cysts.

Mucus from the stools was plated out on 29th and 30th December, and 3rd and 30th January. At the third plating Morgan's bacillus No. 1 was obtained.

No. 575, MESSENGER. Admitted 10th January, 1917.

History.—This man was sent on sick leave from Mesopotamia to recruit his health after an operation for appendicitis in September. Previous to the operation he had been habitually constipated, but after it he noticed that his tendency was distinctly the other way, though he had no definite diarrhoea. No history of dysentery at any time. He reported himself at this hospital on 10th January on account of slight diarrhoea of two days duration. He passed five or six stools per day for the first few days, with a little mucus and a trace of blood, but was otherwise in good health. He states that his bowels had been getting distinctly looser during the last month.

Stool Examinations.—11th January—Soft stool, with a small patch of soft mucus. 19th January—Soft stool. 24th January—Formed stool with a patch of soft mucus. 31st January—Soft stool with much incorporated mucus. No parasites found on any examination.

The stools of the 11th, 24th, and 31st were plated, and a bacillus giving the reactions of the Shiga bacillus, but inagglutinable even after subculture, was obtained at the first plating.
A CASE OF MALARIA SHOWING ATYPICAL PARASITES

BY

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AND

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Since the publication in 1914 of Stephens’s account of *Plasmodium tenue* as a new malarial parasite of man, several papers dealing with the peculiar forms which he described have appeared. These have been of a more or less controversial nature, and although the general verdict appears to be that ‘*Plasmodium tenue*’ is not a new species, but an atypical form of one of the known species, Stephens’s paper has served a useful purpose in directing attention to the occurrence and significance of such forms. It appeared subsequently that they had been seen by other workers, but they had not attracted much interest, the parasites of malaria being described on stereotyped lines, much the same in all text-books, attention being directed specially to the differentiation of the three species from one another, with little reference to variations from the type within the species.

The rarity of these atypical forms is evident from the experience of Stephens and of most of those who have criticised his account of *Plasmodium tenue* as a new species. The present writers, whose laboratory experience during the last three years has included the examination of very large numbers of blood films from men who had contracted the disease in Gallipoli, Egypt, East Africa, Mesopotamia, and India, have met with only two cases in which a considerable proportion of the
parasites were abnormal. They have therefore thought it worth while to record one of these cases, in which, in addition to forms similar to those described by Stephens, there were present also atypical parasites at a late stage of development.

The patient was a Turkish prisoner of war, admitted to this hospital on the 3rd of April, 1917. He stated that four months previously he had been admitted to hospital in his own lines with fever which, from his description, was most probably malarial. He was discharged after two months treatment, and readmitted not long afterwards on account of conjunctivitis, and various secondary complaints which appeared to indicate general debility. On admission to this hospital he was much emaciated and obviously very anaemic; the heart was dilated, some ascites was present, and the liver and spleen were enlarged, the latter reaching two and a half inches below the costal arch. His temperature remained normal for over a week after admission, and subsequently showed slight rises which were not accompanied by rigors. Under full quinine treatment his fever ceased and his general condition improved rapidly. Two months later he was discharged to a convalescent camp. There was nothing to suggest that his illness was anything more than chronic malaria, aggravated by the conditions of service.

The first film, examined at the time of his first rise of temperature while here, showed a heavy infection of malarial parasites, averaging three or four per field. The impression given by the examination of the first few fields was that it was a sub-tertian infection, with here and there a few simple tertian rings. But there was an unusual and unfamiliar look about a large proportion of the parasites which led to a closer examination. Excluding these which could be definitely decided on as simple tertian parasites, successive rings did not, to use Stephens’s phrase, present the ‘regular, almost monotonous contour’ of the malignant tertian parasite, and there was a much larger proportion than usual of rings about which there might be a difference of opinion. Further, there were many forms present which recalled at once the figures accompanying Stephens’s two papers. The general appearance of the film is shown in Plate XLIV, which represents thirty-five infected corpuscles drawn without selection.

These small rings and atypical parasites were almost the only forms to be found. During the course of a thorough examination of two large and evenly spread films, five fully grown but undivided simple tertian parasites, and one mature rosette of the same species, were seen. There
were no crescents. Simple tertian parasites intermediate between the rings and the fully grown ones were conspicuously rare. One would have expected, in a long standing case such as this, and particularly since there were two broods of parasites present, that trophozoites of *P. vivax* would have been found at all stages of growth, and the rarity of the intermediate forms suggested strongly that many of the rings which, from their size and ameboid activity, one would have regarded as simple tertian parasites, were in reality sub-tertian. At least half the parasites showed some ameboid activity, and forms such as those illustrated in the first row of the second plate were common; the very long and twisted processes seen in some of Balfour and Wenyon's figures, and in those of Stephens's first paper, were not seen. The chromatin in most of the rings was much more abundant than usual, and was arranged very irregularly. In three of the figures in Plate XLIV it will be seen that there are definite rings of chromatin projecting into the vacuole. It was noted that the increase in the amount and the irregularity in the arrangement of the chromatin was not peculiar to parasites which one would regard as definitely *P. falciparum*, for these features were also present in larger forms (cf. Plate XLV, fig. 11). In some of the corpuscles there were bodies of the shape of nuclei, but usually larger, taking the stain in the same way as the chromatin of the parasites, but showing no cytoplasm. These correspond to the 'anaplasma-like' bodies, described and figured by MacFie and Ingram, and the present writers are inclined to agree with the interpretation of these authors, that these bodies are malarial parasites without cytoplasm, for in these films it was easily possible to trace all stages between definite parasites and these round or oval particles without a trace of cytoplasm.

The first films were taken at nine in the morning of the 11th, the patient being given his first dose of quinine immediately afterwards. Other films were taken in the afternoon of the same day and on succeeding days. In films made eight hours after the first, the parasites were very much reduced in numbers, and those which were found presented a much more regular appearance than before. The majority present were normal sub-tertian rings, there being only a few with ameboid processes or irregular nuclei. The simple tertian parasites had grown in the usual course, and were well differentiated. On the morning of the 12th there were still a few 'tenue' forms, some small rings, which could almost all be definitely placed as either *P. vivax* or *P. falciparum*, and some half grown parasites of the former species. During the next three
Plasmodium *falciparum* and *Plasmodium vivax*, mixed infection, with degenerative and "tenue" forms:—35 infected corpuscles drawn at random.
Figs. 1—20, "tenue" forms, atypical rings, and "tanaplasma" bodies.

"21, 22, 24, 26, Plasmodium vivax, showing very scanty chromatin!

"23, 25, 27, showing no chromatin.

Fig. 28, a parasite with no pigment.
days the number of parasites got progressively smaller, the sub-tertian rings disappearing first. On the morning of the 15th, rings were very scanty, and were not subsequently found. On that day a single mature rosette of *P. vivax*, and an immature crescent, were found. Crescents appeared in greater numbers on the following few days, and then disappeared with continuous quinine treatment.

A considerable proportion of the large forms of *P. vivax* showed abnormalities in the amount of chromatin and pigment which they contained, and in the irregularity of the appearance of Schüffner's dots. In one of the five found on the first day (Plate XLV, fig. 28) pigment was entirely absent; in only one of the five were Schüffner's dots present, although the staining was perfectly uniform throughout the slide. In many of the fully grown or half grown forms the chromatin was very scanty, occurring only as small dots and bars, resembling minute bacilli, scattered throughout the cytoplasm. In several parasites it was impossible to distinguish any chromatin at all, although the other parasites in the same slide were normally stained. The slides were subsequently re-stained, but this did not bring out the chromatin any more than before. The contrast between the large amount of chromatin in the ring forms and the small amount in many of the fully grown parasites was very striking, when these were seen together on the same slide.

With regard to the interpretation of these atypical parasites, there appears to be little doubt that they are degenerative forms, which occur when successive attacks of fever over a long period have reduced the patient's vitality to a serious degree. The approach of death through gradually increasing weakness as a result of the disease creates for the parasite an unfavourable environment, which reacts on its morphology. The disturbance of the normal relations between nucleus and cytoplasm, which is the main characteristic of these forms, is one of the results which can be brought about artificially in other protozoa by alterations in their environment. The beginnings of the change which produces the 'tenue' forms when it is carried to an extreme degree can, we think, be frequently noted in severe and especially in long continued malarial infections. The plate of *Plasmodium falciparum* drawn by Stephens as a standard with which to compare his *P. tenue* is interesting in this respect, if it be compared, as we have done on several occasions, with films from patients suffering from a first or second attack and in otherwise good health. In such cases, though the parasites may be numerous, they are remarkably uniform; the nucleus is either undivided or consists
462 A Case of Malaria showing Atypical Parasites.

of two approximately equal dots, and the great majority of the parasites are typical 'rings.' In Stephens's heavily infected case, of the fifty-nine parasites drawn, thirty-one have a single nucleus, twenty-two have two, and five have three, while one is represented without a nucleus. The contrast between the 'tenue' forms and normal sub-tertian parasites would have been much more striking if the plate of *P. falciparum* had been drawn from a recently infected case.

The occurrence in our case of atypical simple tertian parasites indicates that the changed conditions of the blood can affect this species also, causing degenerative changes of the same nature. The mildness of most benign tertian affections, as compared with those due to the sub-tertian parasite, accounts for the greater rarity of atypical forms.

We are indebted to Major W. Glen Liston, c.i.e., l.m.s., Director of the Bombay Bacteriological Laboratory, for the services of the artist who drew Plate XLIV.

REFERENCES.


THE MOUTH PARTS OF OCHROMYIA JEJUNA,
A PREDACEOUS MUSCID.

BY

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Ochromyia jejuna is a large and handsome fly, common in Madras, and probably in most parts of India, during the hot weather. It is often seen at rest on walls or on stones, apparently basking in the sunshine, but though it often remains motionless for considerable periods, it is extremely alert, and when disturbed flies off with a direct and rapid flight which renders it difficult to capture. The abrupt manner of its arrival and departure is in striking contrast to its immobility when not on the wing.

The interesting features of its mouth apparatus first attracted the attention of the writer during a study of the mouth parts of the blood-sucking Diptera. To serve as controls to other observations, large numbers of non-blood-sucking flies of different families and genera were examined by dissection, and cleared preparations were made to show the arrangement of the chitinous parts of the proboscis. In contrast to the great variations of structure which are met with in the blood-sucking Muscidae, it was found that, in this family and in others closely allied to it in which the feeding habits are similar, the mouth apparatus is of a uniform type. Such differences as are met with are mainly in the number and arrangement of the prestomal teeth and in the mode of termination of the pseudo-tracheal rings at the prestomum. The teeth vary little in size or strength, and are, as in Musca, delicate slips of chitin which could serve at the most for scratching the surface of the object from which the fly wishes to feed. The terminal rings of the pseudo-tracheal channels, though presenting very great variety in their arrangement, were not found to be developed into a definite interdental
armature such as is present in the blood-sucking forms. In *Ochromyia*, however, both these structures were found to be developed to such a degree as to indicate at once that the manner in which the fly obtains its food is highly specialized. This is in fact the case, for *Ochromyia* is predaceous.

The present writer has not himself seen this species in the act of feeding. He is indebted to Mr. Howlett for the following graphic description:

'The flies have been recorded as attacking swarming Termites and sucking their juices, but I have never seen them doing this at Pusa.

'Here they seem to confine their attention to ants. When the ants are engaged in transferring their larvæ from one place to another and are hurrying along with their burdens, an *Ochromyia* (or sometimes three or four) will often be seen to settle three to six inches to one side of the line of route. From there it will every now and then make a quick dart and seize a larva carried in the jaws of an ant: usually the ant does not leave go, and there ensues a tug-of-war, the fly holding the larva with its proboscis only and straining backwards with its body. Generally but not always the fly wins, the ant releasing its hold of the larva. The fly then retires to its perch and quickly reduces the larva to a flaccid condition, apparently sucking its internal juices, but I have not made sure of the exact nature of the injuries inflicted. There seems to be no specialization as to the species of ant attacked, but I have not seen the flies plundering any quite small species.'

Observation of the fly thus provides an explanation of the presence of the large prestomal teeth, for it is evidently by means of these that *Ochromyia* seizes and holds its prey. They serve a double purpose, however, for they are also used to pierce the larval skin in order that the fly may obtain access to the body fluids. In this action they function in the same way as the corresponding organs in the blood-sucking *Muscidae*. It is therefore of considerable interest to see to what extent the structure of the proboscis is modified from the *Muscæ* type in correlation with the predatory habits of the fly, and whether the modifications are on the same lines as in the forms which feed upon mammalian blood.

To an ordinary examination without dissection there is little to indicate that the proboscis of this fly differs from that of other *Muscids*. It is of the usual shape, is completely retractile within the head, and is therefore not seen when the fly is at rest. It bears numerous large
pigmented macrochaetae of the usual type, including a fringe at the external margins of the labella. When extended it is held perpendicular to the long axis of the body, but is capable of movement in an anterior direction, the movement taking place in the normal manner at the joints between the haustellum and the rostrum and between the rostrum and the head. When examined in a cleared preparation, however, certain small but interesting points are to be noticed with regard to the shape and degree of development of the chitinous parts. The haustellum is distinctly longer than the rostrum, and the mentum is of greater extent and more heavily chitinized, than in Musca. The labella, though large, are much smaller in proportion to the size of the fly than they are in most Muscids. These changes are in the same direction as those which have been described in the three species of the genus Philatomyia, and represent an increase in rigidity and stability about equal to that found in Philatomyia gurnei, the middle one of the three. There is, however, no ‘keel’ to the labial gutter, and no indication of a ‘bulb’ in the labium. It is evident that in Ochromyia the musculature of the proboscis is not developed to any great extent, since there is only a moderate increase in the strength of the areas to which the muscles are attached.

The structures on the inner walls of the labella, however, are very different to those in Muscids of ordinary habits. The teeth are strong and obviously purposeful structures, there is an elaborate interdental armature of the same type as that found in the blood-sucking flies, and the discal sclerite is reduced to a narrow ring having little resemblance to the racket-shaped structure which surrounds the prestomum in Musca.

There are six teeth on each side, all unequal in size and shape, the fourth, counting from the anterior or upper aspect, being much larger than the rest. The shape and arrangement of these structures is shown in the accompanying figure (Fig. 2). Each of the teeth shows evidence of a bifid nature. The first is almost bilaterally symmetrical, terminating distally in a broad incised end, and is attached proximally to the discal sclerite. The second is gently incurved at its distal end but divides proximally into two roots, while the third, although not divided at the proximal end, shows definite lateral thickenings, and has unequal projections distally. The fourth tooth is about twice the size of the third, and terminates in a sharp point; it has, however, a small pointed projection on its anterior side, and has two connected roots. The fifth is about the same size as the third, and is attached to the discal sclerite by two converging roots connected by thin chitin. The sixth is bifid almost
Cragg.—Mouth parts of Ochomyia jejuna.
Fig. 1. The proboscis of *Ochromyia jejuna*, drawn from a cleared preparation, diagramatic. *f.*, the fulcrum. *ap.*, apodeme of the labrum. *p.*, palp. *s. d.*, salivary duct. *s. v.*, salivary valve. *b. c.*, buccal cavity. *l. r.*, the distal end of the lateral rod of the labial gutter, articulating with the discal sclerite; these structures are of course subjacent to the membrane represented as a lightly shaded area. *lbl.*, labella. *fu.*, the furca, articulating with the fork of the mentum. *mt.*, the mentum, forming the posterior and lateral parts of the labium.

2. The teeth and interdental armature of one side. *i. a.*, the interdental armature. *t. p.*, the tooth plate, attached to the discal sclerite. *t.*, the teeth. The posterior of lower teeth are to the right. × 155.

3. The joint between the end of the labial gutter and the discal sclerite, seen from the side. *l. r.*, *m. r.*, the lateral and median thickened portions of the labial gutter. *a. j.*, the anterior joint. *t. p.*, the tooth plate. *lb. r.*, labellar rod, and *axp.*, axial apophysis, forming the discal sclerite.

4. The articulation of the mentum with the external labellar wall. *l. r.*, the lateral thickened border of the mentum. *m. f.*, the fork of the mentum. *fu.*, the furca. *l. v.*, the labellar wall. *p. j.*, the posterior joint.

5. The hypopharynx, dissected from its attachments. *s. d.*, salivary duct. *a.*, point of attachment to the labial gutter.

throughout, and is much the smallest of the set and the least heavily chitinized.

The teeth are attached by their bases to the membraneous portion of the inner wall of the labellum, and are grouped together to fit into the concavity formed by the tooth plate, a projection of the discal sclerite. The third and fourth are attached only to the membrane, while the remainder are also connected by their roots to the chitinous tooth plate, the first and sixth being directly inserted into little cup-like recesses. Though the teeth vary in length, their apices lie about the same level, with the exception of the fourth, which projects beyond the others by about a third of its length.

The interdental armature consists of a series of long and slender blades of chitin, pointed at the tip. They are so numerous and so much compressed together that it is difficult to make certain of the exact arrangement and attachment, especially with regard to those at the ends of the set. There appear to be three pairs of these blades between each two adjacent teeth. They arise from a common stalk which is adherent to the membraneous wall of the labellum and extends nearly to the tooth plate. The first pair is slightly shorter than the others, and passes outward from its attachment in more abrupt curves. The blades corresponding to the innermost and outermost teeth are shorter and narrower than the rest, so that the tips of the blades as a whole form a curved line. The whole of the blades form a brush-like tuft surrounding the prestomum.

The pseudo-tracheal membrane is of the ordinary type, but is rather simpler than usual in the arrangement and number of its channels. There are eleven channels at the periphery of the labellum. The anterior four of these are united to form a single channel at the level of the tips of the blades forming the interdental armature, and the eighth and ninth unite near the middle of the labellar surface. There are thus seven channels at the prestomum, and of these five lie between adjacent teeth and terminate at about the level of the distal and middle thirds of the blades of the interdental armature. The first and seventh channels terminate at the anterior and posterior sides respectively of the first and seventh teeth. The channels terminate abruptly, without much modification of the terminal rings, except that the last ring of each channel has a definite downward projection; to this there is attached a delicate band of fibres which fuses with the common stalk from which the blades arise.
One can see in this fly, perhaps even more clearly than in any of the
blood-sucking Muscids which have been described, that the long blade-
like organs which compose the interdental armature are nothing more
than bifid and elongate pseudo-tracheal rings. The clearly bifid nature
of the teeth, and the close resemblance of the posterior tooth to a pair
of short blades, suggest that the teeth themselves have originated
from terminal rings of suppressed pseudo-tracheal channels.

Between each two adjacent channels, and about the middle of the
surface, there are two short jointed sense hairs of the type usually found
in this situation in the blood-sucking Muscidae. Each hair has a short
stout base about as broad as high, and a terminal joint about the same
length and half the diameter, terminating in a sharp point.

The discal sclerite (Fig. 3) has the form of a narrow horse-shoe-
shaped ring, expanding distally into a thin tooth plate, and attached
proximally to the end of the labial gutter. It is not separated into its
two constituent parts, but has on its lower or posterior side, joining the
two arms of the horse-shoe, a short pointed projection, directed distally
and lying about the level of the apex of the fifth tooth. This represents
the axial apophysis. The line of union between it and the two lateral
parts which represent the labellar rods is shown by a linear area in which
the chitin is much thinner than elsewhere. The articulation between
the sclerite and the end of the labial gutter is almost a fixed one; the
thickened lateral ridges of the gutter are pointed, and fit into notches
near the free ends of the labellar rods, while the thickened median and
ventral portion of the gutter is received into a notch just above the
axial apophysis. The close attachment of the discal sclerite to the labial
gutter ensures stability.

The posterior joint (Figs. 4 and 6), between the furca and the fork
of the mentum, shows a moderate degree of specialization. The furca
is a thickened rod, not a mere flat chitinization of the membrane as in the
non-blood-sucking flies, and is attached to the lateral ridges of the
mentum by a stout band of fibres. The fork of the mentum is developed
on the same lines as in the blood-sucking Muscidae. The proximal
division is fused with the mentum, and is represented by two thickened
areas at its distal borders, corresponding to the ventral sclerites of the
Stomoxys group, which support the middle portion of the furca. There
is no square incision at the distal end of the mentum as in Musca. The
distal portion (corresponding to the distal division of the fork in Musca)
has a thickened free end on each side, hollowed into a cup-shaped
depression into which is received a knob-like projection of the furca, the arrangement constituting a simple ball and socket joint.

The other parts of the proboscis call for little remark. The labrum-epipharynx and hypopharynx are of the usual type, and are concealed within the labial gutter. The hypopharynx is free except at its proximal end, not fused with the labial gutter as is the case in the more highly developed blood-sucking forms. The pharynx and buccal cavity are similar to those of Musca.

With this review of the structural peculiarities of the proboscis, and with a knowledge of the structure and mechanism of the corresponding parts in the blood-sucking Muscidae to guide us, we are in a position to consider the manner in which the fly seizes its prey from the jaws of the ant, and how it obtains the body juices which are all that it can devour. It is clear from Mr. Howlett’s observations that Ochromyia does actually use its proboscis as a prehensile organ with which to seize the larva, and that when the tug-of-war takes place the fly strains backwards, pulling with the leg and body muscles. It must, therefore, contrive to get a firm grip of its prey. Now in the resting position the teeth of the two sides are in contact with one another on the borders of the prestopum, directed in the long axis of the proboscis, with the apices of all the teeth except the fourth in line with one another. They are fitted by their bases into the cup formed by the tooth plate, and can move only in one plane, the movement being a rotation upwards in the long axis of the proboscis, in such a way that the two sets of teeth spread out to form a rosette. This outward rotation is brought about when the outer wall of the labella is rendered taut by the action of the powerful muscles which lie in the cavity of the labium and are inserted into the arms of the furca. The parts are returned to the resting position by the transverse muscles of the labium, aided by the body muscles, which act in such a way as to force the blood of the fly into the labellæ. These two actions can undoubtedly be carried out with a very great rapidity.

We may suppose that the fly approaches its prey with the labellæ closed and the two rows of teeth in their resting position, and that immediately it touches the larva the muscles in the labium contract. The furca is then drawn upwards, its arms rotated backward on the fixed point provided by the ventral sclerites, and, through the tightening of the labellar wall, the teeth are expanded outwards on their bases. The two fourth teeth, which are the only ones which terminate in a sharp point, and which project far beyond the blunter small teeth, will
be forced into and through the skin of the larva, which is at once fixed as it would be by a barbed hook. If the muscles inserted into the furca are sufficiently strong to keep the hook in position, the fly can pull against the ant until it secures its victim. It can then carry it off, still fixed by the pointed teeth, to consume it at leisure. The further procedure of making a puncture in the larval skin is carried out by the repetition of the same mechanism, but here all the teeth come into play as the wound is deepened. The fluid as it exudes is sucked up in the ordinary way by means of the pharynx in the rostrum. The function of the interdental armature, as in the blood-sucking species, is probably that of a filter, to prevent the larger particles and debris from the wound gaining access to the food canal, which, as in all Diptera, is in some parts very narrow.

The life history of *Ochromyia jejuna* is not known. In view of its predaceous habits and the similarity of its mouth parts to those of the blood-sucking Diptera, it is of some interest to note that it is closely related to *Auchmeromyia luteola*, the larva of which is well known as the blood-sucking "Congo floor maggot."

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A CONTRIBUTION TO OUR KNOWLEDGE OF ENTAMOEBA COLI.

BY

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Since the time, now nearly half a century ago, when intestinal amebæ in the human host were first recognised by Lambl, the views held with regard to their significance and identity have undergone many changes. The early observers apparently attached little importance to them, considering them to be harmless parasites, although they were first found in cases of diarrhoea and cholera. In subsequent years the view that some amebæ at least were pathogenic, and the cause of dysentery, gained ground, and in 1891 Councilman and Lafleur described *Amœba dysenterie* as the cause of dysentery. The subject was put on a clearer footing for the time by the publication, in 1903, of Schaudinn's memoir, in which two species, *histolytica* and *coli*, the one the cause of amoebic dysentery, the other a harmless parasite, were described and differentiated, and placed together in the new genus *Entamoeba*.

Schaudinn's description of the morphology and life cycles of these species has become classical, and is found, comparatively unaltered and illustrated by diagrams, in modern text-books. It holds its place, however, rather by the reputation of its author than by the confirmation which it has received from subsequent workers, for although the morphological differentiation which he made between the two forms has been amply confirmed, in general terms, on almost every other point his findings have been called in question. It is now believed that the form
which he described as *histolytica* is in reality a degenerate form, and that he was in error in describing the methods of reproduction by gemmation and sporulation. With regard to *Entamoeba coli*, reproduction by schizogony still awaits confirmation, and no recent observer has seen the ‘autogamy’ in the cysts, on which so much stress was laid. In fact, much of the work which followed the publication of Schaudinn’s paper was of the nature of hesitating but destructive criticism, and though there has been general agreement on the essential points of the duality of the species and the pathogenicity of one of them, the life cycles of the parasites are still imperfectly known.

There are indications in the current literature that our views even on these two points may have to be modified. The recent work of Wenyon and O’Connor in Egypt, and of MacAdam and Keelan in India, has shown that *Entamoeba histolytica* is by no means always pathogenic, for its cysts are to be found in the stools of a remarkably large percentage of people who have never had dysentery. In this connection one cannot but remark that the evidence of pathogenicity so far recorded falls far below the standard demanded, by common consent, in the study of bacteria. The validity of the two species is generally accepted mainly, perhaps, on account of the marked differences in the cysts, but it is also admitted that one phase of *Entamoeba histolytica*, previously described as a distinct species under the name *Entamoeba minuta*, is practically indistinguishable from a corresponding phase of *Entamoeba coli*. Even the distinctions between the cysts have been questioned, for Knowles and Cole, working by statistical methods, arrived at the conclusion that there is but one species, which may be occasionally pathogenic.

The observations contained in the following pages are mainly the result of a long series of routine examinations, and of the subsequent study of the permanent preparations which were made when opportunity occurred. The majority of the men whose stools were examined had been invalided from Mesopotamia on account of dysentery, and were convalescent at the time of examination. *Entamoeba coli* was found to be a common intestinal parasite, the pathogenic species, either in the free or encysted condition, being relatively seldom seen. Individual cases which showed interesting features were followed up as far as circumstances permitted, permanent preparations being made on successive days. One series of preparations has been specially studied, and since all the figures which accompany this paper were made from it, it will
be of advantage to summarize briefly the history of the patient and the notes made at the time of the examination of the fresh specimens.

J. D.—Admitted to hospital in Bombay on 19-11-16, from Mesopotamia, where he had been since March, 1916. He stated that he was in good health until July, when he had an attack of dysentery of sudden onset, his stools amounting to twenty on the first day. He returned to duty at the end of six weeks. After a month, during which he had had no dysenteric symptoms at all, he had another attack similar to the first. Within a month of its onset he was again passing normal stools, and has had no symptoms since. On admission he had no complaint except weakness, and he improved rapidly before transfer. During the seven weeks he was under observation he had no dysenteric symptoms of any kind.

Stool Examinations:—The stools were examined on nine occasions, at approximately equal intervals. On the first two examinations Entamoeba coli, both free and encysted, was found in small numbers. On the third examination the amœbæ were very much reduced in numbers, and only one cyst was seen. On the following day many large amœbæ were found, but no cysts, the stool being loose on this occasion as the result of a saline purge. The number of amœbæ continued to be remarkable on the succeeding examinations up to a month after admission. At the last examination, on January 9th, no amœbæ were found after a long search.

The patient also harboured Macrostoma, which was found twice in small numbers. Subsequent study of stained preparations showed that he also harboured Entamoeba nana.

The history suggests two attacks of bacillary dysentery, from each of which the patient completely recovered. This was confirmed by the fact that his serum agglutinated the Shiga bacillus (coarse clumping) in a dilution of 1—100 (Nov. 29th).

Morphology.

The numerous accounts of the morphology of Entamoeba coli which have been given by different workers since the parasite was first described by Schaudinn display a remarkable want of uniformity and incompleteness in matters of detail. Nevertheless, the majority of those who have written on the subject are evidently recording the results of careful observation, and the lack of uniformity is to be attributed to the great variety of structure which may be met with in this species. Craig says of his own description that it 'is a composite picture which is true of the vast majority of the parasites belonging to this species'; Hartmann, writing in 1913, gives an excellent general account, but does not describe the many unusual forms which he must have met with; James speaks of the cytoplasm and the motility varying with the phases of the cycle. These variations are best explained by the assumption that the several forms, grading into one another as they do, by imperceptible degrees, represent the ill-defined phases of the life cycle
of the amoeba within the human host. With the exception of an attempt to separate off the pre-cystic phase, however, little appears to have been done to resolve the composite picture of which Craig speaks into its component parts.

Size.—The range of size given by different authors varies considerably. Schaudinn, as quoted by Craig, gives the diameter as from 8 to 50 microns. Craig states that as a general rule the parasite measures 10 to 20 microns, though during the different stages of development it may measure from 5 to 50. Hartmann gives the dimensions as from 20 to 40 microns, and states that Werner found forms as small as five microns in diameter. It may be noted that it is not always clear whether the measurements given are those of a resting and spherical amoeba or the maxima in extension.

The above statements are evidently of a general nature, and record the impressions gained by experience rather than the results of exact measurements. They do not indicate, or at most only vaguely, the relative proportions of the amoebae of various sizes, and do not fix the average or mean size of the species. If reproduction takes place by binary fission and schizogony, the obvious inference from the wide range of size is that the large forms are those which are about to divide, and the small ones the result of the division. But it is probable that growth does not take place at a uniform rate, and that there is a certain mean range of size which the cell tends to maintain throughout the greater part of its life. Under the stimulus of an approaching reproductive division it will enlarge, and after division the daughter cells will grow rapidly until the lower limit of the normal range is reached.

With a view to obtaining more exact information on these points the writer has attempted the task of measuring a large number of amoebæ. The measurement presents certain technical difficulties, for it is only possible to measure the superficial area as seen under the microscope, the amoeba being between slide and coverslip, and therefore possibly expanded laterally by the pressure of the latter; motile amoebæ, which preponderate in successful preparations, vary greatly in shape, and to obtain a measure of their size it is necessary to calculate what their diameter would have been had they been resting and spherical. Nevertheless, the obvious fallacies are common to all the individuals measured, and the measurements are comparable. The range of size is so great that the inevitable fallacies in technique are likely to be outweighed.
For this purpose selected slides from the series already referred to have been used. The slides were fixed in Schaudinn's fluid and stained by the Heidenhain iron-haematoxylin method, using an identical technique throughout, the length of immersion in the various fluids being carefully regulated. Four slides were taken, representing the infection on four days, and from each of them two hundred amoebæ were drawn in outline with the aid of a camera lucida. The superficial area of each drawing was then measured by means of a specially prepared piece of tracing paper, ruled in squares after the manner of a Thoma-Zeiss haemocytometer slide. The superficial area of a series of circles of known diameter having been ascertained by actual measurement with the same scale, the size of the amoebæ expressed as a diameter was obtained. The results of these measurements are shown in the accompanying chart, in which the vertical height represents the percentage of amoebæ of the size indicated on the base line. To avoid an artificial exactness the amoebæ are arranged in groups with a limit of two microns, i.e., those 14 microns in diameter and less than 16 are shown together, followed by those 16 and less than 18. The dotted line in the third graph represents the average of eight hundred observations.

The chart brings out clearly the preponderance of amoebæ of a certain range of size. Of the total eight hundred amoebæ measured, ranging in diameter from 5 to 31 microns, 24 per cent were 16 microns in diameter and under 18, and 20 per cent were 18 and under 20. One may say, therefore, that in this race of Entamoeba coli half the individuals were between 16 and 20 microns in diameter, as measured in wet-fixed preparations stained with iron-haematoxylin. In the fresh condition the size would naturally be a little larger. In view of the great range of size between the largest and the smallest, one may take these as the normal limits of size in amoebæ which are mature but not about to divide.

On each of the graphs there is a definite 'shoulder' in front of the apex, very well brought out in the second and fourth. In the second series of measurements 15 per cent of the amoebæ were 14 microns in diameter and under 16, and 14½ per cent from 12 to 14. Of the whole eight hundred, 24½ per cent were 12 microns and under 16 in diameter. Evidently there is included within these limits of size a definite type, representing a phase in the life cycle, and the approximation to the size of the cysts in this species suggests that this is the pre-cystic phase. Cyst formation was in fact going on, for cysts were present in
Chart showing the measurements of 200 amebae on four days. The dotted line on the third graph shows the average of the 800 measured. Abscissa = diameter in microns.
the preparations in numbers equal to 7 per cent of the free forms; all stages were found, the 2-nucleated and vacuolated cysts being the most common.

A graphic representation of the measurements of a large number of amœbæ, therefore, enables one to separate them into four groups. There is a small group, amounting to 7½ per cent in the eight hundred, which measures less than 12 microns in diameter; a fairly well-defined group which includes—but does not consist entirely of—the amœbæ which are about to encyst, and which have therefore become reduced in size below the mean; a large group, comprising about half the total, which measures from 16 to 20 microns in diameter; and an ill-defined group of amœbæ larger than these, the percentage being generally in inverse ratio to the size. The number of amœbæ above the mean size is much greater than the number of amœbæ below it. The very small and the very large forms are of special interest.

**Small amœbæ.**—There are two well-defined types of small amœbæ. One of these, which is very rarely met with, is motile, contains vacuoles in the cytoplasm and may have ingested bacteria, has a nucleus of the ordinary type, and in fact resembles those of the mean size. The cytoplasm frequently contains numerous chromidia. Examples are shown in figures 5 and 6 on Plate I. The origin of these forms cannot yet be explained. They are too small to be the result of binary fission, as will be seen by a comparison with the forms figured on Plate III or with any of the dividing forms figured by other workers. They undoubtedly suggest schizogony, but the most exhaustive search through the preparations in which they occurred has failed to show any other evidence of such a mode of division.

The second type is a very distinct one. The amœbæ are very small, under ten microns as a rule, and are usually spherical or nearly so, and show little if any signs of motility. The cytoplasm is clear, without vacuoles or inclusions, and at the most shows only very fine granules; in a few of the larger individuals of this type small alveolar spaces can be distinguished. The nucleus is relatively huge, and occupies the greater part of the cell. It contains a large amount of chromatin, which is distributed mainly in large granules at the periphery. The karyosome is spherical, oval, or in the form of a bar, and is large and conspicuous. The nuclear network is as a rule not easily distinguishable. In many cases there is a well-marked clear space around the nucleus. It is characteristic of this type that the granules of chromatin at the
PLATE I.
DESCRIPTION OF PLATES.

All figures were drawn with the aid of an Abbé apparatus. The magnification is according to the scale at the foot of Plate IV, i.e., 1,500 diameters.

PLATE I.

Figs. 5 and 6. Minute amœboid forms, the product of schizogony? Such forms are very rare.

,, 1—4, 7—10. Pre-cystic forms. Note the great variability in the amount of chromatin in the nucleus.

Fig. 11. Probably an early pre-cystic form. (Compare the nucleus with that of figure 1.)

,, 12. Pre-cystic form, with large karyosome and no peripheral chromatin.

,, 13. Division of the karyosome in the pre-cystic stage.


Figs. 15—20. Cyst formation. Figure 17 shows commencement of the vacuole. Figures 18 and 19 show reduction bodies. (Compare with the figures on Plate II.)

,, 21—26. Small amœbæ with a large amount of chromatin and a small amount of homogeneous cytoplasm. (Compare with the small amœba in figure 61 on Plate III.)

Fig. 27. A *free* nucleus.

Figs. 28—35. Degenerating amœbæ.
periphery of the nucleus are not closely and evenly approximated to the nuclear membrane; some appear to lie close to it without touching it, and their outer borders do not take their shape from the contour of the nuclear membrane, as is usually the case. Further, some of the granules appear to lie partly within and partly outside the nucleus. Chromidia are frequently present, either as delicate linear fragments which tend to lie parallel with the periphery of the nucleus or as larger blocks which are either spherical or oval in shape. Amœbæ of this type are illustrated in figures 22 to 26 on Plate I.

During a prolonged search through the preparations in which amœbæ of this type were present a single specimen was found—that shown as figure 61 on Plate III—which throws some light on the origin of these peculiar forms. The figure represents a large amœba just about to divide unequally. The parent form is of the ordinary vegetative type, and is in the act of setting free a small amœba of the type just described. Though one would not wish to lay undue stress on a single observation, the writer considers that the finding of this small amœba in the act of leaving the parent form is sufficient to negative the view, to which he was previously inclined, that the type is a form of degeneration. One does not know what part these forms play in the life cycle of the species, but the liberation of a small amœba, remarkably rich in chromatin, from a large individual, is surely a significant event.

'Free nuclei' have been described and figured by James, who states that Elmassian has also seen free nuclei in Entamoeba blattæ and had actually witnessed the expulsion of the nucleus in the living organism. Such free nuclei have been seen by the writer, but in most cases, if the appearance of the nucleus was not indicative of degeneration, careful examination with a properly adjusted light showed the presence of a delicate layer of cytoplasm surrounding the nucleus, and it appears probable that these free nuclei are related to the forms described above. Structures resembling degenerate nuclei are frequently met with; many of these are the product of the reduction division to be described in a succeeding section.

The pre-cystic stage.—The majority of the amœbæ between 12 and 16 microns in diameter belong to this type, which is illustrated in figures 1 to 4 and 7 to 10 on Plate I. The cytoplasm stains evenly and fairly densely, is alveolar in structure, and has no large vacuoles or inclusions. The nucleus is usually rich in chromatin and does not show a well-marked reticulum. Though 2-nucleated forms with commencing formation of
the vacuole (figure 17, Plate I) have been found, the writer has not been fortunate enough to see nuclear division at this stage.

Amoeba from 16 to 20 microns in diameter.—It is to amoebae of this size that the current descriptions most closely apply. Perhaps the most striking feature is the extreme variability of the nucleus, in the amount of chromatin which it contains, and in the size and arrangement of the particles. All the stages which have been described as making up the 'cycle of the karyosome' in Entamoeba histolytica (tetragena) can be seen equally well in this species, and it is in fact impossible to draw any useful distinction between the nuclei of the two species. Such differences as there are, are differences of degree; on the whole the nucleus of coli is larger and contains more chromatin than that of histolytica, but nuclei which might be regarded as characteristic of the pathogenic species, judging from some of the descriptions, can be found in amoebae from normal stools.

Chromidia are not infrequently met with. They tend to assume the form of long filaments, thickened at one end and tapering at the other, and bent about the middle, a form which the writer has not seen in the pathogenic species. Their presence in the cell does not appear to be related to any special type of nucleus, for they have been found in amoebae in which the nucleus is rich in chromatin and in others in which it has been difficult to distinguish the nucleus.

Enucleate amoebae have been noted by several observers, notably by James, who states that he has found, as many as 15 per cent of amoebae in some preparations to be without nuclei. The writer has not been able to satisfy himself of the existence of virile and motile amoebae in which the nucleus was absent; in a small proportion of amoebae the peripheral chromatin is extremely scanty, and in very fine grains, so fine that had the decolorisation in iron alum been carried very little further they would have been indistinguishable, and only the karyosome would have remained, within a circular space which might easily be taken for a vacuole. Figure 70 on Plate IV is an instance of such a nucleus. This amoeba was on the same slide as that figured on the right of it, which shows a normal nucleus and many very densely stained chromidia. Degenerating amoebae are of course frequently without nuclei, but here the appearance of the cytoplasm is a sufficient indication of the condition.

Amoebae over 20 microns in diameter.—The majority of the larger, amoebae do not differ from those of average size. It is among them,
however, that one finds the processes of nuclear division. These will be discussed in the next section.

**MULTIPLICATION IN THE VEGETATIVE STAGE.**

*Binary Fission and Reduction Division of the Nucleus.*

*Entamoeba coli* is stated to multiply in the vegetative phase by binary fission and by schizogony. Opinion is practically unanimous regarding the occurrence of the former; schizogony, which was figured by Casagrandi and Barbagallo and later described in detail by Schaudinn, has not been confirmed by later workers.

In binary or simple fission the nucleus is supposed to divide in a simple manner to produce two equal and similar nuclei, the cytoplasm following suit. Craig, who evidently followed Schaudinn closely, gives a simple description of the process. He states that the nucleus elongates, the nuclear membrane becoming thinner and less refractile, while the chromatin, which is visible as brightly refractile dots or granules, becomes concentrated at the poles of the nucleus. The nucleus then elongates and becomes constricted at the centre, and finally divides. Coincidently with this the protoplasm becomes less granular, a constriction appears, and finally division becomes complete after division of the nucleus.

Craig states that these phenomena are not infrequently observed, and from his account, and from the precise statements on the subject which one finds in text-books such as that of Castellani and Chalmers, one would suppose that the observations could be easily confirmed, and the details followed in stained preparations. Such, however, has evidently not been the experience of subsequent workers. Kuenen and Swelengrebel state that they did not see nuclear division, though they saw forms with two nuclei. Hartmann says that it is not known how nuclear division takes place. James failed to demonstrate the complete phases, and his numerous and clear illustrations do not show any forms in which the cytoplasm is dividing. He states that the scanty number of individuals which show what may be interpreted as the later stages of nuclear division, even in heavy infections, is in marked contrast to what is seen in the pathogenic forms in acute dysentery.

One must admit, therefore, that this part of the life cycle, so long generally accepted and so precisely defined in the text-books, still awaits full confirmation and detailed description. The rarity of all stages of
binary fission will be readily admitted by all who are familiar with this parasite, and is indeed a striking fact in the very heavy infections which are sometimes met with. Though the early stages of nuclear division might easily be missed in the routine examination of stools, it is difficult to explain how the final and obvious stage, that of division of the cytoplasm, is not more frequently seen, if binary fission is a usual mode of reproduction. It is possible, of course, that the process is so rapid that at any given moment only very few amœbae are in the act of dividing or it may be that division occurs on the mucous membrane of the gut and not in the lumen, while the amœba is in a resting condition and adherent to the membrane.

The writer has succeeded in finding many amœbae showing division of the nucleus and cytoplasm, but has not been able to reconstruct from them an explanation of the normal mode of division. Indeed, the difficulty of following this part of the cycle appeared to increase with succeeding observations, and he has been led to the view that binary fission is a much more complex affair than is generally supposed.

First as regards the size of the dividing forms. It has been already explained that of the eight hundred amœbae which were measured, roughly one quarter were between 12 and 16 microns, and one half between 16 and 20. Of the remaining 25 per cent, seven and a half were below 12 microns, and the rest above 20. It will be seen from the chart that the percentage of large forms is fairly regularly in inverse ratio to the size. It is mainly in the largest forms that the stages of nuclear division are found.

A comparison of the figures on Plates I and II with the chart brings out an interesting point. Figures 54, 56, and 59 show amœbae in the act of dividing, and each individual of the three pairs is at least 20 microns in diameter. The young amœbae, in fact, are already of the average size when they are produced from the parent cell. This has been the case in all the amœbae which the writer has seen, in which the cytoplasm was actually dividing into two halves. Similarly with those amœbae in which the nucleus has divided, but in which there is no indication of division of the cytoplasm; the majority are very large, quite large enough to produce two full size amœbae. If, therefore, the binary fission shown in these preparations is a normal mode of multiplication it follows that the seven and a half per cent of very small amœbae are not young forms produced by binary fission, but represent some as yet unrecognised phase, or phases, in the life cycle.
Fig. 36. An amoeba with two normal nuclei.

37. A small form with two nuclei. Not a pre-cystic form. (Compare with figure 17 on Plate I.)

Figs. 38-40. 2-nucleate *amoebae*, with nuclei of irregular shape.

41-43. 2-nucleate *amoebae*, one nucleus of each pair having very little chromatin.

44-46. 2-nucleate *amoebae*, one nucleus of each pair degenerating.

47 and 50. *Amoebae* with one nucleus and two reduction bodies.

48 and 53. *Amoebae* with one nucleus and three reduction bodies.

Fig. 49. A nucleus in the act of dividing.

51. A large *amoeba* with one nucleus and one old reduction body.

52. An *amoeba* showing a cell inclusion, simulating a degenerate nucleus.
PLATE II.

Cragg.—A Contribution to our Knowledge of Entamoeba coli.
Though all the forms in which the cytoplasm is dividing are large, division of the nucleus is met with in amœbæ of all sizes from 15 microns upwards. The smaller 2-nucleate forms, which are very rare, are readily distinguished from early stages of cyst formation by the characters of the nucleus and the cytoplasm; the latter is alveolar and looser in texture than in the pre-cystic forms, and takes the stain less deeply, while the nuclei are not of the cystic type.

The mode of division of the nucleus has not been determined. According to Schaudinn it divides amitotically; Hartmann, who had evidently not seen a clear demonstration of the process, thinks that from the analogy of the nearly allied tetragena and ranarum one should expect a mitosis or a promitosis, an opinion to which James also inclines. Craig states that clear evidences of a mitosis are to be observed.

In the writer's preparations one amœba, that shown as figure 49 on Plate II, has been found, in which the nucleus is in the act of dividing. There is no evidence of mitosis or promitosis to be made out. The two nuclei are of normal size, and the chromatin is arranged in fairly large granules, most of which are at the periphery of the nucleus. One of the karyosomes is spherical and regular, the other irregular in shape. The nuclear network could not be distinguished. Figure 36 shows an amœba containing two nuclei, normal in appearance, except that they are oval in shape with their long axes in the same line, suggesting a recent division.

In most of the multi-nucleate amœbæ met with in this series of preparations one at least of the nuclei showed a deviation from the ordinary type, and in most cases this was definitely in the direction of degeneration. The largest number of nuclei seen was four, and of these two or three were evidently degenerating and about to be cast out of the cell. The appearances indicate that binary fission may be associated with a reduction division of the nucleus.

It has unfortunately not been possible to find examples of all the stages of this process, and so furnish a complete demonstration. The stages found, however, are sufficient to justify an attempt to arrange the events in an ordered sequence and to form a working hypothesis. As will be seen from the figures on Plates II and III, the nuclei show a great diversity of structure. Some of them are of the ordinary type with the chromatin arranged in granules at the periphery and just under the nuclear membrane, the karyosome being distinct and usually surrounded by a narrow clear zone; the nuclear network may be well shown. Other nuclei are remarkable for the small amount of chromatin which
they contain; although the karyosome is clearly shown, the peripheral chromatin may be represented by a few very fine grains only. Such nuclei are of the usual size and preserve their spherical contour. In addition to these there are nuclei showing every grade of degeneration down to bodies which would hardly be recognizable as nuclei were it not for the presence, often in the same cell, of the intermediate stages. The changes which occur are a gradual reduction in size, a diffuse staining of the nuclear sap, the collection of the chromatin into larger clumps which do not remain at the periphery of the nucleus, but become irregularly distributed, and a disappearance of the karyosome; while these changes are going on the spherical contour is lost, and the nucleus becomes elongated or pear-shaped, while at the same time it passes to the periphery of the cell, and is eventually, we may safely assume, extruded. One may readily recognize in these bodies the products of two successive reduction divisions of the nucleus, the process being much more clearly shown in these large amœbæ than it is in the cysts, in which a similar reduction is believed to take place during maturation.

The details of the division process have not yet been followed out. Judging from the example figured as figure 49, the first division is amitotic, and results in the formation of two nuclei equal in size and with a normal amount of chromatin. Theoretically each of these may again divide, both the daughter individuals from one and one of the two from the other degenerating, or one may divide and the other degenerate as the first reduction body, the second and third being produced by subsequent divisions of the surviving nucleus. The present observations are not complete enough to decide by which means the result is arrived at, but the latter is the more likely. As will be seen from figures 47, 48, and 53, two of the nuclei are at an equal stage of degeneration, while in the two last mentioned figures there is in addition an older one. One at least of these divisions is associated with a marked reduction in the amount of chromatin in the nucleus, and results in the production of nuclei of the type described above, and shown in figures 43, 47, 48, 50, and 53.

Now a reduction division of this type results in the production of one active nucleus and three reduction bodies, and in this case, as figure 56 on Plate III clearly shows, it is associated with division of the cell as a whole. A further division of the nucleus must therefore take place, the two daughter individuals both persisting, each to become the nucleus of one of the daughter amœbæ. The processes of reduction division, extrusion of the reduction bodies, and the final division of the persisting
Figs. 54, 56—59. Dividing amœbæ. In figure 56 two of the reduction bodies are still retained within the cell. In figure 57 the final division of the nucleus has not yet taken place. In figures 58 and 59 there is a large mass included at the periphery of one of the daughter cells, taking the haematoxylin stain diffusely.

Fig. 55. The final stage of binary fission. The cytoplasm of the two amœbæ was a little more definitely in continuity than is brought out in the drawing. Note the difference between the two nuclei.

,, 60. A degeneration form.

,, 61. A large amœba dividing unequally, and thus giving origin to a form of the same type as those in figures 21—26 on Plate I.
Cragg.—A Contribution to our Knowledge of Entamoeba coli.
nucleus followed by the division of the cell as a whole, do not appear to take place in a uniform manner. Figure 56, for instance, shows an amoeba in which the final nuclear division has taken place and division of the cytoplasm is imminent, while two of the reduction bodies still remain in one of the daughter cells. In figure 57 the reduction bodies have been extruded and division of the cytoplasm already begun, before the final division of the nucleus has taken place. Of all the forms which the writer has seen in which the cytoplasm was in the act of dividing, only a few, of which figure 55 is an example, conformed closely to the general idea of simple binary fission.

The possibility of reduction division was considered by Hartmann in Entamoeba tetragena, but this observer finally dismissed the idea, concluding that what he saw were degenerating forms, a conclusion which his figures certainly support. It is hardly necessary to discuss the possibility that the amoebae represented on Plate II are degenerating forms; for, except for the reduction bodies, which are essentially degenerate structures, there is nothing suggestive of it. Dead and degenerating amoebae were present in the preparations, and conformed closely to the description which Hartmann gives of the degeneration processes in Entamoeba tetragena.

The significance of this reduction division is obvious. It occurs in many protozoa, and is recognised as a process preparatory to syngamy. Syngamy in Entamoeba coli will be discussed in a succeeding section. One may add, that the occurrence of a form of division preceded by reduction division of the nucleus does not necessarily negative the occurrence of simple binary fission as a mode of reproduction.

Schizogony.

The exact statements made with regard to this mode of division by the earlier workers are, as in the case of binary fission, in striking contrast to the findings of more recent observers. Originally it was stated that the chromatin of the nucleus becomes separated off into eight little masses at the periphery, each mass eventually becoming a nucleus and taking its proportion of the cytoplasm to form a daughter cell. Subsequently it has been stated that eight nuclei are produced by the repeated division of the original nucleus. The presence of eight small masses of chromatin at the periphery of the nucleus is often seen, but schizogony as a means of reproduction in the vegetative phase is practically without confirmation. As has been already noted, small
amœbæ which might be the result of schizogony are occasionally seen (figures 5 and 6).

The description Craig gives of the eight daughter nuclei, 'visible as brightly refractile masses of granules,' and the figures which he reproduces from Casagrandi and Barbagallo, suggest very strongly that a not uncommon parasite of the amœba has been mistaken for a nucleus. This organism consists of a minute particle of chromatin surrounded by a thin cytoplasmic layer, the size being about that of the karyosome of the nucleus of E. coli; these minute bodies are collected together into spherical masses each having about the same diameter as the nucleus, or not more than twice as large, and from one to eight such masses may be present in the same cell. The size of the individual organisms is fairly uniform, but the masses of them vary, being generally largest when there are only one or two in the amœba. The intensity with which the haematoxylin stain is retained is in direct proportion to the size of the mass. In the fresh condition these bodies are extremely refractile, and appear to be surrounded by a membrane, the whole having an appearance not unlike that of a large nucleus unusually rich in chromatin. Craig's figure II, No. 11, facing page 86 of his book, and copied from Casagrandi and Barbagallo, appears to represent an amœba containing eight such parasites, the true nucleus of the amœba not being shown. No. 16 on the same plate shows an amœba containing two masses of parasites and a nucleus.

The writer is not aware of any description of this parasite, which he found quite commonly in amœbæ in the stools of men from Mesopotamia. Wilmore and Shearman figure what is apparently the same organism.

The idea of schizogony has recently been revived in a different form by Mathis and Mercier, who, by measurement of a number of cysts,
were led to the conclusion that three groups should be recognised. Two of these, measuring fourteen and fifteen and a half microns in diameter respectively, are of the familiar type, and are regarded as male and female forms, giving rise in the new host to male and female gametes. The third type is much larger, may have more than eight nuclei, up to sixteen, and has a more delicate membrane. This form they regard as giving rise to a-sexual individuals. The authors state that these large cysts may be mature when passed in the stools, and that it is not rare to see such cysts in which the membrane has been ruptured and the little amoebae are escaping; such an event is in fact shown in one of their illustrations.

The appearances described and figured by these authors are evidently capable of a different interpretation. The rôle of the true cyst in the life history of the amoeba is the transference of the infection from one host to another, and the conditions demand that the wall of the cyst should be of such a strength and consistence as to protect the organism from adverse influences during transit. When the proper environment is reached, and not till then, the cyst wall ruptures. If, as Mathis and Mercier state, the wall of this kind of cyst is so delicate, and the young amoebae so mature, that schizogony actually takes place in freshly passed stools, it appears that this schizogony is designed for the reproduction of the species in the same host, and not for the transference of the infection to another host. It is of course quite likely that an amoeba about to undergo schizogony should surround itself with a temporary protecting envelope.

**SYNGAMY.**

The occurrence of sexual phenomena in the *Entamaeba* is a priori probable. The copulation of gametes liberated from cysts has been described by Mercier as a part of the life cycle of *Entamaeba blatta*, and, according to Hartmann, Prowazek found in *Entamaeba williamsi*, which is regarded as at most a variety of *E. coli*, small amoebae with two nuclei, which appeared to be different and to copulate, suggesting a fertilisation process similar to that of *E. blatta*. Hartmann states that it is not known if the young amoebae liberated from the cysts of *E. coli* copulate, but thinks it probable that they do. Castellani and Chalmers introduce the process into their diagrammatic representation of the life cycle of *Entamaeba coli*, representing two small amoebae from the same cyst fusing with one another. In the diagram showing
the life cycle of the amoeba of the cockroach Mercier indicates the union of two gametes from different cysts, and in a recent paper, written in collaboration with Mathis and already referred to above in connection with schizogony, he describes and differentiates male and female cysts in *E. coli*. Copulation is believed to take place when the young amöebae are liberated from these cysts in their new host. It will be observed that those authors who have considered the matter regard the sexual union as taking place at the commencement of a new cycle, immediately on the liberation of the young amöebae from the cysts. As the cysts which give origin to the male and female gametes may come from different hosts, the opportunity occurs for the mingling of two races of the species, a proceeding which may be supposed to enhance the vitality of the offspring of the pair. So far as *Entamoeba coli* is concerned, however, the occurrence of syngamy has not hitherto been demonstrated.

In the preceding pages the writer has drawn attention to certain observations which point to the existence of a sexual process in this species—a process which takes place during the vegetative phase of the life cycle, and is in no way connected with encystment. We have to note the existence of a well-defined type of amöeba, which, if one may reason from one observation, is produced by the unequal division of a large amöeba, one individual of the resulting pair receiving a large amount of chromatin and a small amount of cytoplasm. Further, division of the large amöebæ is associated, in some instances at least, if not in all, with a reduction division of the nucleus, an event which is generally recognised as preliminary to syngamy in many protozoa. On Plate IV are figured a series of amöebæ which offer more convincing evidence of the occurrence of syngamy in *Entamoeba coli*.

Figures 62 to 67 represent large amöebæ, in each of which is included a smaller individual. The large amöebæ, as one may infer from their shape, are motile individuals, and the number and size of their vacuoles show that their metabolism is active. The nuclei vary, but within normal limits, and do not show any unusual features. The small amöebæ are spherical, from 10 to 14 microns in diameter, and show a more compact cytoplasm, which is condensed a little at the periphery. The nuclei are all very small, and show marked deviations from the ordinary type; that in figure 62, for instance, has no karyosome, or the karyosome, if present, is closely approximated to the peripheral chromatin; that in figure 67 has two rod-shaped karyosomes; the small nuclei in figures 63 and 65 have very little chromatin. The separation of
Figs. 62-67. Syngamy. These six pairs of amœbæ were all found in one series of slides made from the same stool.

68 and 69. Conjugation? The pairs were in much closer apposition than was possible to represent in the drawing.

Fig. 70. An amœba with a small secondary nucleus? Several such forms were seen in the same series of slides, the portion containing the small nucleus being in each case separated from the rest of the cytoplasm by a sharp constriction.

72. Another example similar to the above, but with a very large karyosome.

73. A greatly elongated amœba, ruptured during mounting. In figures 71 and 73 note the presence of chromidia, and their characteristic shape.

All figures are drawn to the scale at the foot of this plate.
Cragg.—A Contribution to our Knowledge of Entamoeba coli.
the substance of the small amœbæ from that of the large ones which enclose them is clearly brought out by the shrinkage which has taken place in the fixing and staining of the preparations.

These six amœbæ were all found in a series of slides made from the same stool, and the most careful search through many other preparations from the same and other patients has not resulted in the discovery of other stages. Such forms have not been recognised in fresh preparations, though the writer is inclined to think that they would serve to explain many appearances which have puzzled him in the past. A full demonstration of the process must therefore await further observations. That the phenomena observed are in reality a part of a sexual process there can be little doubt. The alternative explanations are that the small amœbæ are of another species, ingested by the large amœbæ, or that it is a phase of division, the daughter amœbæ being fully formed before they are liberated from the parent.

Conjugation?—Figures 68 and 69 represent two pairs of amœbæ in intimate contact with one another. By careful focussing one could make out quite clearly that the cytoplasm of one was actually enveloping a part of the cytoplasm of the other in an intimate manner which it has not been possible to represent in the drawings. In life the contact must have been very close indeed, for it would be undone to a certain extent by the shrinkage during fixation and staining. It will be noted that in the larger of the two amœbæ in figure 69 there is a small secondary nucleus, oval in shape and with a large karyosome, and that in both the amœbæ shown in figure 68 chromatia are present. The interpretation of such forms is not possible with our present knowledge, and the figures are left to speak for themselves.

NUTRITION AND MOTILITY.

The mode of nutrition of the entamœbæ appears to have received little consideration from those who have studied these parasites. Most text-book and general accounts contain the statement that Entamœba histolytica ingests red blood corpuscles, and that the non-pathogenic species ingests only bacteria. One is left to infer that these constitute the normal food of the two species.

A closer examination leads one to doubt whether this is an adequate statement of the case, and whether it is consistent with observed facts. The presence of red blood corpuscles in the amœbæ which are found in cases of acute dysentery is striking and obvious, but the organisms
Entamoeba coli.

These under such circumstances represent only one phase in the life cycle, and we know that this species exists and multiplies in the absence of red corpuscles. Similarly in the case of Entamoeba coli, though bacteria are undoubtedly present in a certain proportion of the amœbæ, actual observation of large numbers of fresh specimens and the examination of stained preparations leave one unconvinced that the ingestion of bacteria is the normal means of nourishment.

Large numbers of active amœbæ contain no bacteria, and when these are present they usually retain their normal appearance. Diplococci, for instance, are frequently seen in amœbæ, but the two individuals of the pair are equal and retain their characteristic positions in relation to one another. Many bacteria remain actively motile after ingestion, and in quiescent amœbæ they may often be seen to move actively within the vacuole which encloses them. There is nothing, in fact, to suggest that they are undergoing digestion. In examining stained preparations the number of bacteria taken up appears at first sight to be greater, but a close scrutiny shows that the majority are really on the surface of the amœba, to which they have become adherent during fixation, and are not enclosed by a vacuole. The impression which the writer has gained from his own experience is strengthened by consulting the numerous figures of Entamoeba coli in the literature on the subject. Although of course the artists were usually illustrating some other point, one would expect that bacteria would have been shown if they had been present in the specimen which was being drawn; in the majority of instances, however, only a few scattered cocci or no bacteria at all are shown.

The disproportion, on the average, between the size of the amœba and the mass of bacteria ingested by it, the fact that many large and evidently flourishing amœbæ contain no bacteria, and the doubt as to whether the ingested organisms are digested, lead one to question whether Entamoeba coli is entirely, if at all, holozoic, and to enquire whether it does not obtain its nourishment from the medium in which it lives in some other manner. It may be a true but harmless parasite, living upon material which would otherwise be absorbed by the host, or, as is more probable, a saprophyte, living upon the waste products of the host.

Whether the amœba is actually moving across the field, or carrying out those curious and characteristic heaving and wave-like expansions of the ectoplasm, the movement appears singularly ill-adapted for a
holozoic mode of nutrition. A lobose pseudopodium could at the most only capture non-motile organisms, and although the writer has frequently watched motile amoebæ for considerable periods with this point in view, he has never seen a bacterium taken up by a pseudopodium, nor is he aware of any statement by any observer that this occurs. The observation would not be very difficult, since the ectoplasm which forms the advancing edge of the pseudopodium is clear of contained particles. Nevertheless one can hardly doubt that the principal object of the movement is the search for food.

A consideration of the nature of amœboid movement provides an explanation of the rôle it plays in the nutrition of the amœba. The normal shape of an amœba, as of other protozoa which possess neither a stiffened ectoplasmic layer nor an internal supporting structure, is that of a sphere. Movement consists in an alteration of the spherical contour—in the case of Entamœba coli the alteration may be merely a transitory wave-like expansion, first on one side and then on the other, or the whole amœba may be elongated till its length is several times greater than its breadth, as in progressive movement. Now the property of a sphere is that the superficial area is the least which will enclose the given amount of space, and in any movement of the amœba the superficial area is increased in proportion to the degree of movement. If we regard the ectoplasm as an absorbing surface, it follows that amœboid movement here signifies an exposure of the absorbing surface to the fluid in which the food substances are held in solution, and the purpose of the movement is explained.

If the saprophytic mode of nutrition of Entamœba coli is admitted, there remains to be explained the occasional but undoubted presence within the amœbæ of bacteria and other organisms. Here one must give due weight to the fact that many of the enclosed organisms are still motile; the writer has seen spirochætes very actively motile within a vacuole, and Gauducheau has recorded a spirillar infection of amœbæ. Taking into account the relative motility of an amœba and a motile bacillus or spirochæte, it is much more likely that the latter organisms enter the amœbæ through their own motility, than that the amœba captures them. In other words, these organisms are in all probability parasitic within the amœbæ. This does not, of course, apply to flagellates, which are occasionally seen within amœbæ. The writer has never seen motility in such enclosed protozoa, and thinks it most probable that they are dead or degenerating when ingested.
It may be noted that the two kinds of bacteria most commonly seen within the vacuoles of this amoeba are a short diplo-bacillus and a rather large motile bacillus with rounded ends. The former has a rather characteristic appearance, and is heart-shaped rather than round. The latter might be any one of a large group. Streptococci, though they are often seen adherent to the surface of the amoebae in stained preparations, have not been seen enclosed within vacuoles in the cytoplasm.

SOME REMARKS ON THE LIFE CYCLE.

A critical examination of the literature on the subject leaves the reader convinced that the life cycle of *Entamoeba coli* is as yet not fully understood. The simple cycle of growth, multiplication by binary fission or schizogony, and encystment as a means of propagation to other hosts, is neither fully confirmed by recent work nor is it in itself sufficient to explain many of the observed facts. It must have been the experience of most of those who have studied this interesting parasite to have met with many forms which could not be allocated to any part of the life cycle, and also, as the literature indicates, to have failed to find stages which theoretically ought not to be uncommon.

The variations in the intensity of infection are as yet unexplained. The majority of the infections met with in the routine examination of stools are fairly light, and are maintained at a uniform level from day to day. If one regards *Entamoeba coli* as a harmless parasite, in which the adaptation between parasite and host is perfect, this is what one would expect, judging from the analogy with other infections, such for instance as those of trypanosomes in animals which are the natural hosts of the species. But now and then one meets with infections so heavy that the amoebae must constitute an appreciable proportion of the mass of the stool, and it has repeatedly been observed that such infections, persisting for a time, may become suddenly reduced to such an extent that it is only after long search that one finds a single amoeba. The case from which most of the observations recorded in this paper were made was an instance of this. Similarly with regard to cyst formation; a few cysts, in various stages of maturity, according to the consistency of the stool, are to be found in practically all infections at all times, their numbers, in proportion to those of the free forms, being fairly constant. But at certain times cyst formation appears to receive a special impetus, and the proportion of cysts to free forms is greatly
increased. This was noticed by James, who, however, does not offer any explanation of the phenomenon.

Other considerations lead one to suspect that the life cycle of this organism is as complex in reality as its representation in the diagrams is simple. Granting for the moment that multiplication in the vegetative stage takes place by binary fission and by schizogony, one is at once met with the question as to what relation these two modes of reproduction bear to one another and to encystment. Do the daughter amoebae, which are the product of binary fission or of schizogony, continue to reproduce themselves by the same method, or do the two methods alternate? We have as yet no information bearing on such points, nor do we know which mode of division gives rise to the generation which is destined to encyst. Further, one has to account for the form which are rarely met with. Consider, for instance, the forms which have been described by Mathis and Mercier as schizogonic cysts. Though the interpretation which these authors have placed upon their observations is open to argument, the observations themselves can be safely accepted as accurate, and it is a remarkable fact, if these forms are a normal phase in the life cycle, that they should not have been seen before. The double amoebae which the present writer has described and figured in this paper, and which he regards as a phase of a sexual process, are another instance of a similar nature. As has already been stated, all the examples seen were in the same series of preparations, a most exhaustive search through other slides from the same patient, but made on different occasions, having proved fruitless.

The most likely explanation of the rare forms appears to be that they represent phases in events which are not always included in the cycle of growth and multiplication, but take place only at rare intervals, when, perhaps, the virility of the race has become impaired through a long period of asexual multiplication.

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THE MAGGOT TRAP: A MEANS FOR THE SAFE DISPOSAL OF HORSE MANURE AND SIMILAR REFUSE.

BY

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While relatively small quantities of horse litter can be disposed of in incinerators, for which it provides a useful supply of fuel, the disposal of large quantities, such as have to be arranged for in the case of mounted troops, is always a matter of difficulty, and close and constant supervision is required if the fly nuisance is to be kept down. The difficulty arises partly from the fact that stable manure is a commodity of some value for agricultural purposes and as a padding for the floor of riding schools, and there is a natural reluctance to destroy it. Further, as a manure, its value depends largely on the progress of the same fermentation processes which render it so suitable a breeding place for flies. The interests of the farmer, who wishes to keep it in heaps and to allow fermentation to go on, are exactly opposed to those of the sanitary officer, who, unless he adopts the method of 'close packing,' wishes to have it dry and harmless as soon as possible. The compromise so often adopted, of removing it to a distance from the lines, where it is supposed to be innocuous, is not usually very successful in practice.

The purpose of this communication is to invite attention to a means of disposal in which these conflicting interests are reconciled. Fermentation is encouraged, but the fly larvae are caught and destroyed as soon as they are full grown. The method has been successfully used in America, but has not, so far as the writer is aware, been tried in this country. From its simplicity of construction, cheapness, and ease of working, and the small amount of skilled supervision required, the maggot trap would be a useful means of disposal.
appear to be specially suitable for Indian conditions, particularly in
cantonments where there are mounted troops.

The principle underlying the construction and use of the maggot
trap is dependent on a well-known fact in the bionomics of the house
fly, namely, the migratory habit of the larva. Moisture and warmth,
such as are produced by the fermentation of organic matter, are essential
for the development of the larva, but as soon as the latter has become full
grown and ready for pupation it leaves such an environment, and seeks
out a dryer place for the final stage in the metamorphosis. The
majority do not travel very far, and are to be found within a foot or two
of the heap of manure, or in the dry portions round its edges, but
they may, as has been shown by Gordon Hewitt, travel considerable
distances, and even penetrate into the soil to a depth of two feet. Pupae
are practically never found in the portions which are still warm and
moist. The trap is essentially a device to catch the larva during this
migration.

The maggot trap is constructed in the following manner. A shallow
concrete trough is built on the ground level, with sides a few inches high,
and having an outlet at one end, to which the floor slopes a little, and an
arrangement by which water can be let in at the other. On this is placed
a moveable wooden platform of a slightly less length and breadth, and of a
convenient height. The floor of the platform is formed of strips of
wood set an inch apart, so as to leave a series of long, narrow intervals.
The sides of the platform above the level of the floor are formed of open
woodwork. The actual details of construction and the dimensions would
of course vary according to local circumstances. The American workers
used a trough 22 feet by 12 feet, with sides 4 inches high, and a platform
20 feet by 10 feet, on legs a foot high, and with sides 3 feet high.

The method of use is as follows. The trough is first filled with
water, the outlet being plugged. The manure is brought straight from
the stable and stacked tightly on the platform, commencing from one end,
carrying on from day to day until the platform is as full as it will hold.
Each day the pile is watered thoroughly, to keep it suitable for the larva
during their growth, while at the same time keeping it unsuitable for
pupation. Flies oviposit without hindrance on the manure, and the
larva develop under very favourable conditions, but when full grown the
imigratory instinct forces them to endeavour to leave the manure, and
in endeavouring to do so they either crawl through the sides and so fall
into the water, or drop through the crevices in the floor of the platform.
Every two or three days the water in the trough is changed, the drowned larvæ being collected in a grating at the outlet. When the platform is full the pile of manure is allowed to stand until fermentation is complete and the conditions therefore unfavourable either for oviposition or for the development of the larvæ—a period, under American conditions, of about ten days. The manure is then harmless from the point of view of the fly nuisance, and can be stacked with safety until it is convenient to remove it.

Certain precautions are necessary in the use of the trap. It is most important that the pile be watered daily, or the oldest portions may become dry enough to allow the larvæ to pupate there. The water in the trough must be changed regularly, or it may become a breeding ground for mosquitos. It was found advantageous to place a layer of straw on the floor of the platform before piling on the manure, to prevent as far as possible fragments falling through the spaces intended for the larvæ.

The American workers collected the water from the trough in a concrete cistern, and had an arrangement for pumping it back again when the larvæ had been removed by the grating. Under Indian conditions it would generally be easy to arrange an inlet from the pipe supply, and to dispose of the used water in a sullage garden in the ordinary way.

The traps are best used in pairs, of such a size that one will be filled up while the other stands to allow of the completion of fermentation. It would of course be necessary to determine the period during which the pile remains suitable for fly breeding under the local climatic conditions, and to allow a reasonable margin over this for convenience in working. As a guide to the size required under particular circumstances, a platform of the dimensions already stated would take the manure from fifty horses for a period of ten days. The amount per horse may be estimated at two cubic feet per day.

One of the advantages which such a method promises over those now in use is the small amount of supervision it is likely to require once it has been started. The syce must remove the litter somewhere, and if the trap is conveniently placed, it is as easy to pile it there as elsewhere. The daily watering and the periodical changing of the water and cleansing of the trough would become a part of the ordinary routine of the stable and could be supervised satisfactorily by a quite unskilled person. Any slackness in carrying out these simple tasks would be evident at once to an inspecting officer.
As regards the efficiency of the trap, the figures given by the originators are very remarkable. In one trap, in one week, more than 800,000 maggots were caught, and from a careful examination of the pile for puparia and a comparison of the number found with that of the maggots destroyed in the trap, it was found that over 99 per cent of the total of the larvae which developed in the manure were caught in the trap.

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A NOTE ON RELAPSING FEVER IN INDIA,
WITH SPECIAL REFERENCE TO ITS
SEASONAL PREVALENCE.

BY

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ALTHOUGH relapsing fever has long been known to be endemic in India, our knowledge regarding its geographical distribution, seasonal prevalence, and its importance as a cause of mortality is, from a combination of circumstances, very limited. It is a disease without any of those conspicuous features which make it possible for even an unskilled person to diagnose plague, cholera, and small-pox with reasonable accuracy, while the sudden and acute onset of the malady, and its relatively short duration, will in a large proportion of cases prevent the patient seeking medical aid unless it is close at hand. Outbreaks of the disease among the rural population are not likely to attract the attention of persons competent to diagnose its nature unless they reach serious proportions, and there is little doubt that a not inconsiderable number of cases are included annually in that heterogeneous mixture which the statistician is compelled to tabulate as undifferentiated ‘fevers.’ It is only when the disease occurs in an organised body of men under care and control that minor outbreaks are recognised, and, from the very factors which make up the etiology of the disease, so far as we know them, it is just among such communities that outbreaks are least likely to occur. The small epidemics which are reported from time to time among troops and followers, tea garden coolies, etc., are merely an indication of the continued prevalence of the disease among the general population; they afford only an unsatisfactory indication of the degree of the prevalence.

Relapsing fever is known to occur over the greater part of this country, though, according to Sir Leonard Rogers, it apparently does not occur in Assam, Bengal, or Madras. It is believed to have a special endemic focus in the Kumaon hills, from which area it has spread now and then to the plains below. The Reports of the Sanitary Commissioner
with the Government of India contain many records of isolated cases and small outbreaks among troops and followers, and in jails, in Northern and Central India and the Deccan. It is to be noted that its occurrence is not determined by climatic conditions, for it is not an uncommon disease in stations so dissimilar as Bombay and Quetta. Of special interest are the serious epidemics in the United Provinces; to judge from the available records, the disease is more serious here than elsewhere. It is evident from the writings of Steen and Townsend, and of Bisset, that the disease in this area was no new thing at the time of their observations in 1912 and 1913, but had been present in the locality for many years, though not in epidemic form. There is reason to believe that further outbreaks of the same nature have occurred since those which were followed by these observers.

The special feature of the diseases seen in India, to which it is the purpose of this communication to invite attention, is the seasonal prevalence in its relation to transmission by the louse. Since this insect was first incriminated by Mackie at Nasik, its rôle in the transmission of the relapsing fever of Europe and North Africa has been proved to the hilt, and if the seasonal prevalence of the disease were the same throughout India as it is in those countries, it would not be necessary to question the part played by the louse. But the epidemics in the United Provinces appear to occur mainly in the hottest part of the year, in the months of March, April, and May, and when we come to consider the part played by the louse in epidemics such as these difficulties at once present themselves.

The attention of the writer was first drawn to this question by an experience of a series of cases, hardly to be called an epidemic, at Ahmednagar in the summer of 1918. The total number of cases was not large, but the circumstances under which they occurred made him, as sanitary officer of the brigade, very apprehensive of trouble to come. At the time existing lines and camps were being expanded as rapidly as possible, and new ones being erected, to accommodate recruits for the new army then being formed. The units of the brigade consisted almost entirely of recruits, with only a nucleus of trained soldiers. Almost all were in camps, and in the early days some overcrowding was inevitable. Famine conditions prevailed in the Ahmednagar district, and water was a valuable commodity in camp. Owing to the water scarcity, the rapid rate of recruiting, and the great difficulties with which the engineers had to contend, it was not possible at the start to provide
really satisfactory arrangements for the ablutions of the men and for the washing of their clothes. Parties of recruits were arriving almost daily, and there was a continual coming and going of recruiting parties between the camps and the recruiting areas.

The cases were almost all imported, occurring in newly arrived men within the incubation period of the disease, or were directly traceable to such imported cases. Several men apparently contracted the disease in the segregation camp in which new arrivals were accommodated. What constituted the serious feature of the situation in the mind of the writer at the time was that enquiries made from the recruiting parties on their return revealed the fact that a 'fever' was raging in many of the districts, notably Gurgaon, from which the recruits were drawn. In view of the season it could hardly be malaria, and the arrival of sick men among the parties, in whose blood the spirillum was found, pointed strongly to its being relapsing fever. If this were the case, there was a great danger of the introduction of the disease in the constant stream of new arrivals at the camps, and if the disease once got a hold, it would not have been easy to cope with it under the conditions which prevailed at the time.

No epidemic, however, developed. Cases continued to occur in two units during the hot weather, one or two at a time, and the disease showed none of that extremely rapid spread which ordinarily characterises epidemics of relapsing fever. As a cause of sickness among the troops as a whole it was negligible, and the mortality was very low in the cases which developed the disease after arrival. Two of the camps, each containing up to eight thousand men, remained without a single case, though the medical officers were on the watch for the disease. From the end of the hot weather no more cases occurred either among the new arrivals or the troops in camp. In the early months of the following year, however, the writer, then transferred to Poona, found that cases were beginning to appear in several scattered units. From conversations he had with various medical officers, he gained the impression that it is at this season of the year that relapsing fever may be expected to crop up. A transfer from Poona prevented further observations, as the hot weather advanced.

This series of cases presented two interesting features, namely, the season at which they occurred, and the failure of the disease to become established when circumstances appeared to be favourable to it. The season coincided with that at which epidemics have previously been
recorded from the United Provinces. In view of the rôle assigned to the louse in the transmission of the disease, the occurrence of epidemics at such a season is very remarkable, and the circumstances should be subjected to a critical examination.

In Europe, America, and North Africa, relapsing fever, like typhus, is most prevalent during the winter. Once the mode of transmission was known, it was not difficult to account for this fact. Many circumstances combine to make the winter season the most favourable for the louse, both as regards the heaviness of infestation of individuals and the opportunities for the transfer of lice from person to person. Obviously, unless personal cleanliness is a deep-rooted habit, its degree will vary in inverse proportion to the incidental discomfort. A change of apparel at night—a most important point in the limitation of the infestation, as Nuttall has shown—is not so likely in the case of a poor person in cold weather as in hot, for his inclination is rather to put on all the clothes he has. There is further a greater tendency for men to crowd together in their houses, giving increased facilities for the spread of the parasites. Actual observations have in fact shown that the degree of lousiness among a population is greater during the winter than in summer. The louse itself is little affected by a fall in the air temperature, since this has only a slight effect on the temperature of its habitat, the close neighbourhood of the skin of its host.

Unfortunately there are too few data available to enable one to determine whether such factors cause a rise in the prevalence of relapsing fever during the cold weather in India, in those parts where the seasonal variation is sufficiently marked to affect the habits of the people. In the considerable number of cases recorded by the Sanitary Commissioner with the Government of India the time of occurrence is not stated. Mackie, writing of the relapsing fever of Bombay, states that its seasonal prevalence is a marked feature, and that very few cases occur during the hotter months of the year. Brown, in a valuable unpublished report which will be referred to later, is inclined to attribute to the disease a constant rise which he noted in the mortality in the United Provinces during the month of December.

On the other hand there is the definite fact of the occurrence of epidemics in certain districts of the United Provinces in the hottest months of the year, March, April, and May. During these months the climate is hot and dry. In April 1914, for instance, the highest maximum temperature at Bareilly for the month was 107.2° F., the lowest minimum
59·0° F., with an average maximum of 96·0° F. and an average minimum of 67·9° F. May was a little hotter, with an average maximum of 100·75° F. for the month. June shows much the same maximum temperature, but a consistently higher minimum. The atmospheric humidity increases steadily as the rains approach; the average figures are 53 for April, 61·6 for May, 65·6 for June, and 85·5 for July.

A consideration of the habits of the people and the conditions under which they live convinces us that any high degree of louse infestation at this season is most unlikely. The Indian ryot, never at any time addicted to over burdening himself with clothing, wears the scantiest of garments during the summer heat. Clothing discarded while at work is left exposed in the fields, where it is subjected to the action of sunlight and heat of an intensity which is likely to be speedily fatal to lice. Among many numerically important castes it is the custom of the men to wear the hair cut short, so short as to place the head louse at a serious disadvantage. There is no huddling together at night, for as often as not the peasant seeks the cooler open air for his night's rest, and sleeps on a charpoy in his compound. Lice, undoubtedly, exist throughout the year, but, from what one knows of their biology and the habits of the host, the hot weather is the season when one would confidently expect their numbers to be the lowest.

It must be admitted that our present knowledge of the biology of Pediculus has been gained almost entirely in temperate climates; the observations made do not necessarily apply without modification to the Pediculus of tropical and sub-tropical climates. But what evidence there is regarding the seasonal prevalence of lice in hot climates supports the argument set out above. The writer, whose fortunes during the war did not take him to Mesopotamia, has been told by several observers on whose testimony he would place much reliance that during the hot weather there the lice disappear. Sergent and Foley state that in Algeria, where relapsing fever is definitely a winter disease, the degree of louse infestation among the population among which it occurs is much less in summer than in winter, corresponding to the scanty attire worn during the hot season. The several writers who have recorded their observations on the epidemics of relapsing fever in the United Provinces have noted the presence of lice, but one is struck, on a critical reading, by the fact that none of them remark on any unusual prevalence of these parasites. Brown, in the report already alluded to, states that lice were less numerous in March and April in the Meerut district than in the colder months, and records the fact that on one occasion, on the 15th of May, he
carefully searched the bedding of five cases of relapsing fever and found only one louse. He also records some experiments on the effect of exposure of lice to sunlight, which indicate that such treatment is very speedily fatal to them.

It may be stated, then, that from the facts, as far as we know them, the louse appears very unlikely to be the transmitting agent in the hot weather epidemics of relapsing fever.

The writer thinks it probable that herein lies the explanation of the failure of the disease to take hold in the Ahmednagar camps, the situation in which has been explained. It was not that lice were not present, but that they were not efficient as carriers of this form of the disease. The best possible facilities for personal ablutions and for the washing of clothes were, of course, provided at the segregation camps, but it would have been too much to expect that this would have entirely eliminated the pest. Lice were, in fact, present in the camps, though their numbers were never very considerable. As the normal length of life of the louse is three weeks, there must necessarily have been a constant importation of them from the affected districts with the incoming recruits, and had the louse been acting as a carrier in the villages from which the men came, the introduction of a considerable number of infective individuals would have been almost a certainty. It must be remembered, in this connection, that an extreme degree of infectivity is characteristic of this disease; it does not pick out an individual here and there, but affects often the whole household at the same time.

This view is borne out by a perusal of the notes of cases in the reports of the Sanitary Commissioner with the Government of India. In no case does he record a serious outbreak among troops, even in the earlier days when the rôle of the louse was not suspected, and consequently no special measures were taken to combat it. Most of the cases apparently occurred singly or in small groups, and it is frequently noted that one or more of these was definitely imported. The highest number of cases recorded for one unit is 15, in the 18th Lancers at Meerut in 1914. The 38th Central India Horse had 13 cases at Goona in 1911, two units had 9 cases, three units 7 cases; the 41 cases which are recorded in 1914 were distributed in eighteen stations. These figures are in sharp distinction to those of the outbreaks in the Bijapur Jail in 1901, with 323 cases, and in the 'Deccan Gang' in 1902, with 210 cases. These two may well have been the classical relapsing fever transmitted by the louse. It must, of course, remain a matter of opinion as to how far the relative immunity of troops
Relapsing Fever in India.

from relapsing fever is due to the greater cleanliness of their habits, as compared with those of the rural community from which they come.

The possibility of eliminating the louse as a carrier of the relapsing fever of the hot weather introduces us to another side of the question, namely, the differentiation of the types of this disease. This is, of course, no new matter. Much work has been done on the differentiation of the North African, European, and American strains, all of which are transmitted by the louse. In India, Browse has described a form from Quetta, believed to be endemic there, and which he thought might be transmitted by a tick. Jukes has described a form occurring in Darjeeling, and possibly imported from Tibet, differing clinically from the classical fever. These two forms appear to be quite distinct, and different from the form met with in Bombay. If the form described by Steen and Townsend, Bisset, and Brown can be shown to be transmitted otherwise than by the louse it must, notwithstanding the clinical similarity and the apparent identity of the organism, be regarded as a separate entity. Brown himself, as a result of a considerable amount of work on the subject, was not at all convinced that transmission by the louse was in accordance with the epidemiological facts, and suggested as a possible agent a Pentatomid bug, Bragada pieta. He points out that the disease affects the rural population rather than the urban, and is associated with the cutting of the crops. It may be added that, if some arthropod other than Pediculus is proved to be the carrier, the occurrence of a few cases after the first one imported into a unit might well be explained by the conveyance of the infective carrier in the clothes or on the person of the first case; it might live long enough to infect two or three persons, but would be unlikely to breed in such a changed environment.

The writer concludes with a request that delegates who have had experience of this disease will state their observations, particularly with regard to the seasonal prevalence.

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FURTHER OBSERVATIONS ON THE REPRODUCTIVE SYSTEM OF CIMEX, WITH SPECIAL REFERENCE TO THE BEHAVIOUR OF THE SPERMATOZOA.

BY

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In two remarkable papers dealing with the reproductive system of the bed bug, published in 1898, Berlese gave an account of the curious structure which bears his name, and described, under the name of ‘hyperganesis,’ a process by which the spermatozoa received by the female, in excess of those required for the fertilization of the eggs, were digested and absorbed, to serve as additional nourishment during the oviposition period. The process as described by Berlese may be summarised as follows. The special organ is unpaired, is situated on the right side of the abdomen, and is not connected with the reproductive organs proper by any duct or defined channel. During the breeding season the presence of spermatozoa within it is practically constant, and sometimes they are present in very large numbers. They reach it by passing through the haematocele, having penetrated the walls of the spermatheca, and come to lie within the cells of which it is composed, and there undergo a complex process of digestion, the assimilable portion of their substance being absorbed, the remainder excreted. Other spermatozoa pass into the oviduct, and are taken up by its epithelial cells, which have a secretory function similar to that of the cells of the insect mesenteron. Those spermatozoa which do not pass into the haematocele and are not taken up by the epithelium of the oviduct pass
upwards in the genital tract, and come to lie within a mass of nucleated protoplasm, undifferentiated into cells, which lies below the follicle in the ovariole.

The special organ—which we may for convenience continue to call the organ of Berlese—is, so far as we know, unique among insects, and no one had previously suggested that spermatozoa could have such a function as that ascribed to them by Berlese. One would have expected the matter to have at once excited the interest of entomologists, and, because of the wider aspect of the question and the far-reaching possibilities which Berlese’s theory of ‘hypergamesis’ suggests, of physiologists also, more especially since the main facts are easily confirmed by a few simple dissections of a common insect. Little notice, however, was taken of Berlese’s work. The only paper which appeared on the subject was one by Carazzi, who took up the matter at Berlese’s own request, and examined some of the latter’s preparations, as well as others which he made himself. Carazzi’s criticism was very damaging, and may well have led those reading it, if they had not seen the original papers, to regard Berlese’s theory as finally disposed of. It referred, however, to only a part of Berlese’s work, and Carazzi did not question the facts regarding Berlese’s organ and the presence of spermatozoa within it and in the haematocele.

The failure of Berlese’s work to attract the attention which it certainly merited may be attributed partly to the circumstance that it was published in a journal which does not ordinarily contain papers of this nature. It was brought more prominently to notice by the publication, in 1907, of his book, Gli Insette, in which Berlese, uninfluenced by Carazzi’s criticism, gave an account of the phenomena in Cimex and in other insects in which he had observed a similar course of events. It was this account which first led the present writer to interest himself in the subject. A methodical investigation by means of controlled experiments has shown that certain of Berlese’s statements are incorrect, and those which have been confirmed are hardly enough to sustain such a theory as that of hypergamesis; on the other hand, some of the new facts which have come to light are such as to throw doubt upon the generally accepted assumptions with regard to the spermatozoa in insects, and to emphasise the existence of a lacuna in our knowledge of the process of fertilization. The special organ present in the female bed bug, in which the spermatozoa are found, is certainly a most exceptional structure, but one cannot affirm that the manner in which the spermatozoa reach the
ova is exceptional, for want of an adequate investigation of the same process in other insects.

Two difficulties arise at once when one begins to consider the mechanism of fertilization. In the first place, there is an almost entire lack of information as to what happens to the spermatozoon which does not succeed in finding its counterpart in an unfertilized egg; secondly, and particularly with reference to insects, our knowledge of the precise time and place at which fertilization of the eggs takes place is very unsatisfactory. On the first point, the general text-books do not go further than to teach that the function of the spermatozoon is to fertilize the egg, and to explain at length the associated nuclear changes; the enormous disproportion in numbers between the spermatozoa and the eggs which they have to fertilize is regarded as an instance, as Starling puts it, of the prodigality of nature, and the ultimate fate of the vast majority of the spermatozoa is left unexplained. It is assumed that only one spermatozoon enters the egg, and it is stated that in some cases a covering is formed over it after fertilization, with the special object of preventing the entry of more spermatozoa; instances of the penetration of more than one are known, but they are regarded as exceptional. The chances against any spermatozoon reaching an egg are supposed to be so great that large numbers are necessary to ensure that one shall reach each egg, and it is assumed that the unsuccessful ones simply degenerate. The more one thinks of it, the more one is inclined to doubt the correctness of this assumption—for, as far as can be ascertained from the literature which has been available to me, it is an assumption, not a demonstrated fact. Nature is prodigal, perhaps, in the sense of making abundant provision for so necessary an object, but not wasteful. If we admit, as we surely must, that some attraction of the nature of chemotaxis exists between the spermatozoon and the egg, and take into account the fact that in so many cases, cases in which the spermatozoa undoubtedly enormously outnumber the eggs, the easiest course for the spermatozoon is that which leads it directly to the egg, the great excess of spermatozoa seems unnecessary. It is a little curious, in view of the advanced state of our knowledge of the processes of fertilization and development generally, that so little attention should have been paid to this point. The large excess of spermatozoa is an almost universal rule in the animal kingdom, and if we once admit the possibility of such a theory as that of hypergamesis, it will be necessary to consider also the possibility that something of the kind is of universal occurrence.
In this connection the shape and structure of spermatozoa are pertinent facts. If their end is not a mere wasteful death, it is not at all a difficult assumption that they should penetrate into the cells of the body. We know that spirochaetes, which resemble spermatozoa in the general outline of their structure, and are similarly designed for great motility, can penetrate intact tissues. As will appear in the course of this paper, the spermatozoa of Cimex have very great powers of penetration, and normally pass through several layers of cells in their course to their final destination. Many instances of a similar power of penetration have been brought forward by Kohlbrugge with reference to the spermatozoa of the higher animals.

As regards the second point, it is generally assumed that the spermatozoa received from the male insect at the time of copulation are stored in the spermatheca, from which they issue as the eggs pass down the oviduct, and that fertilization is accomplished by the entry of the spermatozoon through the micropilar apparatus, which is usually situated at the anterior pole of the egg. The spermatozoa lie in wait for the eggs as they emerge, so to speak, and fertilization is the final act in the intracorpororeal life of the egg. I have been unable to find records of any cases in which this assumption has been proved to be correct. How little certainty there is regarding it may be gathered from the writings of entomologists of the first rank. Packard states that 'fertilization* of the female takes place once for all a long time previous to oviposition; the semen in the receptaculum seminis passes out as the eggs slip down the egg-passage, and a spermatozoon gains entrance into the interior of the egg through the micropyle.' But he goes on to quote the case of Oecanthus, in which, according to Ayres, fertilization probably takes place while the egg is passing into the vagina, 'since it is hardly possible that the male element could gain access to the follicles before the chorion is secreted.' Miall and Denny, in their account of the cockroach, state that 'the convex surface of the chorion is perforated by numerous micropyles, fine pores through which it is probable the spermatozoa gain access to the interior of the egg.' Lowne states that in the blow-fly 'fertilization is effected as the ovum passes through the oviduct,' but

* There is some confusion in the use of the term 'fertilization' as applied to insects. It would be preferable to restrict its use to the actual fertilization of the egg by the entry of the spermatozoon, and to use the word 'impregnation' to denote the reception of the sperms by the female.
his lack of justification for so positive an assertion betrays itself in a subsequent paragraph, in which we read that 'the egg next passes into the uterus, and rests with its micropyle in immediate relation with the ducts of the spermathecae, from which no one doubts the sperm-cells enter the egg.' 

Lowne was aware that developing ova are frequently found in the oviducts; his explanation of the fact is not at all a convincing one, and shows a disposition to make the facts fit with the theory which one would not have expected from him—it must be remembered that such eggs must have been impregnated, and have evidently therefore been in the uterus, hence they must have passed the orifices of the ducts of the paraovaria in their descent; and the fact that they are found in the oviduct shows that they have been passed back into the oviduct, instead of having been deposited.' 

Miall and Hammond make the clearest statement on this point, for in their account of Chironomus they state definitely that it is not known when fertilization takes place. One must further note that an explanation which would fit in with the anatomical facts in the majority of insects, which have spermathecae and deposit fertilized but undeveloped eggs, will not serve for the very numerous exceptions. The conditions in the pupiparous flies, for instance, present obvious difficulties, as do those in the Tachinids, Sarcophaga, and other larviparous flies. In such insects as Pediculus the usual explanation fails because there are no spermathecae, and it is interesting to note that in this insect, according to Doncaster and Cannon, some of the eggs 'appear to begin their development in the oviduct, and are already segmenting when they are laid.'

The study of the processes of fertilization in insects derives a special importance from the similarity which exists with the corresponding processes in the higher animals. In each the spermatozoa enormously outnumber the eggs, and reach the latter by their own motility within the genital tract of the female. If our knowledge of these matters in insects is unsatisfactory, there is reason to doubt whether we are in a much better position with regard to the higher animals. In an extremely interesting paper published shortly before the war, Kohlbrugge showed that the generally accepted teaching is far from a complete statement of the case, and is not altogether correct. His observations, which were made on bats, rabbits, mice, fowls, and on the shark, show that the spermatozoa do actually penetrate into the mucous membrane of the female genital tract, and are found, not only between the cells, but
actually within them. He further showed, and cites the work of others in support of his own observations, that, in some animals at least, the spermatozoa penetrate into the already fertilized and developing eggs.

His results with reference to the location of the spermatozoa in the fowl are directly at variance with the text-book statements, for he failed to find them near the ovary at the upper end of the oviduct, where they are assumed to be; according to his observations they do not rise much above the enlarged part of the oviduct in which the egg-shell is formed, and fertilization takes place after, not before, the egg receives its coating of egg-white. He also found spermatozoa within the cells of the mucosa at the lower end of the oviduct. In the rabbit he found spermatozoa, some of them so altered as to simulate nuclei, lying between the nuclei of the mucosa cells at the lower end of the oviduct.

Kohlbrugge’s work will doubtless be confirmed and extended, but for the present there appears to be no reason to doubt that the spermatozoa penetrate into the body cells of the female. The phenomenon opens up wide possibilities, and, as Kohlbrugge says, we are only at the beginning of the explanation; a union of the male sexual cells with the body cells of the female may well be expected to influence the latter; from further investigations in this direction may in time come the explanation of many problems, such as telegony and the peculiar incidence of certain diseases, which have long remained obscure. As regards telegony, it would provide a physical basis, the lack of which has stood so much in the way of the acceptance of the evidence.

The observations recorded in the following pages are offered as a contribution to a large and almost a new subject. Before any definite conclusions can be reached regarding the secondary functions, if any, which the spermatozoa of insects fulfil, an extensive comparative study of different types, representing the different ways in which the reproductive system carries out its function, will be necessary. At present we are unable to say to what extent the course of the spermatozoa in Cimex is exceptional, though it will be readily admitted that it is quite an unexpected one; as will be explained in detail, they make their way from the spermathecae to the ovariole, not in the lumen of the oviduct, where one would naturally expect to find them, but through the substance of its walls. As regards the organ of Berlese, we as yet know of no other similar structure; a search among other bugs, and also among the Lepidoptera, might yield interesting results.

Kohlbrugge found that the large number of sections which he had to cut and examine was a serious impediment, and made the investigation very laborious. Insects present no such difficulty, for the whole of the reproductive system of an insect of the size of the bed bug can be arranged on a single slide, even if cut in very thin sections, and the whole insect cut in serial sections does not occupy more than four or five slides. The study of the behaviour and ultimate destination of the spermatozoa of insects might well precede the much more laborious investigation in the large animals.

REVIEW OF PREVIOUS WORK.

Of the few papers dealing with this subject, three have appeared in the Rivista di Patologia vegetale, a journal mainly devoted to economic entomology, and one in which, as Carazzi remarks, one would hardly expect to find papers of this kind. As these papers are not readily accessible a somewhat full account of them will be given.

The first to see the structure herein referred to as the 'organ of Berlese' was Ribaga. His attention was attracted by the irregularity of the border of the fourth sternite of the bed bug, and beneath the sternite he found this structure, and determined that it is peculiar to the female. He confined himself, however, to an examination of the chitinous portion at the external aperture, thinking that this was entirely separate from what we now term the organ of Berlese, though the cells from which the chitinous filaments arise are embedded in it. Ribaga gave an excellent account of the anatomy of the part. Arguing reasonably that since this organ was present only in the adult female, it must have some connection with the function of reproduction, he saw in the complex arrangement of spines surrounding a cavity a stridulating organ, and was at great pains to devise some means to render audible the sound produced. He does not appear to have seen spermatozoa inside Berlese's organ, and was of course ignorant of the true purpose of the apparatus which he described with such minuteness and care.

Berlese's two papers were published in the following year. In the first he deals with the organ which bears his name and with the migration into it of the spermatozoa from the spermatheca. He refers to Ribaga's organ, but adds no new observation or criticism, and evidently, like Ribaga, regarded the chitinous structures at the entrance as distinct from
the rest of the organ. It is to be noted that Berlese throughout suggests no doubt regarding the course of the sperms, and had evidently never seen bed bugs in the act of copulation. During the months of March and April (in Italy) he constantly found large masses of spermatozoa within the organ and within the spermathecae, the right one of which was the most distended, and also found large masses free in the haematocele; these latter he regarded as having passed through the wall of the spermatheca of the right side and as being on their way to enter the special organ. He explains the greater distension of the spermatheca of the right side as being due to the position of the penis of the male during copulation; the penis is falciform and directed to the left, and this, according to Berlese, will bring its terminal part into the opening of the right spermatheca, when the two sexes are in the position normally occupied by bugs in the act of copulation, i.e., with their posterior ends in apposition. He does not specifically state that he has seen Cimex in this position.

He describes the organ as consisting of a mass of cells, not enclosing a cavity, surrounded by a delicate capsule and separated from Ribaga's organ by an elastic pellicle. During the cold months the cells are rounded, or sub-polygonal from compression, have a homogenous cytoplasm, and measure at most twelve microns in diameter, with a round or oval nucleus measuring five microns; they are firmly united to one another, and are not easily separated in dissection. In March, however, the organ contained abundant non-capitate spermatozoa, mainly collected in a large central mass, but partly infiltrated between the cells and invading the periphery, and when this was the case the cells showed remarkable modifications.

When the dispersion of the spermatozoa from the central mass has taken place the majority of the cells, according to Berlese, contain each one spermatozoon. Whether the cell ingests the spermatozoon or if the latter enters the cell by its own motion he is not prepared to say. For a time the sperm remains distinctly filiform, but later it twists upon itself to form a round or oval skein; at the points at which the filament crosses itself in the skein it becomes swollen, either from an alteration of its substance or by the deposition of some substance from the cell, until the spermatozoon forms a rounded mass which comes to assume the aspect of another nucleus. In sections stained with borax-carmine and haemalum this male nucleus stains an intense rose red, while the other nucleus stains violet. At this stage cell-division, usually amitotic but sometimes by
mitosis, takes place, and multi-nucleated cells are frequently seen. In the subsequent course of events there is a gradual fusion, the details of which Berlese did not make out, between the male and female nuclei, and the contents of the cell are transformed into a spherical droplet, while the rest of the cytoplasm disappears, leaving only the droplet in the middle of a walled cavity. In the final phase the parts useful to the insect are absorbed and the waste products eliminated. The droplet breaks down to minute portions which pass to the periphery of the organ and are there taken up by a layer of large cells resembling those of the fat body. The waste products are passed out through the organ of Ribaga, between the chitinous rods of which Ribaga had already noted the presence of pigment granules. He notes that the spermathecae, in addition to spermatozoa, contain free cells which are similar to those of the special organ, but which are derived from the epithelium of the wall of the spermatheca. These cells take a part in the destruction of the spermatozoa.

Berlese concludes his first paper with a series of tabulated conclusions, and a disquisition on the function and eventual disposal of the spermatozoa, pointing out that the number is very greatly in excess of that required for the fertilization of the eggs. He considers that they fulfil a double function in addition to fertilization viz., to provide the female with an abundant supply of nourishment at the time of oviposition, and to provide a stimulus to the development of the sexual organs. He states that notwithstanding abundant food the sexual organs do not develop fully until copulation has taken place.

In his second paper Berlese deals with the phenomena observed in the spermathecae and oviducts. One may note his introductory remark, that he recognizes the necessity of proceeding with the greatest care in questions so minute and delicate as this, and that he has confirmed the observations recorded in his first paper, and has nothing to alter, but on the contrary something to add. He notes with regard to the free cells in the spermathecae that these do not show the cycle of changes observed in the special organ, though they contain spermatozoa. The free cells pass into the oviduct and were not traced further.

The spermatozoa, either as received from the male or issuing from the spermathecae, pass upwards into the oviduct and become reduced in numbers. They are ingested by the cells of the epithelium of the oviduct
and ootheca.* He states that the epithelium of the upper part of the oviduct is composed of high cylindrical cells arranged in pedunculated groups, and compares them with the cells of the mesenteron of insects in full functional activity, affirming that they produce droplets of secretion which are set free in the lumen, and sometimes entirely fill it. These cells take up spermatozoa, which are subjected to a digestive process, the products of which, destined for the nourishment of the female, are passed out into the hæmatocele through the bases and sides of the cells, and appear in sections as a substance which stains intensely with hæmatoxylin.

Below the first, or lowest, egg, there is a mass of nucleated protoplasm, undifferentiated into cells, in which three layers can be distinguished; the lowest consists of a single transverse row of nuclei, in the second the nuclei are densely packed, and in the highest they are very scanty. Spermatozoa are found in the upper two layers of this mass. Berlese concludes that before fertilization of the egg—he does not indicate at what stage this takes place—the greater part of the spermatozoa which have not passed into the body cavity on their way to the special organ are taken up by the cells of the oviduct and by the cells in these layers, and utilised as nourishment for the female. He suggests that the epithelium of the oviduct will be similarly active in those insects which have no spermathecae.

Carazzi’s criticism refers mainly to the observations of Berlese on the special organ. It is undoubtedly very severe. He confirms Berlese’s general description of the organ, but neither in his own preparations nor in those of Berlese, examined at the latter’s request, could he find spermatozoa within the cells. He is convinced that what Berlese took for spermatozoa were nothing more than vacuoles within a cell. As regards the terminal stage of the large droplet, he points out that if Berlese is right then at this stage the spermathecae should be empty, and there should no longer be spermatozoa in the organ, whereas the reverse is the case. The objection that, when the cells are found in this condition and the spermathecae are also full, the latter have been filled by a second copulation, is met by the argument

* I do not understand exactly what Berlese means by the word ‘ootheca’ in this connection. From the context it might be inferred that it refers to the epithelium of the ovariole in its undifferentiated condition, but the word is not ordinarily used with such a meaning, and is not so used by Berlese in his book. The spermatozoa do actually penetrate into the marginal epithelium of the egg follicle after it has secreted the chorion of the egg, but Berlese did not know of this.
that a second copulation is unlikely, and that it is improbable that
the cells of the bursa can survive to function a second time. He
points out that Berlese has taken no account of a circumstance frequently
noted, viz., the collection of the sperms into a central mass. Carazzi found
the greatest variety in the appearance of the cells of this organ. Some
of them contained a large mass which stained partly with acid and partly
with basic stains; others show a small nucleus and only a small quantity
of cytoplasm surrounding a large vacuole. He affirms that he has never
seen spermatozoa which were without doubt within the cells, and
thinks that Berlese has been unduly biased by his own theory of
'hypermegasis.'

In his book Berlese re-affirms his statements, withdrawing nothing,
and dismisses Carazzi's criticism with a remark to the effect that the
latter had not examined the matter with sufficient care. He accords
the subject of hypermegasis a separate heading, and notes its occurrence
in certain other insects.

The subject was referred to briefly in a publication by Patton and
the present writer in 1913. At the time of writing the original papers
were not available, and a detailed examination of the structures could
not be attempted for lack of time. The essential facts regarding the
mode of copulation in Cimex and the relation of the spermatozoa to the
organ of Berlese were, however, recorded. It was shown that during the
act of copulation the penis of the male is introduced into the opening
of Berlese's organ, and not into the median genital opening, and that
Berlese's description of the behaviour of the spermatozoa was therefore
essentially wrong; the entry of the spermatozoa into the organ of Berlese
was proved by the examination of the organ removed immediately
after the act had been seen to take place. In the autumn of 1914 the
present writer contributed to this Journal a preliminary note on the subject;
giving in brief outline the main facts regarding the course of the
spermatozoa subsequent to their reception by the female, as
demonstrated by the examination of bugs in series at known periods after
copulation. At the time the note was written circumstances did not
permit of the thorough examination of the large amount of material which
had been prepared, and no attempt was made to describe the structures
concerned in detail, or to review the previous work on the subject.

It would serve no useful purpose for the writer to go into all the
points at which he finds his own observations at variance with those of
Berlese and Carazzi. Berlese's methods are open to much criticism, for
his observations were made on bugs of the history of which, as regards age and sexual history, he knew nothing. His theory of ‘hypergamenesis’ was arrived at by fitting together disconnected observations, some of which were certainly inaccurate. The writer is inclined to share the opinion expressed by Carazzi, that Berlese was led astray by his own theory. Though one does not wish to lay too much stress on the matter, it will perhaps not be improper to mention two suggestive examples of the kind of thing which invites criticism. One of his admirably executed illustrations shows an ovariole in longitudinal section, and in this he represents the first egg as nearly mature, and the second about half the size of the first, and already containing much yolk. This is not the way in which the eggs of Cimex develop. At the time when the lowest egg is fully mature the one above it is very small. As regards technique—a very important matter in the interpretation of the changes in delicate secreting cells—Berlese states that, when studying the organ as dissected out of the bug and teased on a slide, he got good fixation, better than by the use of chemical reagents, by heating the preparation between slide and coverslip until a bubble appeared, that is, practically, by the use of boiling water.

The only other recent publication* on the anatomy of Cimex is one by Hay Murray, whose paper was written ‘with special reference to uncorrected errors of previous investigators,’ and who devotes a long appendix to the correction of ‘some of the more outstanding errors’ in the account of the bug in the Text-Book of Medical Entomology written by Patton and the present writer. Hay Murray, who devotes a considerable part of his communication to a criticism of Landois’ well-known paper, is distinctly unfortunate in his expressed intention, for his own contribution of errors is a substantial one. He devotes a paragraph of a dozen lines to the organs of Berlese and Ribaga, and shows that he has confused the two, probably from an imperfect understanding of the account given by Berlese in his book, and through not having seen the original papers in the Rivista. His description of the reproductive tract of Cimex, which is apparently original work, since he had not Landois’ paper to guide him, is far below the standard of the distinguished entomologist he sets out so assuredly to correct, and his diagram of the tract can only be called grotesque. He quotes Southall to the effect that

* With the possible exception of certain German papers, not yet been obtained, which may contain anatomical notes.
about fifty eggs are laid in each batch, and states that all the ova mature at about the same time. No reference to his authority is given, but he apparently relies on the treatise of that Southall who, in the reign of George the Second, made a living by ridding the houses of the nobility and gentry of bugs, by his special secret ‘nonpareil liquor,’ at a charge of half a guinea a bedstead, tapestry included. Southall’s pamphlet is delightful reading, but one hardly expects, in these days of accurate methods, to find him solemnly quoted as an authority on the biology of Cimex. What he really said on the subject was—‘They are hot in nature, generate often, and shoot their spawn all at once, and then leave it, as Fish do. They generally spawn about fifty at a time, of which spawn about forty odd in about three week’s time usually (but sometimes two or three days more or less, according as the weather proves more or less hot), come to life; the Residue proving addle, as do often the Eggs of Hens, etc.’ Hay Murray refuses to believe that copulation can take place in the manner in which we stated that it does, and even maintains that it would be a matter ‘of great difficulty, if not utter impossibility,’ because the opening of the female is on the right side and the penis is bent to the left. As independent confirmation of our statements has already been published, and since they can be confirmed by anyone prepared to carry out the simple experiment, there is no need to deal with his argument.

**Technique.**

The object of the experiments was to follow the course of the spermatozoa from the moment of copulation, in order to find out the relation of the remarkable changes in the cells of the organ of Berlese to the passage of the spermatozoa through it, and to ascertain when and how the latter reach the spermatheca, and in what manner they are eventually disposed of. Bugs for experiment were bred from the early stages, and in order to get virgin females the fully grown nymphs were isolated, each in a separate tube, while they accomplished the final moult. When required for experiment each was placed with a male, and the pair given the opportunity—which they rarely failed to take advantage of—of a meal of human blood, by inverting the tube in which they were contained on the skin of the forearm. Copulation usually takes place immediately the pair have fed. After the act was completed the male was removed, and the female kept for the required period. Throughout the experiments the bugs were kept in an incubator at 30°C., and were fed every second
or third day. Bugs are extremely easy to rear and handle, and casualties were few. In some experiments the female was dissected, and the isolated organ embedded and cut in serial sections; the ovaries were either mounted as whole preparations and stained with borax carmine or Delafield's haematoxylin, or were sectioned. In other experiments the whole bug was fixed and embedded by the combined paraffin-celloidin method. In dealing with the whole insect the legs and elytra were pulled off in the fixative, and, after allowing time for the latter to act, the whole of the three or four anterior tergites was dissected off to allow freer penetration of the fluids. Many fixatives were tried, and of these the ones which gave the best results for the delicate cells of Berlese's organ when functionally active were Zenker's fluid and Schaudinn's. Bles's fluid is excellent for whole insects.

Experiments were carried out with C. lectularius and C. rotundatus, both of which were obtained locally.* No notable differences were found between the two either in structure or in the behaviour of the spermatozoa.

There was one uncontrolled factor in the experiments, which was probably responsible for certain apparent inconsistencies in the results, viz., the amount of sperms received by the female at one act of copulation. From sections of the organ of Berlese, fixed immediately after the act has been seen to take place, one learns that the amount may vary considerably. Unfortunately the importance of this factor was not recognised in time, or steps might have been taken to control it by ensuring that all the males used were of the same age and had been reared under the same conditions. The error due to this factor would tend to conceal the difference, if there is any, between the two species used in the experiments.

**The Organ of Berlese. Its External Opening. Penetration of the Spermatozoa and the Associated Activity of the Cells.**

_The Organ in the Virgin Female._

The Organ of Berlese is situated on the right side of the abdomen of the female bed bug, in the region of the fourth and fifth sternites.

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* Kasauli is situated on the edge of the Himalayas, about 6,000 feet above sea-level, and within easy reach of the Punjab plains below. C. rotundatus is of course the common Indian species.

(Plate XII. Fig. 50). On the fourth sternite there is a short longitudinal slit which lies immediately external to the opening into the organ, as shown in the accompanying text figure. The organ is spherical in shape when dissected out, and measures about one millimetre in diameter, more or less according to the amount of spermatozoa it contains and according to the stage of functional activity of its cells; the organ reaches its greatest size about eight to twelve hours after copulation.

![Text Fig. 1.]

The (apparent) fourth sternite of *Cimex lectularius*, dorsal view. c.o., the incision leading to Ribaga's organ. m., membraneous portion of the sternite. sp., spiracle. The dotted line indicates the limit of the investiture of bristles, which are present only on the posterior half of the segment.

has occurred, when the cells are distended with secretion. When *in situ* it is compressed by the surrounding organs, and its anatomical relations vary according to the state of distension of the alimentary canal and the size of the ova in the ovaries. Usually its upper (dorsal) surface is compressed against the mid-gut (Plate V, Fig. 2), with the right lateral oviduct lying between the two. The organ has little rigidity, and assumes the spherical form when not compressed. Its only attachment is to the ventral wall of the abdomen, by means of the structure to be described as the organ of Ribaga. It is not connected with any of the other organs, and receives only a small tracheal supply. Around the organ there is a layer of fat body, generally composed of two or three rows of cells, continuous with the fat body elsewhere in the abdomen.

Each of the writers who have dealt with this subject has emphasized the fact that this structure is present only on the right side. One of the most remarkable things about this remarkable organ is that it is occasionally present on both sides. In one of my series of sections of *lectularius* I found two organs, each with the organ of Ribaga fully
developed, side by side in the fourth segment. Unfortunately neither was functionally active or contained spermatozoa. On another occasion, being then on the look-out for it, I found the paired organs in a dissection, also of _lectularius_ (Plate XII, Fig. 48). In what proportion of females it is present on both sides it is impossible to say, for in the earlier work one accepted it as an unpaired structure. It is not likely, however, that it was missed very often, on account of the method of dissection employed; this was to isolate the fourth and fifth segments, drawing the alimentary tract out in front and the ovaries behind, and subsequently to separate the tergites and sternites of the isolated segments, exposing the internal aspect of the sternites. The organ is then quite conspicuous, and easily distinguished from fat body by its colour, and one is inclined to think that if the paired condition were at all common it would have been noticed before. Berlese states that he examined several hundred female bugs, but makes no mention of having seen this organ on the left side. When first commencing this study I examined a long series of females with the object of determining if there were traces of the organ on the left side, but found nothing.

The organ consists of a mass of cells surrounded by a delicate capsule. There is no lumen, and in the unimpregnated condition the cells are uniform in structure and are regularly arranged. They are not, however, closely compressed together to form a solid organ; the union between adjacent cells seems to be mainly by the anastomosis of short, blunt processes which might be compared to the pseudopodia of an amoeba. The cell is not connected to all those which surround it, but to only one or two, though the union is a firm one, and the cells are not easily dissociated in dissections. The mode of union is well shown in preparations in which imperfect fixation has led to some shrinkage of the cells, and to an exaggeration of the spaces between them. The whole mass of cells, one may say, constitutes an irregular network, of which the cells are the nodes. In life the intercellular spaces are presumably filled with fluid.

The capsule is extremely delicate and by no means easy to see, for it is closely united to the cells which it encloses and is in intimate relation to the surrounding fat body. It consists of very thin and flat cells with small nuclei, and is similar to the investing membrane of the ovaries and is almost as delicate. Occasionally, and particularly in preparations representing a stage subsequent to the passage of the spermatozoa through it, the capsule is much thicker and more definite.
possibly on account of a recent multiplication of its cells. No openings
or pores have been found in this capsule, and there is no connection
between it and the peritoneal investment of the ovary.

In the newly moulted and virgin female the cells (Plate VI, Fig. 6)
are spherical or oval, or polygonal from compression, and measure about
twelve microns in diameter. The cytoplasm is finely granular and
presents no special characters. A few small vacuoles are present in
some of the cells. The nucleus is spherical or oval, slightly eccentric
in position, and has a well defined spherical or rod-shaped nucleolus,
the remainder of the chromatin being arranged in small grains about the
periphery. In some preparations many of the cells contain a small deeply
stained particle resembling the nucleolus, situated midway between
the nucleus and the border of the cell. This very definite body is
sometimes present in cells when functionally active; why it should be
sometimes present and sometimes absent has not been ascertained. It
does not appear to have any connection with cell-division.

The Organ of Ribaga.

It is convenient to retain, for the present at least, the name given
by Berlese to the structures which constitute the entrance to the organ
of Berlese. Ribaga's description of it is very minute and careful, as
regards the anatomical detail, but he was naturally influenced by his
misconception of the function. Much credit is due to him for having
found a structure which had escaped the notice of so many observers,
including so accurate and painstaking a worker as Landois.

On examining Berlese's organ in the fresh condition, and when
it is empty of spermatozoa, one sees a denser and more opaque white area
in the centre, running transversely across the upper part of the slit in the
fourth sternite, and within this area one can distinguish a central axial
part which is deeply pigmented. The mass extends through the middle
two-fourths of the transverse diameter of the organ, and is situated at
its most ventral part; it is intimately attached to the two sternites and the
membrane which connects them with one another. It lies, in fact, in
the intersegmental membrane. When examined in sections of the part
under a low magnification it is found to consist of four clearly defined
layers: an internal layer of cells, a layer of faintly stained fibrous tissue,
a layer of pigmented chitinous rods, and a series of free spines, which
project into the space bounded by the fifth sternite and the overlapping
portion of the fourth. Under a high magnification (Plate V, Fig. 1)
it becomes evident that the whole structure consists of a mass of equal and similar cell-units, each of which is a chitinogenous cell with a long and highly specialized process; the cells are bound together at their bases, while their free ends project on the body surface. The cells are more compactly arranged than are those of the organ of Berlese in which they are embedded, and therefore show up as a denser white mass in fresh preparations, while the external spines and rods are pigmented, and appear as the darker axial mass, which is rendered more conspicuous by the presence in this region of granules of pigment.

The chitinogenous cells are arranged in a single layer, which is continuous with the corresponding hypodermal layer of the adjacent parts of the sternites and the intersegmental membrane. This layer is invaginated within the substance of Berlese's organ, leaving beneath the fourth sternite a much larger space than exists beneath the others (Plate XII, Fig. 50.) The bases of the cells, which are much wider than their apices, form an arch or dome limiting the fold, while the chitinous free extremities converge together and project into the central space between the fourth and fifth sternites. The general relations of the parts to the organ of Berlese and to the integument are shown in the second and third figures on Plate V, which, it may be noted, are not diagrammatic, but represent portions of actual sections of whole bugs.

When one examines the cells under a high magnification and in sections cut at an appropriate angle, the separate regions corresponding to the layers of the organ as a whole are easily distinguished. A small portion of the structure is represented in the first figure on Plate V. The innermost part, or cell proper, is pear-shaped, the end corresponding to the stalk of the pear being much drawn out. The cells are arranged in a regular layer, and the nucleus lies in the expanded part. They are separated from the cells of the organ of Berlese by a thin basement membrane, in which no nuclei can be seen. The next layer of tissue consists of delicate fibrous bands which run somewhat irregularly towards the periphery; it is not possible to follow the course of an individual band through the whole thickness of the layer, but the bands undoubtedly commence in the attenuated ends of the cells and terminate by uniting with the elements of the next layer. The chitinous rods are arranged in parallel rows and are packed closely together—much more closely than has been represented in the drawing. Careful focussing shows that the rods are rather irregularly flattened, especially at the proximal ends. At their distal ends they are abruptly thickened, so that each appears to come

into close contact with its neighbours, the whole having the appearance of a palisade, the separate elements of which are thus united together distally by a transverse band. From this point each rod is continued into a free process or spine, which is rounded at the base, produced distally into a very fine point, and slightly curved in its distal half.

So far we are no nearer a solution of the question as to how the spermatozoa enter Berlese’s organ. The layer of chitinogenous cells is continuous, and the most minute examination of sections stained with saffranin, which gives a clearer definition of the parts than any other stain, has failed to reveal any pores or minute openings, while the binding together of the chitinous rods rather suggests an effective barrier. A close examination of the terminal parts of the free processes with the highest available magnification, however, affords some light on this point, though the structures are so small—the breadth of the spines does not exceed 2.5 microns—and the optical difficulties are so great that it is necessary to be very cautious in interpreting the observations.

On examining a detached row of blades one notes that the distal half (Plate V, Fig. 5) of each is slightly broadened, and appears flatter than the proximal portion. In suitably stained sections two fine lines can be seen, arising from the margins of the blade at its broadest point and running proximally and inwards to converge at an acute angle. From the point where they meet a still more delicate line can be traced for some distance up the blade. The comparison which at once suggests itself is that with an opening blade of grass. If my interpretation is correct, the blades are not simple pointed cylinders, but are hollow tubes, opening distally as if cut in the oblique manner in which one first cuts a quill to make a pen. Each blade constitutes a separate pore for the entry of the spermatozoa.

The chitinous rods of which these blades are the continuations and free extremities are far less regular in appearance than the blades. The distal part, where each rod is in contact with its neighbours, is cylindrical, but more proximally they become flattened; the flattening is not always in the same plane, and it is only by careful focussing that one ascertains the cause of the apparent irregularity. The extreme proximal end of each rod unites with a band of fibres from the internal layer.

When examined in sections taken at right angles to their length the blades and the distal portions of the rods appear as simple rings, very closely packed together. No intervals can be distinguished between the separate elements. We know from experiment that the spermatozoa do
actually penetrate into Berlese's organ by this route, and it is clearly more likely that they should pass through the open rings than through the potential but certainly extremely narrow and rigidly bounded intervals between the chitinous elements. Each chitinous rod and the blade into which it is continued represents, according to my view, a highly modified hair, originally flattened to a long and narrow scale, but having its sides bent inwards till they meet to form a cylindrical tube, opening obliquely at the free end, more gradually and less regularly at the proximal end. The tunnel thus formed does not of course lead into the substance of the cell of which the hair is a process, but opens out into the intercellular space in the fibrous layer. The spermatozoa pass into the obliquely cut free end and emerge in the fibrous layer, and thereafter work their way inwards until they collect as a mass within Berlese's organ.

In the distal parts of the organ of Ribaga there are constantly present certain pigment granules. These are of a reddish brown colour, and vary in size and number in different preparations. Usually they are larger and most numerous in the region of the chitinous rods, the largest of the granules being rather larger than the diameter of a rod; there are smaller granules of the same kind in the distal part of the fibrous layer, the larger granules being nearest the rods, as if they arose by the agglomeration of small grains passing outwards. The majority of these granules are definitely between the chitinous rods, but in some cases they are so closely pressed against them as to give the impression that they lie in a groove in the side of the rod. In the free blades they are definitely within the lumen, and a small round granule is frequently present on the expanded end. Occasionally the whole of the terminal blades are obscured by the presence of an irregular mass which may be of the same nature as the granules. I have not been able to make out any relation between the amount of granules present and the different stages of the functional activity of the cells of Berlese’s organ. Berlese considered that these granules are waste products, produced during the digestion of the spermatozoa, and excreted through the organ of Ribaga. This cannot be the case, for similar granules are present in the newly moulted and virgin female.

If these grooved or tunnelled hairs form the true entrance for the sperms, the space produced under the fourth segment by the invagination of this very specialized part of the integument constitutes an antechamber. The fifth sternite is sharply bent just below the point at which the ordinary chitin joins the specialized layers of cells, and here it is provided
with a series of minute cog-like teeth, the points of which are directed towards the tips of the superpendent blades. (Plate V, Fig. 4.) The function of these teeth is not very clear, but the possibility suggests itself that they assist in retaining the penis in position during copulation.

Ribaga's explanation of the function of the organ which he described is certainly ingenious and interesting, and it must be admitted that it is quite in keeping with the structure of the parts as he described them. He saw in the numerous delicate blades a comb, which was drawn across the scraper formed by the cog-like teeth on the fifth segment, to produce the sound by which the male was to be summoned; the space between the fourth and fifth segments was the sounding box, and the motive power was provided by the intersegmental muscles. Ribaga was at great pains, though with no success, to render this sound audible to the human ear, and had a microphone specially constructed for the purpose. He naively remarks that his failure to hear the sound is no evidence that the structures do not act as he supposed, for the experimental conditions under which his captive bugs were placed were not such as to be likely to promote the exercise of a sexual instinct.

**Copulation.**

The act of copulation in *Cimex* has already been described. Briefly, the male places himself upon the female in an oblique position, head to the left, abdomen to the right, and retains his hold with his legs. The pair remain in this position for a varying period, from a few seconds to five minutes or more, and if they are conveniently arranged one can see, with the aid of a binocular microscope, that the penis is actually inserted into the slit (Text Fig. 1, page 46) in the fourth sternite. The penis of the male is falciform, and is directed to the left when at rest, and is thus in the most suitable position to enter this slit. It may be noted that in a somewhat extensive experience of breeding bed bugs neither Major Patton nor I have ever seen them in the position which Berlese assumed they adopt when copulating, i.e., attached end to end after the manner of plant bugs. The male of *lectularius* adopts a more nearly transverse position than that of *rotundatus*.

The position of the sexes in relation to one another in copulation was noted independently by Hay Murray in 1914, but he did not recognize the fact that the penis enters the slit in the fourth sternite, and moreover declined to believe it when it was pointed out by us.
The Spermatozoa.

The spermatozoa of *Cimex* (Plate VII, Fig. 15) are extremely long. With the lowest power of the microscope by which the delicate filament can be distinguished they extend through several fields, and measurement is difficult and a little uncertain. As measured by fitting together the portions traced from each field with the camera lucida and estimating the length by means of a map measurer, I find the length to be about 0.8 millimetre, or 800 microns, i.e., about the same as the diameter of Berlese’s organ. The head of the spermatozoon is in the form of a rod, much thicker than the rest of the filament, and more rigid, to judge from its relatively small movements in fresh preparations and from its straightness when fixed. The head measures about 30 to 50 microns, stains a deep crimson with Geimsa stain, and is of uniform thickness except at its anterior end, where it tapers to a blunt point. Its thickness is about the same as that of the typhoid bacillus, i.e., 0.5 micron. The remainder of the spermatozoon is an extremely delicate thread, a little thinner, perhaps, than the spirillum of relapsing fever as seen in blood films. The above comparisons refer to osmic-acid-fixed preparations stained with Geimsa, which tends to exaggerate the thickness. Some of the filaments in dry-fixed preparations split into two or sometimes three longitudinal portions in a way which suggests that the structure of the spermatozoon is not that of a single axial strand surrounded by a sheath; occasionally the split extends to the extreme end of the filament.

During movement the whole length of the spermatozoon is thrown into short coils in the manner typical of the spirochaetes. Some of these coils are preserved in successful osmic acid preparations, as shown in the figure on Plate VII, which represents a spermatozoon drawn with the camera lucida, slightly rearranged to fit conveniently on the plate. The movement is so very like that of a spirochaete that one can readily believe that the spermatozoa will have equal powers of penetration.

The great length of the spermatozoa makes one a little cautious in regard to the possibility of their becoming enclosed in a cell of 10 to 15 microns diameter. Berlese does not describe or figure them, and was apparently unaware of their length.

The Migration of the Spermatozoa and the Associated Changes in the Cells of Berlese’s Organ.

When the female is dissected immediately after copulation has been seen to take place, the organ of Berlese is found to be distinctly distended.
and of a dense white colour, and when opened on the slide large numbers of active spermatozoa are found, massed together like a ball of thread. In sections (Plate XI, Fig. 41) they are found as a rounded mass which may occupy as much as two-thirds of the section. The mass is compact, and nothing can be made out but interlacing threads and a few more distinct and thicker points which represent the heads of the spermatozoa cut in various planes. In some preparations the threads appear to be arranged in whorls or bundles, some of which are cut in their length and some across, but this arrangement is never well defined at this stage. Spermatozoa have not been found in or about the organ of Ribaga, although they have been very carefully looked for in specimens dissected and fixed with the greatest possible despatch after the completion of copulation; evidently the spermatozoa pass almost directly from the penis of the male into the tunnels formed by the modified hairs, and are not first deposited in the space between the segments; the passage to the centre of the organ is a rapid one, with little or no straggling.

Around this central mass the cells of the organ are opened out so as to form large spaces, or there may be an area around it which is entirely free from cells. Towards the periphery the cells tend to lie with their long axes parallel with the capsule, and further out the organ is as compact as it is in the virgin. The appearance suggests that the cells have been compressed outwards from the centre, possibly by a fluid injected with the spermatozoa, and coming from the large accessory glands of the male (Plate XII, Fig. 47).

Within a short time this central mass breaks up, and the spermatozoa make their way to the periphery of the organ. Two hours after copulation (Plate XI, Fig. 43) they are found in thick bands running in various directions, thrusting aside the cells and spreading out to a looser arrangement near the capsule. The cells near the capsule have still much the same structure as those in the virgin, but the vacuoles are more defined. Those which are enclosed within and between the bands of spermatozoa, on the other hand, always contain large vacuoles, and some of them consist of little more than a cell-space surrounded by the cell wall, against which the nucleus is compressed. Only a few of the spermatozoa are detached from the bands and come to lie between the cells.

The exact relation of the vacuolation of the cells to the entry of the spermatozoa is not very clear. The formation of vacuoles certainly takes place to some extent independently of the presence of the spermatozoa,
for it is found in virgin bugs a week or more after the final moult. (Plate VI, Fig. 7.) Females will usually copulate readily two or three days after the last moult, and on the whole the formation of vacuoles within the cell and their distension with secretion seems to be a preparation for the reception of the sperms, rather than the result of their presence. It does not occur in the newly moulted virgin, but is always present to a greater or less degree in preparations which contain recently injected spermatozoa.

At the end of four hours the majority of the spermatozoa have reached the capsule of the organ, and lie in thick clumps against it, while only a few small bands and some isolated spermatozoa remain in the central parts. The condition of the cells varies in different preparations, according to the extent to which the vacuoles have coalesced with one another. In most preparations representing this stage the majority of the cells are reduced to single large vacuoles, with a little cytoplasm, containing the nucleus, compressed at the periphery; a few cells containing only small vacuoles are intermingled here and there with the distended ones. A few spermatozoa have already penetrated the capsule.

After about eight hours the spermatozoa begin to emerge from the organ into the haematocele in large numbers. Exactly how they get through I have not been able to find out. Inside the organ there are dense strands which have thrust aside the cells (Plate XI, Fig. 44), while outside there is a small tuft lying between the true capsule of the organ and the investing layer of fat body, and between the two details cannot be made out. The spermatozoa do not emerge singly, but always in clumps at particular points, corresponding to the bundles into which they are collected within the organ.

Practically the whole of the cells have now become fully vacuolated, and the interstices between them are filled up with spermatozoa. (Plate VI, Fig. 8, and Plate XI, Fig. 44.) At this stage a change in the cells, noticed in a small proportion at earlier stages, becomes conspicuous, more so in some preparations than in others. A granular mass appears within the large vacuole, almost filling it up, and this takes on the eosin stain very intensely. In some of the cells containing this eosinophil mass a further change takes place; the mass contracts, becomes rounded, and so alters in its staining reaction that the central part becomes basophil, and stains intensely with haematoxylin. The result is a spherical body with a deep purple or black centre and a bright pink margin. Such structures
are well illustrated in Berlese's drawings, the pink staining body being his male nucleus, supposed by him to be derived from the spermatozoon, the dark body the female nucleus. The true nucleus of the cell, however, is always present, though it is compressed and not always seen in thin sections. At this stage the acidophil body may be fairly common, while the basophil one is rare.

In twelve hours (Plate VI, Fig. 9) after copulation there are still spermatozoa present in the organ, though they are now not nearly so numerous. The majority of the cells contain the eosinophil body, and in some of them it has contracted and changed its staining reaction. The vacuoles are now not so large, and around many of them there is a thick rim of cytoplasm.

Twenty hours (Plate VI, Fig. 10, and Plate XI, Fig. 45) after copulation the organ presents a very striking and complex picture in sections. Many of the cells show the eosinophil body only, an equal number the violet staining of the central part, to a greater or less degree; the mass is not always spherical or regular, but may be split up into several parts, or may be elongate, and it may fill up the whole of the cavity of the cell or only a part of it. In fact, there is every grade from a swollen cell filled up with a very finely granular and very vividly stained pink mass to a cell containing a few rounded masses stained violet or black, with a faint pink rim at the margin. In addition there are cells which show no trace of either body, but have a regularly vacuolated cytoplasm, which may be produced into the pseudopodia-like processes by which the cells are attached to one another; other cells contain nothing but one large vacuole with fragments of cytoplasm around it. In the more regular cells, which have well defined vacuoles, there are indications of a commencing cell division. Some of the nuclei show two nucleoli, or the single nucleolus may be dumb-bell shaped; in others the nucleus is constricted in the middle, or two or more nuclei may be present in the same cell. Cells containing as many as six nuclei are not uncommon. According to Berlese, division is in some cases by mitosis, but I have never seen anything but amitotic divisions, initiated by division of the nucleolus.

From this stage there is a rapid return to the resting condition, as seen in the organ in the virgin bug. The large vacuoles disappear within the first two days, and the cells resume their compact appearance. The eosinophil masses disappear early, but the basophil masses are present for several days after the vacuoles have resolved, though they are
gradually reduced in size. Within three days (Plate VI, Fig. 11) after the act of copulation the cells have a cytoplasm with well-defined small vacuoles, a round or oval nucleus in which the chromatin is regularly arranged, and, except for the presence of the basophil substance in some of the cells, the organ resembles that of a virgin a week or so old. The basophil substance is collected together as a compact, round, and very densely stained mass, lying in a regular vacuole only a little larger than itself.

How far the cells can recover after having been once so greatly distended, and to what extent they degenerate and are replaced by new cells resulting from the cell division which has been noted, it is impossible to say. Certainly the number of nuclei present in the stage of regeneration, in the 2-nucleated and the multi-nucleated cells, is very great, but on the other hand a very considerable proportion of the cells contain small basophil masses at the end of two and three days, and do not then show any signs of degeneration. The organ is again ready to function a second time within forty-eight hours of the first copulation, as has been shown by several experiments.

I have examined this organ in a long series of preparations representing periods up to three weeks after one act of copulation, and up to six weeks after several, and have found no changes other than those described. Once the cells have resumed their resting condition, and the last traces of the basophil body have gone, no further change takes place until spermatozoa are again received into the organ.

By far the greatest part of the spermatozoa leave the organ of Berlese within the first day. A few stragglers are found in some preparations several days, even to a week, or more, after copulation, so that under natural conditions the organ is probably never quite free from them, as the bug receives a second supply before the first has been entirely disposed of. As has already been noted, the amount of spermatozoa received at each act of copulation varies considerably in both species. The variation in the number of spermatozoa is accompanied by a varying degree of change in the cells, and these uncontrolled factors have made it impossible, in my experiments, to appreciate any difference there may be in the behaviour of the two species, either as regards the rate at which the spermatozoa pass through the organ under the definite temperature and food conditions adopted in the experiments, or in the degree of the functional activity of the cells. The function is undoubtedly of the same kind in the two species.
In addition to the controlled experiments a considerable number of 'wild' bugs have been examined by the same methods. All the changes in the cells and the conditions as regards the spermatozoa and ovaries which have been found in the controlled series have also been found in bugs taken at random while living their natural life, and with one exception nothing has been found in the wild bugs which had not been found in the experiments. This exception is the presence, in one preparation (Plate VI, Fig. 13) out of the many made from wild bugs, of a body contained within cells which have a uniform cytoplasm. It is present in about ten per cent of these, many of which have two or more nuclei. Its size varies from a quarter to half that of the cell, and it is round or oval in shape, sometimes a perfect sphere, sometimes irregular, but always with indefinite margins. It is embedded in the substance of the cell and not enclosed in a vacuole, and shows a very faint striation, longitudinal when the body itself is elongate, and stains faintly and diffusely with hematoxylin, assuming a purple or violet colour which is in marked contrast to the sharp staining of the cell nucleus. The organ in which these bodies were found shows all the stages of the secretory process—cells containing a single large vacuole, others containing the eosinophil body, etc., and also the small and very densely stained masses lying in small vacuoles which represent the terminal stage, thus showing that copulation had taken place recently and had taken place more than once. The preparation is a series of sections of the whole bug, and shows two mature eggs, each containing an embryo. Spermatozoa were present in the organ of Berlese, in the hæmatocele, and in and around the spermathecae. No explanation of the nature of this body is forthcoming. The preparation is well fixed (Muller's fluid) and stained, and the appearance is not likely to be an artifact. With Berlese's work in mind one naturally considers the possibility of its being an ingested, perhaps partially digested, spermatozoon, but the striation is very faint, and there are in none of the bodies any deeply stained points which might be the heads of spermatozoa cut in section; well stained spermatozoa between neighbouring cells are available for comparison, and one would have to allow his imagination very free play to regard these intracellular bodies as spermatozoa. They have been studied all the more carefully because they are the only cell-inclusions seen in the organ of Berlese which could possibly be regarded as spermatozoa. Nothing like them is represented in any of Berlese's drawings.
The Secretory Function of Berlese’s Organ.

From the foregoing it is evident that the cells of Berlese’s organ constitute a gland of a most unusual type, and have a function which is intimately related to the passage of spermatozoa through the organ. It is possible to trace generally the course of events from the virgin condition to the restitution of the cells in readiness for the reception of a second quantity of spermatozoa. In the newly moulted virgin the cytoplasm is compact and free from granules or vacuoles. During the first few days of adult life a secretion appears in the cells, and is contained in vacuoles which tend to coalesce as they increase in size. The vacuolation is continued after the reception of the first batch of spermatozoa, and results finally in the distention of the cell to such an extent that almost the whole is occupied by one large vacuole, surrounded by a rim of cytoplasm in which the nucleus is compressed. In this condition the secretion is not coagulated, or at least not rendered granular or stainable, by the reagents used in the preparation of the sections, and the vacuole therefore appears as a clear space. By the time the spermatozoa begin to leave the organ a change in the constitution of the secretion occurs, and it now appears in sections as a finely granular and strongly eosinophil mass, which, on account of the inevitable shrinkage during coagulation, occupies less than the whole cavity. Later a further change occurs, by which commencing at the centre, the mass assumes a basophil character, and at the same time begins to contract, and often to split up into smaller portions, while at the same time the cytoplasm of the cell begins to resume its position around the vacuole as this becomes smaller. The basophil substance gradually disappears, doubtless by absorption into the hæmatocoele as represented by the spaces between the cells of the organ, until eventually nothing of it is left but a small black mass in a vacuole little larger than itself. While this is going on, nuclear division takes place, and many multi-nucleated cells are present in the organ. How far the cells which have functioned once can serve again it is not possible to say.

These cells, therefore, produce an internal secretion which is thrown into the circulation at the time when the spermatozoa are passing through the organ and are on their way to the ovariole. It may be inferred that the function of this secretion is in some way connected with reproduction.

The Homology of the Organ of Berlese.

While one is ignorant of the function and of the development of the organ of Berlese, it is hazardous to speculate on its homology. One
turns naturally for guidance to the general plan on which the reproductive system of insects is built up, and to the instances which are known of the persistence of primitive conditions. Having these in mind the organ of Ribaga, easily recognised as an ectodermal invagination, might perhaps be regarded as the survivor of a series of bilateral and segmental genital openings, having retained the function of the reception of the spermatozoa, but having lost all connection with that of oviposition. The organ of Berlese is possibly a mesodermal gland, which has lost, or never had, the secondary connection which is normally formed between such glands and the oviduct. The occasional persistence of the organ on the left side as well as on the right is strongly in favour of an original bilateral arrangement, and the search for similar structures in other species of Cimex and its near allies promises to be of great interest. The rest of the reproductive system has developed on more normal lines, but there are several points which suggest that Cimex is not far removed from a primitive type. The testes, for instance, are definitely lobed, the seven lobes being equal in size, and corresponding in number in a general way with the abdominal segments. The ovaries contain the same number of ovarioles, and also show a remarkable persistence of a tissue which is embryonic in type.

The existence of two apertures, one for the reception of the spermatozoa and one for oviposition, is of course not peculiar to Cimex, but is found in the Lepidoptera, though here the copulatory opening is said to be connected with the oviduct by means of a duct along which the spermatozoa pass.

**The Spermatozoa in the Haematocele.**

The spermatozoa first appear in the haematocele about four hours after copulation, as a small cluster lying below Berlese’s organ. During the next few hours the number increases rapidly until the end of the first day, by which time most of those received into the organ have passed through its capsule. When lying free in the haematocele they are arranged in little whorls or bundles, which correspond to those present in Berlese’s organ at an earlier stage. These clusters are easily recognised in dissections, and are large enough to be seen with the naked eye. When seen by reflected light they have a dense white colour which at once distinguishes them from fat body. By transmitted light one can distinguish a denser central part, which has a yellowish tinge. The marginal fringe is composed of very actively motile filaments, which keep up a
constant and regular spiral wriggling movement. The denser central part may be formed by the collection together of the heads of the spermatozoa. Such free clusters are always present in the hæmatocele eight hours or more after copulation, and are practically constant, during the breeding season, in wild bugs which have lived in free association with the other sex.

These clusters pass backwards towards the posterior end of the abdomen, and take up a position around the junction of the lateral oviducts and the spermathecae (Plate IX, Figs. 34 and 35, and Plate XII, Fig. 50) entirely obscuring the structures in this part when they are present in their maximum amount. The most careful examination of many series of sections has failed to reveal any kind of surrounding capsule, or any duct connecting the spermathecae with the organ of Berlese. The masses become adherent to the structures they surround, and by careful manipulation the entire reproductive system can be dissected out with the spermatozoa still adherent to the organs. They are of course easily detached, and allowance must be made for this in the interpretation of the figures on Plates IX and X, which show the accumulation of the spermatozoa around the oviducts and spermathecae at different stages in their progress.

Having become collected around the spermathecae the spermatozoa proceed to penetrate the wall just as they did that of the organ of Berlese, and it is equally impossible to find out exactly how they do it. Sections show masses within and without the single layer of cells which make up the wall, and there is no pore or definite aperture to be found. The accumulation of spermatozoa reaches its maximum in about twenty-four hours, and from this stage there is a gradual diminution in the size of the free mass and a corresponding increase in the size of the spermathecae, which become more and more distinctly outlined as large rounded bodies as the chamber is distended by the entry of the sperms. (Plate X, Figs. 37 and 39.) Two days after copulation the spermathecae are very large, and only a few small masses remain free outside them. As a second copulation may take place about the second or third day, one finds, in wild bugs, spermatozoa in the organ of Berlese, free in the hæmatocele, accumulated around and attached to the spermathecae, and within the latter. The amount present in some preparations is astonishing. The posterior end of the abdomen is sometimes literally packed with them, partly inside the distended spermathecae and partly free.

Whether, as Berlese affirms, the spermatozoa are used up as nourishment for the female during the oviposition period, or not, it is certain that the actual bulk of spermatozoa received is very large. The densely
Observations on the Reproductive System of *Cimex*.

packed mass found in the organ of Berlese after a single act of copulation may measure half a millimetre in diameter, and before the whole of this has reached the interior of the spermathecae a second copulation may take place. The total bulk is equivalent to a considerable fraction of that of the eggs laid—one might estimate it roughly at a third. Had Berlese carried out any exact experiments on this point he would assuredly have found strong support for his theory of hypergamesis. One must note, however, that the spermatozoa are very large, and that the actual number of individuals is not so great as the size of the mass might suggest.

**The Spermatozoa in the Female Reproductive Organs.**

*The Structure of the Parts.*

In addition to the presence of Berlese's organ the reproductive system of *Cimex* shows many interesting features which have not been noted in other insects. How far these are really peculiar to the bed bug it is impossible to say at present, for our knowledge of the histology of the female genital tract in insects is very limited. The tract in *Cimex* consists of paired ovaries, lateral oviducts, a short common oviduct or vagina, and paired spermathecae. It will focus attention on the essential points if I anticipate a subsequent section and state that the spermatozoa, when they leave the spermathecae, do not pass into the lumen of the oviduct as one would naturally expect them to do, but make their way upwards through the substance of its wall, and come to lie in a mass of nucleated protoplasm immediately below the first egg; and that I have found nothing to confirm Berlese's statements regarding the digestion of the spermatozoa by the epithelial cells of the oviduct.

*The Ovaries.*

Each ovary is composed of seven ovarioles, which are of the meristic type, that is to say, each growing ovum has its own group of vitellogenous or 'nurse' cells. The young ovariole in the newly moulted female (Text Fig. 2, page 63) is a small spindle-shaped body, tapering above into an apical filament, and attached below to the lateral oviduct by a short and stout pedicle. Its upper half consists of large germ cells, from which are developed the ova and the nurse cells, its lower half of epithelium, a delicate layer of which is prolonged over the germinarium and on to the apical filament. Only one egg proceeds to full development in each ovariole at one time, the second remaining small and round; when the first egg is ripe a minute clear space, which may be the third egg, can sometimes be distinguished above the second. The development of the
ova proceeds to a certain point independently of fertilization. The first egg takes to itself a group of nurse cells, becomes surrounded by the differentiated portion of the epithelium which is destined to secrete the chorion, and begins to assume the elongate form of the ripe egg. A constriction develops between the egg with its nurse cells and the terminal portion of the tubes, which consists of the second egg and the

**Text Fig. 2.**

**Ovarioles at different stages of growth.**

I. From a newly moulted and virgin female. *g.*, the germarium, from which the ova and nurse cells arise. *ep.*, a solid mass of epithelial cells, forming the pedicle. II. The smallest ovariole from a female two days old, twelve hours after copulation has occurred. *e.1.*, the first egg. *p.*, the upper division of the pedicle, containing protoplasm with free nuclei. *p'.*, the lower part of the pedicle, a continuation of the oviduct. III. The largest ovariole from the same ovary. *e.2.*, the second egg. *m.ep.*, vitellogenous or "nurse" cells. *m.ep.*, marginal epithelium. At this stage the formation of the chorion has not begun. IV. The terminal portion of an ovariole, in which the lowest egg is almost ripe. *e.3.*, possibly the third egg at a very early stage. *ch.*, the chorion of the lowest egg. Spermatozoa are found in the space between the operculum of the lowest egg and the second egg, where there are indications of the formation of the protoplasmic mass.
germarium, not yet separated from one another. In virgin bugs a week or more old all the ovarioles show about the same stage of development, each having an elongate egg about half grown and a smaller round one above it. (Plate IX, Fig. 27.) Once fertilization has taken place development proceeds in rotation in the several tubes of each ovary, so that more than one completely ripe egg is seldom found on each side; this is of course in correspondence with the egg-laying habit of the female, the average number of eggs being about two per day. There are no signs of the formation of the chorion by the choriogenic portion of the epithelium until the stage is reached at which spermatozoa are present in the protoplasmic mass, already referred to, below the first egg; from this we may safely infer, as will appear more clearly later, that the chorion is not produced till after the fertilization of the egg which it surrounds.

The ovary is surrounded by a loose investment of connective tissue, the so-called peritoneum, which fits over the ovarioles like the fingers of a glove, and is attached to the lower end of the lateral oviduct. At the upper end this sheath is continued over the apical filament.

Occasionally one of the ovarioles fails to develop with the other, and remains as a small round mass of tissue attached to the median oviduct by a short stalk. (Plate IX, Fig. 33.) The separate ovarioles can be distinguished, and the germarium is distinct from the mass of epithelial cells, but no ova can be seen. Such immature ovaries have been found in females several days old, even when the other ovary is fully grown and contains mature eggs.

The cells of the marginal epithelium, at first cubical, soon become columnar and regularly arranged. (Plate VIII, Fig. 25.) Their nuclei are very uniform and characteristic, and have a single large central block of chromatin and a peripheral layer of fine granules, with few or no particles of intermediate size. Nothing suggestive of cell-division has been seen in these cells. The chorion is first seen, in haematoxylin preparations, as a black lining on the inner surface of the cells. In sections of bugs containing mature eggs (Plate XII, Fig. 49) it is very conspicuous on account of the intensity with which it retains the haematoxylin stain. The action of the reagents used in the preparation of the sections usually causes it to split longitudinally, and the separated fragments curl inwards away from the epithelium, as shown in the photograph on Plate XII. The chorion is formed in two layers, an endo- and an exo-chorion, of which the former is the thicker. The layers (Plate VIII, Fig. 26) are united to one another by numerous short stout columns which run in a
radial direction, and project on the outer surface of the egg. One is at first inclined to regard these columns as a micropilar apparatus, but there is no indication of a central channel, and moreover they are formed at a time when the egg is already fertilized and the embryo within it developing. They are probably nothing more than processes which serve to anchor the egg in position when it is laid, and correspond to the sculptural markings so commonly met with on the eggs of insects.

As the egg attains its full growth the marginal epithelium is stretched in the tangential plane, the columnar cells becoming flattened. (Plate VIII, Fig. 26.) The nuclei lose their characteristic structure and show several chromatin particles of various sizes. When the chorion is fully formed its thickness is almost equal to that of the flattened cells.

The mature egg, when laid, has a long ovoid shape, its axis being slightly bent at the cephalic or anterior end. Its surface, in addition to the processes mentioned above, is marked out into polygonal areas which are the impress of the ends of the choriogenic cells. The anterior end is flattened and fitted with an operculum or lid, which is thrust off by the young larva as it escapes. The operculum is fully formed within the ovariole, and previous to the deposition of the egg there is above it a narrow area in which the epithelium is represented by undifferentiated cells. Active spermatozoa can be seen in this area in the fresh condition.

Contrary to what is believed to be the case in insects as a general rule, the embryo of Cimex undergoes the early stages of its development while the egg is within the ovariole. Stages such as that shown in the photograph on Plate XII, Fig. 49, are commonly met with in sections of wild bugs. The development of the embryo commences before the formation of the chorion is complete.

It has been stated that the ovariole is attached to the lateral oviduct by a short and stout pedicle. This pedicle is unlike anything I have seen, or seen described, in other insects. In the very early stages it appears to consist of a simple mass of epithelium (see Text Fig. 2, page 63), but by the time the first egg is commencing to grow the pedicle is clearly differentiated into two parts. The upper half, or rather more than the half, is constricted above and below so as to leave a spherical portion, while the distal part is a simple column of a uniform diameter, running into the lateral oviduct.

This upper portion of the pedicle consists of the mass of nucleated protoplasm already referred to. It is bounded by a thin membrane in which there are a few scattered nuclei; above, this membrane is
continuous with the basement membrane of the choriogenic epithelium, while below it dips inwards and separates the upper portion of the pedicle from the lower, thus shutting off the ovariole from the oviduct. The protoplasmic mass may therefore safely be assumed to be undifferentiated epithelium, similar in origin to the marginal epithelium of the follicle and to that which invests the germarium and is continued over the apical filament. The nuclei of this mass are concentrated in the centre of the spherical portion leaving the peripheral part free. According to Berlese, who, it may be noted, offered no explanation of the function or ultimate disposal of this remarkable tissue, the nuclei are arranged in layers, as indicated in the summary of his second paper. I have not been able to confirm his description of this part of the ovariole.

The spherical mass of nuclei, surrounded by a clear space, is easily seen in suitably stained dissections, and can be recognised in the majority
of the photographs on Plates IX and X. The details are best studied in serial transverse sections. (Plate VIII, Figs. 23 and 24.) Below the level of the nuclei, and between them and the distal part of the pedicle, there is an area in which the section shows nothing but a finely granular cytoplasm with a few small nuclei belonging to the basement membrane at the sides. Sections through the lower part of the mass show densely packed nuclei in which the chromatin is most irregularly arranged in blocks of various sizes, and does not stain well; a nuclear membrane is present, and takes on the stain diffusely. The nuclei are for the most part oval in shape, and have a curiously ‘shrivelled’ appearance which calls to mind that of a tissue which has been over-heated in the paraffin bath. A little higher up the nuclei are less closely packed, and show a different structure and a marked difference in their staining reactions. The change in the staining is very striking when one follows the same ovariole in a series of sections, for at the lower part the nuclei take on a light purple tint, much the colour one gets with Delafield’s stain, while at the middle and above, only a few sections away they take on the intense and sharply defined black which one expects with a well-matured hematoxylin. The structure of the nuclei in this part of the mass is irregular in the extreme. A few are round or oval, of about ten microns in diameter, and have the chromatin arranged in round granules of various sizes, usually distinctly large; the nuclear membrane is very faintly defined, and no nuclear reticulum can be distinguished, the nucleus consisting of sharply defined granules in a clear space. Other nuclei are much smaller, and contain only a few grains of chromatin, while some are very large, and contain many fine granules in addition to large discrete particles. In none of the cells can a definite nucleolus be distinguished. Obviously profound nuclear changes are going on in this region, but I have not been able to define what they are. In a few individuals stages which are presumably part of a process of division have been seen, such for instance as that represented in Fig. 23 on Plate VIII, where two pear-shaped nuclei are seen to be connected by a long rod. I have not found simple amitotic division, nor have I seen stages of mitosis on defined lines, and will make no attempt to reconstruct a cycle of nuclear change from the complex picture which this tissue presents.

At the upper end of the mass the nuclei show the same variety of structure, but here there are indications that each nucleus is taking to itself a portion of the protoplasm. A cell wall is formed, and may include
one or two nuclei, which are generally large, sometimes very large. The ultimate disposal of these nuclei now becomes evident. The wall of the tube, which below this point is formed only of the delicate basement membrane, already described as being continuous with that of the choriogenic epithelium, here begins to show much larger nuclei, and in successive sections the transition between the nuclei of the central mass and those of the wall can be followed. The peripheral cells become more regularly arranged on the basement membrane at the higher levels, until they assume the same position and character as the cells of the marginal epithelium of the follicle. The nucleated mass of protoplasm in the pedicle, therefore, serves as a reservoir from which the choriogenic epithelium is added to as the egg grows and elongates. The epithelium is in fact prepared for the egg as it grows downwards into the pedicle of the ovariole. It will be recalled that cell-division is not found in the marginal epithelium once it has become regularly arranged and of a columnar type; the additional cells required to cover the increasing surface of the egg are provided by the tissue below the egg.

The above description applies to the pedicle as seen in an ovariole in which the egg has not yet attained its full size. As the egg grows to full size the nuclei and protoplasm in the pedicle are gradually used up, and by the time the chorion, with its transverse columns, is fully formed, and the embryo has begun to develop, the distal (caudal) end of the egg reaches as far as the lower division of the pedicle. The egg reaches the oviduct, one may presume, by the rupture of the basement membrane which surrounded the original undifferentiated mass of protoplasm.

This protoplasmic mass must of course be used up before the egg is laid, for it blocks the exit. It is, however, constantly present below the growing eggs in ovipositing bugs, and must therefore be re-formed after the first egg is laid. The details of this re-formation, and the manner in which the stretched out and flattened epithelium left after the completion and deposition of the first egg is reconstituted, have not been fully worked out. As the eggs in the several ovarioles come to maturity in rotation it is extremely difficult, in studying sections of bugs, to select an ovariole which has certainly contained a ripe egg a short time before fixation. Such ovarioles can be easily recognised in dissections by their length, which is approximately that of the egg; they contain large numbers of large nuclei, probably produced by a rapid multiplication of the epithelium. A small area of undifferentiated protoplasm is always left immediately above the operculum, and it is here that the
re-formation of the protoplasmic mass begins, about the time the first egg is nearly ready to leave the ovariole.

The distal part of the pedicle, below the basement membrane of the epithelial cells, is a simple tube, the thick cellular wall of which surrounds a lumen which is continuous with that of the oviduct. This part of the pedicle is clearly an outgrowth of the oviduct, and resembles it in structure and in the behaviour of the spermatozoa within it.

The Lateral Oviducts.

The lateral oviduct is a tube of uniform diameter and rather thick walls, formed by the convergence about one point of the pedicles of the ovarioles, and runs obliquely backwards and ventrally at the side of the mid-intestine, against which it is compressed, to unite with its fellow of the other side a short distance in front of the genital opening. The wall of the duct is composed of epithelium in which the distinction between individual cells is wanting, or at any rate is indistinguishable. The cytoplasm is uniform and presents no special characters. The nuclei (Text Fig. 4) are large, averaging about ten microns in diameter, and are round or oval; when oval the long diameter is in the tangential plane, not the radial. The nuclear membrane is very delicate, and no nuclear network is seen. The chromatin is characteristically arranged in a few large and sharply defined masses, which may be peripheral or central, and there are few or no smaller particles. In thin sections the several fragments into which the nucleus is cut appear as isolated granules scattered throughout the tissue in an apparently irregular manner.

This epithelium is thrown into folds (Plate VII, Fig. 19), the number of which corresponds with that of the ovarioles. The folds are deep, and entirely fill up the lumen as seen in transverse sections, but there is no difference in structure between the apex and the base of a fold; the object of the folding of the epithelium is to allow of expansion during the passage of the egg, rather than to provide an additional secreting surface.
According to Berlese the epithelium has a structure and a secretory function comparable with that of the epithelium of the mesenteron in insects; its cells ingest and digest the spermatozoa, and also give off into the lumen of the oviduct globules of secretion resembling those given off by the cells of the mid-intestine. I think he was altogether in error in this, for I have never seen anything at all comparable with the masses of globules which have such a characteristic and universal form in secreting mid-gut cells, nor have I seen the deep staining of the bases and sides of the cells which, according to Berlese, indicates the passage of the assimilable parts of the spermatozoa into the haematocele.

The internal border of the epithelium is very ill-defined, and takes on a delicate pink colour with eosin stain. In it there are numerous clear spaces which might be mistaken for vacuoles, but which, I think, are nothing more than the spaces left after the shrinkage of a coagulable fluid during fixation. Their arrangement is not at all like that of the vacuoles of a secreting cell. (Plate VII, Fig. 21.) The ill-defined eosinophil layer bordering the epithelium I take to be a glutinous secretion, the purpose of which is to provide the egg, as it passes down the tube, with a coating of cement, by means of which it is fixed to the substance on which it is laid. It will be noted that the female bed bug has no accessory glands specially designed for this function. The effectiveness of the fixation secured by this cement and the short spines which cover the surface of the egg is realized when one is dealing with eggs laid on filter paper in experimental work. The paper can be freely handled without much risk of the eggs becoming detached, although they are only in contact with the fibres of the paper at one point, and the layer of cement is so thin as to be imperceptible.

The Spermathecae.

The spermathecae of Cimex differ so much from the usual types that one has some hesitation in retaining the name. In the young and virgin female they are ill-defined, and are nothing more than slight lateral expansions of the common oviduct at the junction of the lateral oviducts. (Plate IX, Fig. 27.) As the bug grows older, whether it is fertilized or not, the spermathecae enlarge until they form definite pouches of some size. In ovipositing bugs the size of the spermathecae depends on the amount of spermatozoa they contain, for their walls are non-chitinized and have no rigidity. They may be very small, or reach a size almost equal to that of Berlese's organ, and either the one or the other may be
the larger. I have not found that, as Berlese states, the right spermatheca becomes more distended than the left. It may be noted that, as Berlese points out, paired spermathecae are rare in all orders of insects, The spermathecae are closely attached to the wall of the oviduct without the intervention of a neck or pedicle.

Their structure (Plate VII, Fig. 16) and mode of development are altogether exceptional. In the virgin bug the spermathecae are practically solid bodies, composed of cubical or rounded cells of ten or twelve microns in diameter, with a homogenous cytoplasm and a nucleus which has no special characters. Here and there, and especially at the periphery, there are spaces between the cells, and the wall may be reduced to a single layer. At a later stage the cavity expands at the expense of the thickness of the wall, but all the cells of the original mass are not taken up; clumps of them remain as apparent ingrowths from the wall, connected with it only by a narrow pedicle, and some of the cells remain free within the lumen of the chamber which is eventually formed. After copulation has occurred and the spermatozoa have penetrated into and distended the spermatheca, the wall is stretched out and becomes very thin, but some free cells remain intermingled with the spermatozoa. According to Berlese the cells of the spermatheca take up spermatozoa, but I have never found this to be the case. Berlese regarded the cells in the interior of the spermatheca as ingrowths from its wall, whereas they are really unexpanded portions of it.

One would ordinarily assume that the lumen of the spermatheca is communicated with the lumen of the oviduct by a duct or channel. There may be a minute constant opening, but after a most careful search through a considerable number of series of sections of this part, both in the transverse and longitudinal planes, I have failed to find it. There is certainly no channel lined by regularly arranged cells, though there are of course in the earlier stages many crevices between rows of cells, in the part next the oviduct as in other parts. Since the spermatozoa do not pass to the lumen of the oviduct a channel is really unnecessary.

The cells of the spermathecae have no distinguishable chitinous intima. This, taken with other points, leads one to doubt whether these organs in Cimex are similar in origin to those in other insects. Ordinarily the spermathecae of insects are developed as ectodermal invaginations, either primarily connected with the common oviduct or acquiring a secondary connection with it, and have a well-defined chitinous intima which gives them rigidity. Pending a study of the
development of the parts in the nymph it would seem more proper to regard those of *Cimex* as outgrowths of the terminal end of the lateral oviducts, and as of mesodermal origin.

*The Common Oviduct.*

The common oviduct is a very short channel leading directly from the junction of the lateral ducts to the genital opening. It is a typical ectodermal structure (Plate VII, Fig. 18), lined by small cells with a thick chitinous intima, and surrounded externally by muscle fibres. The epithelium is puckered and crumpled into innumerable small folds, but not thrown into villi. The intima follows the intricate folds of the cells, and is thrown into coils which might easily be mistaken for spermatozoa.

The spermathecae are situated at the point at which the flattened epithelium of the common oviduct gives place to the higher and larger cells of the lateral ducts. The upper end of the common oviduct is divided into two by a short median partition.

*The Passage of the Spermatozoa to the Ovariole.*

A detailed description of the histology of the tract between the spermathecae and the ovum having been given, the further course of the spermatozoa presents little difficulty in explanation. From the spermatheca to the ovum the spermatozoa are intercellular or intracellular throughout, and there is reason to suspect, though there is no definite proof of it, that something of the nature of 'hypergamesis' occurs, though the spermatozoa are not absorbed either at the place or in the manner which Berlese indicated.

From the spermathecae, which, as already noted, have no duct leading to the oviduct, the spermatozoa pass into the substance of the wall of the lateral oviduct, and are here arranged in bundles of parallel fibres, an arrangement which is preserved until they reach the ovariole.

Their course is best studied in longitudinal sections of the duct. In such sections (Plate VIII, Fig. 22) representing a stage twenty-four hours or more after the last act of copulation, every fold of the epithelium of the oviduct shows portions of a bundle of spermatozoa, running in the long axis of the duct. On account of the irregular folding of the epithelium one cannot of course see more than a small part of the bundle in any one section. The bundles follow all the folds of the epithelium; when a
nucleus is encountered the bundle splits up, part going on one side and part to the other, to reunite beyond it. Transverse sections of compact bundles simulate nuclei very closely. They have about the same size and outline, and the individual filaments which are cut exactly across resemble minute chromatin particles, while those which happen to be convoluted at the point of section are cut twice, and simulate delicate rods of chromatin. The spirochaete-like coils which the spermatozoa assume during their upward progress are often very clearly shown in sections.

Here and there in a series of sections one finds a nucleus apparently enclosed in a loose and wide mesh of filaments, as if a spermatozoan had been arrested in its course and had coiled itself around the nucleus. The nuclear membrane, always indistinct in the oviduct, is obscured by the surrounding filaments, and the spermatozoan has every appearance of being in intimate relation with the nuclear chromatin, the large particles of which appear to be entangled in the network. Whether this is significant of an interaction between the spermatozoon and the somatic nucleus, or not, it is difficult to say. I have never found the head of the spermatozoon in such a network, though it is frequently seen, and easily distinguished by its greater thickness, in sections. The extreme length and thinness of the spermatozoon make it impossible to determine whether such a network is formed by one spermatozoon or several. Berlese described and figured spermatozoa in the oviduct cells, one neatly coiled up in each cell and quite distinct from the nucleus, but I have seen nothing which would conform to his description, or which resembles his figures.

The spermatozoa thus pass, unimpeded by intercellular membranes, through the wall of the oviduct up to the ovariole. From the lower part of the pedicle, which is in reality a part of the oviduct, they pass into the ovariole, and by the end of twenty-four hours after the first act of copulation they are to be found in considerable numbers in the mass of nucleated protoplasm which occupies the upper part of the pedicle. They are most numerous in the upper part of the mass, where the nuclei are scanty, and are here readily seen in fresh preparations, for they are actively motile. At this stage they lose their regular arrangement in bundles, and are found scattered about the protoplasm in various directions. The head of the spermatozoon is retained, and is very conspicuous in sections of the area which is free from nuclei, on account of the intensity with which it stains.
Here the course of the spermatozoa ends for the time. They do not penetrate into the marginal epithelium surrounding the young egg, at least not in bundles or in an amount sufficient to be recognizable. But when the egg is fully grown and the chorion has developed its spines, and when the choriogenic epithelium is stretched and flattened, they resume their progress, and pass upwards through the thin marginal epithelium towards the second egg. In transverse sections (Plate VIII, Figs. 26 and 26a) through ripe or nearly ripe eggs, in which the embryo is already developing, and from which the marginal epithelium is beginning to detach itself, one constantly finds bundles of spermatozoa at all points of the circumference of the egg, some cut exactly in transverse section and closely simulating nuclei, but easily distinguished from the nuclei of the epithelium by the large chromatin blocks of the latter, others cut into short portions in longitudinal section. I have not succeeded in tracing them beyond this point in sections. In fresh preparations they can be seen, and recognised by their motility, in the narrow area above the operculum of the egg, where the protoplasmic mass which will eventually lie below the second egg is already forming.

I have never found spermatozoa in the germarium, nor have I been fortunate enough to obtain a preparation showing the entry of the spermatozoon into the egg. It is clear, however, that fertilization takes place previous to the formation of the chorion, and that the spermatozoa which pass upwards through the choriogenic epithelium, after the latter has produced the chorion for the first egg, are not destined to fertilize this egg, for its embryo is already at an advanced stage, but are on their way towards the second and succeeding eggs. I have never found spermatozoa within the eggs at any stage. It is most likely that the egg is fertilized while it is very small, before the marginal epithelium has become regularly arranged and has taken on its characteristic columnar shape.

Does ‘hypergamosis’ occur?

Under the term hypergamosis Berlese included two distinct ideas, viz., the stimulus to the development of the reproductive organs which he believed to be provided by the spermatozoa, and the utilization of the excess of the spermatozoa as nourishment for the female during the oviposition period. The first of these he did not elaborate, and he gives little beyond the statement that in the absence of the spermatozoa the female reproductive organs do not develop; he records no breeding
experiments, and did not enquire whether the female bug will lay eggs in the absence of the male. His main thesis, the intracellular digestion of the spermatozoa in the special organ which bears his name and in the epithelium of the oviduct, has been shown to be founded on error. But the problem still remains, as to how the excess of spermatozoa, constituting a very considerable mass of protoplasm in relation to the size of the insect, is disposed of. The factor of bulk brings the problem into sharp relief. It is not a case of an attempt to trace a minute spermatozoon in the substance of a large organ, which might perhaps be compared to a hunt for a needle in a haystack, but of the necessity of accounting for the disposal of a large mass, easily visible to the naked eye, which enters the reproductive tract of the female and does not leave it. By following the course of the spermatozoa from the moment they enter the body of the female we find that there are no indications whatever that they stray from their direct path, or that they undergo any degeneration while on their journey. Exact estimations are of course impossible, but taking the facts as they stand it is fair to assume that the great majority of the spermatozoa received in successive acts of copulation find their way to the mass of undifferentiated protoplasm contained in the upper part of the pedicle of the ovary; they are there intermingled with nuclei which are in an unstable condition, and, as this mass is taken up in the formation of the choriogenic epithelium and finally disappears, the spermatozoa then present pass upwards above the ripe egg to the corresponding mass of tissue below the second egg.

The final phase, whether it be some sort of digestion and absorption, or a union between the spermatozoa and the nuclei among which they lie, eludes one. But the ovariole is not a bottomless pit, and we are compelled to assume that the spermatozoa are in some manner used up within it, during the period when the follicular cells are producing the chorion and the nurse cells supplying the ovum with its yolk, when, in fact, the metabolism of the ovary as a whole is very active. The number of spermatozoa received at a single act of copulation is infinitely greater than that required to fertilize all the eggs which could be produced by the most long-lived bug; during the period of sexual activity copulation is frequently repeated, and there is a constant stream of spermatozoa on their way to the ovariole; is it reasonable to suppose that so large an amount of protoplasm is wasted, that this is in reality an instance to use Starling's words again, of the prodigality of nature? I think, not.
We know little indeed of the biology of the bed bug, and as yet practically nothing of the relations of the sexes with reference to oviposition. Experiments in this direction are now in progress, and there is reason to hope that they will throw some light on the interesting problem of 'hypergamsis.'

**Summary.**

1. Two problems in connection with fertilization in insects are pointed out, *i.e.*, the difficulty of accounting for the disposal of the enormous excess of spermatozoa over the amount required for the fertilization of the eggs, and the question as to where and at what stage the eggs are fertilized. The bearing of the first on the wider problem of the function and behaviour of spermatozoa in general is pointed out, with reference to the work of Kohlbrugge, who has shown that in the higher animals the spermatozoa penetrate into the body cells, and probably exercise an influence upon them; the current assumption with regard to the second, that the spermatozoa remain in the spermathecae and issue thence to fertilize the eggs as they pass down the oviduct, is shown to rest on argument rather than on evidence, and further, it fails in many instances to explain the known facts.

2. An account of Berlese's observations and the theory of 'hypergamsis' which he founded on them is given, and reference made to other work on the subject.

3. The technique adopted is described. Bred insects were used throughout in order to obtain females in the virgin condition; they were kept under controlled conditions, and examined by section and dissection in series representing all stages from the final moult to oviposition and after. 'Wild' bugs were examined as controls.

4. The account of the mode of copulation in *Cimex* given by Patton and Cragg is confirmed.

5. The spermatozoa are described, their great length and motility emphasized.

6. The organ of Berlese in the female bug is described, with special reference to its structure in the virgin female.

7. The organ of Ribaga is described, and is shown to be an ectodermal structure composed of very highly modified chitinogenous cells, each of which is produced to a long process hollow at its free end. The spermatozoa are probably conducted into the substance of Berlese's organ during the act of copulation by means
of these tunnelled hairs. There is no other channel leading into the organ.

8. The passage of the spermatozoa through the organ of Berlese, into which they are received at the act of copulation, and the changes in the cells of that structure during the passage of the spermatozoa, are described. The organ is a gland of an unusual type, and gives off its secretion into the hæmatocele when the spermatozoa are passing through it on their way to the spermathecae.

9. The passage of the spermatozoa in large masses through the hæmatocele, and through the wall of the spermathecae, is described.

10. The anatomy and histology of the female reproductive tract is described. The ovarioles are of the meroistic type, and below the largest egg there is a mass of undifferentiated protoplasm containing many free nuclei in an unstable condition. The epithelium of the lateral oviducts secretes a cement by means of which the eggs are anchored when laid. The spermathecae are without a chitinous intima, and this and other points in their structure and development throw doubts upon the homology between them and the spermathecae in other insects.

11. The further course of the spermatozoa is entirely inter- or intracellular; they do not enter the lumen of the oviduct, but pass upwards in bundles through the substance of the epithelium of the lateral oviducts. This epithelium shows no differentiation into cells by the formation of cell membranes. The spermatozoa eventually reach the protoplasmic mass in the pedicle of the ovariole. Later, when this mass has been taken up in the extension of the choriogenic epithelium around the growing egg, and when this epithelium has become flattened and stretched around the almost ripe egg, they pass upwards through it to the corresponding mass of protoplasm forming below the next egg. They do not stray from their course and do not show any signs of degeneration en route.

12. The significance of these events is discussed. Though Berlese's theory of 'hypergamesis' has been shown to be founded on error, the necessity of accounting for the enormous excess of spermatozoa which penetrate eventually into the ovariole remains. The spermatozoa have been traced to a mass of protoplasm containing free nuclei which are in an unstable condition. The enormous mass of protoplasm received by the female during successive acts of copulation, in the form of spermatozoa, leads one to doubt whether the latter have not some function other than that of the fertilization of the eggs.

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PLATE V.

Fig. 1. The chitinogenous cells of Ribaga's organ, with their terminal spines.

2. Berlese's organ, as seen in transverse section. m.g., wall of the midgut. ov., lateral oviduct. m., muscle passing between tergite and sternite. V. st., fifth sternite. IV. st., fourth sternite. ch.r., chitinous rods with their terminal spines projecting into cv., the space between the two sternites. × 75. C. rotundatus.

3. The same, in sagittal section. c., the chitinogenous cells. f., the fibrous layer.

4. The teeth on the fifth sternite. C. rotundatus.

5. One of the terminal spines of the chitinogenous cells of Ribaga's organ, very highly magnified, to show the infolding of the lateral edges to form a hollow tube.
PLATE VI.

Cells from the Organ of Berlese.

Fig. 6. From a young female, fixed while the chitin was soft and white C. lectularius.

7. From an unimpregnated bug ten days old, not fed since the first moult.

8. The periphery of the organ eight hours after the entry of the spermatozoa, showing the vacuolation of the cells and the masse of spermatozoa between them. c., capsule. The whole section of which this figure represents a part is shown in Fig. 44 on Plate XI. C. lectularius.

9. Twelve hours after the entry of the spermatozoa. The finely granular intracellular mass is the eosinophil body, e. Note the reappearance of the cytoplasm around the contracting vacuole. C. lectularius.

10. Twenty hours. e., eosinophil body. h., basophil body, taking on an intense black with haematoxylin.

11. Three days after the entry of the spermatozoa. h., the basophil body, much reduced in size. C. lectularius.

12. Cells from a 'wild' bug.

13. Cells from a 'wild' bug. x., the intracellular body described on page 58.

14. Nine cells drawn from the same section as that from which Fig. 12 was drawn. a., a resting cell. b., c., d., commencing vacuolation. i., a fully vacuolated cell. e., f., showing the eosinophil body g., h., the basophil body.

Magnification, all figures, 800 diameters.
Plate VII.

Fig. 15. A spermatozoon from the receptaculum seminis of C. lectularius. Fixed by osmic acid vapour and methyl alcohol, stained by Giemsa. × 800.

,, 16. The wall and the peripheral portion of the lumen of the spermatheca, two days after copulation and four days after the final moult. Bundles of spermatozoa are shown on the left, the single layer of cells forming the wall on the right. C. lectularius. × 800.

,, 17. Wall of the spermatheca when stretched by the distension of the chamber with spermatozoa. C. rotundatus. × 800.

,, 18. Wall of the common oviduct. Note the well defined chitinous intima. × 800.

,, 19. Lower part of lateral oviduct, transverse section. From the same series as Fig. 16. The position of the spermatozoa, which are mainly cut in transverse section, is indicated. Note that there are none in the lumen. × 700.

,, 20. A portion of Fig. 19, showing the head of a spermatozoon. C. lectularius.

,, 21. The apical part of one of the folds of the wall of the lateral oviduct, showing the secretion at the inner border of the cells, and some bundles of spermatozoa. × 800.
PLATE VII.

Fig. 16.

Fig. 17.

Fig. 18.

Fig. 19.

Fig. 20.

Fig. 21.

Fig. 15.
Fig. 22. One of the folds of the wall of the lateral oviduct at its upper end, in longitudinal section, two days after copulation, showing strands of spermatozoa within the cells. From a very thin section, in which only parts of many of the nuclei are included. The fragments appear as isolated chromatin particles. Note the absence of intercellular membranes. *C. lectularius.* × 800.

23. Transverse section through the upper half of the mass of nuclei in the upper division of the pedicle of the ovariole, three days after copulation, showing the irregular nuclei and the spermatozoa among them. The capsule is continuous with the basement membrane of the choriogenic cells, shown in figure 25. *C. lectularius.* × 800.

24. Transverse section through the upper end of the same. The nuclei are taking to themselves portions of the protoplasm.

25. The follicular epithelium. Note the characteristic structure of the nucleus. × 800.

26. The chorion and connected structures in an almost ripe egg. The parts are slightly separated from one another by the splitting and bending of the chorion (see Fig. 49 on Plate XII) which takes place under the action of the fixative. The curvature of the chorion is exaggerated from the same cause. *v.*, the vitelline membrane. *end.* *c.*, the endochorion. *b.m.*, basement membrane of the follicular epithelium. *ch.*, the follicular or choriogenic epithelium. *exo.*, *c.*, the exochorion. *sp.*, spermatozoa. *p.*, peritoneal investment of the ovariole. *s.*, one of the spines on the chorion.

26a. A portion of the epithelium from the same section, showing three nuclei and one bundle of spermatozoa, the latter cut exactly in transverse section and simulating a nucleus.
Fig. 22.

Fig. 23.

Fig. 24.

Fig. 25.

Fig. 26a.

Fig. 26.
PLATE IX.

Dissections of the reproductive tract of Cimex rotundatus at successive stages after copulation. Stained with borax-carmine. It is possible that some of the spermatozoa, which are not very firmly attached, have been detached and lost in dissection. Magnification, × 19. (Photographs.)

The organ of Berlese from the same bugs are shown on Plate XI, as follows:— Figure 29 = figure 41, figure 30 = figure 42, figure 31 = figure 43, figure 32 = figure 44, figure 35 = figure 45.

Fig. 27. From a newly moulted female.

" 28. From a female ten days after the moult, but unfed since the nymphal stage.

" 29. Immediately after copulation.

" 30. One hour after.

" 31. Two hours after copulation. Only one ovary was developed in this bug.

" 32. Eight hours after. The re is small cluster of spermatozoa attached to the lateral oviduct on the left side.

" 33. An undeveloped ovary from a bug three days old, and one day after copulation. Developing eggs were present in the ovary of the other side.

" 34. Twelve hours after copulation. Large masses of spermatozoa have now reached the spermathecae, and many of them have penetrated into its lumen.

" 35. Twenty hours after copulation. The lateral oviducts and spermathecae are entirely hidden by masses of spermatozoa. The amount in this preparation is exceptional, but a correspondingly large accumulation has been seen in sections.
Dissections of the reproductive tract of *C. rotundatus* (Photographs).

Fig. 36. Two days after copulation. No eggs have been laid.

,, 37. Three days after. Two eggs were laid. The tubes from which they came are on the outside of the row in each ovary.

,, 38. Four days after. Two ripe eggs, one of which is shown, were detached in dissection.

,, 39. From a ‘wild’ bug. The mass of nuclei in the pedicle can be distinguished. Many spermatozoa were detached during dissection. The arrangement in whorls of those within the spermathecae is indicated by the outline of the latter.

,, 40. From a ‘wild’ bug. *C. lectularius*. Six of the ovarioles on the right and four on the left have recently discharged their ova. The apparent large size of the ovary as compared with the preceding figures is due partly to stretching during dissection. A large mass of spermatozoa is collected round the spermathecae.
Sections of Berlese's organ at successive stages after the entry of the spermatozoa. (Photographs.) Magnification, × 75.

Fig. 41. Immediately after copulation. The spermatozoa are collected in a large central mass.

,, 42. One hour after. The organ of Ribaga is shown in the lower part of the section.

,, 43. Two hours after. The spermatozoa have arranged themselves in bundles and are commencing to work their way towards the periphery. The vacuolation of the cells is well advanced.

,, 44. Eight hours after. Almost all the cells are reduced to large vacuoles, the interstices between which are packed with spermatozoa.

,, 45. Twenty hours after. The basophil masses within the cells are now very conspicuous.

,, 46. Four days after. The organ is now restored to the original condition, except that in some of the cells the basophil mass is still present, though much reduced in size. The difference in size in these figures is partly due to the fact that the section selected for illustration is not always one through the centre of the organ.
Fig. 47. The testes of *Cimex lectularius*. One lobe of the testis on the left was detached in opening out the lobes. \( \times 19 \).

48. The (apparent) fourth sternite, showing the presence of a Berlese's organ on each side. *C. lectularius*.

49. Portion of a transverse section showing a ripe or nearly ripe egg, containing an embryo. The chorion has split and is bent and displaced inwards. The spines on its outer surface are shown. \( \times 75 \).

50. Berlese's organ *in situ*, from a sagittal section. Seven days after the last copulation. The black mass to the right is the midgut, containing the residue from the last meal. Below the organ, on the right, the lateral oviduct; in the centre below, masses of spermatozoa arranged in whorls and free in the hematocoele. Note the difference between the junctions of the third and fourth, and fifth and sixth sternites, with that between the fourth and fifth, where Ribaga's organ is situated on the intersegmental membrane. \( \times 75 \).


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THE GEOGRAPHICAL DISTRIBUTION OF THE INDIAN RAT FLEAS AS A FACTOR IN THE EPIDEMIOLOGY OF PLAGUE: PRELIMINARY OBSERVATIONS.

BY

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The geographical distribution of plague in India has not as yet been fully explained. If we consider, from the facts now known regarding the etiology and epidemiology of the disease, what might have been expected to be the history of the invasion from the time of the introduction of plague at Bombay in 1896, we find a very marked divergence between the theoretical conclusions and the actual course of events. Everywhere in India there are rats, and all the rats harbour fleas; practically all parts of the country have, for a part of the year, a climate favourable, more or less, for the establishment and spread of plague; communications are free, and there are no considerable natural barriers. It would have been reasonable to forecast that the disease would spread from Bombay as from the apex of a fan, until it reached the mountain ranges which separate India from the rest of Asia; that the progress would be on the whole a steady wave but with a more rapid advance along the main lines of communication and where the climatic conditions were the most favourable or the greatest length of time in each year; it would not have been reasonable to forecast that any part of the country would escape.

Within a few years of the first introduction of the disease it spread in all directions, but, in spite of the widespread nature of the epidemics,
large tracts of country escaped altogether, or suffered very lightly. The inequality of the distribution of the disease is very strikingly brought out in the map accompanying the annual Report of the Sanitary Commissioner with the Government of India for 1917. This map shows that the southern portion of the Madras Presidency, east and west of Cape Comorin, large tracts of the eastern coastal area, of the Central India Plateau, and of Eastern Bengal and Assam, and the western half of Burma, have remained free from the disease, although they are near to, and in free communication with, regions which have experienced severe epidemics. Other tracts of country, as for instance that to the west of the Indus, and that on the eastern side of the Madras Presidency, are shown as having suffered little from plague. Although in a general way the epidemics have been most severe in those parts in which the conditions are, according to our present knowledge, most favourable, there are many striking exceptions. The Madras Presidency offers many striking instances of places in which, though the conditions are apparently favourable, the disease has never become established.

It is the absolute failure of the disease to appear in certain areas which is the striking fact. One would not expect the mortality from plague to be evenly spread over the whole country; as the Plague Research Commission have pointed out, the rat population is denser in some parts than in others, and the number of fleas per rat is a variable quantity; climatic conditions may be more or less favourable to the rat flea in its rôle as the vector of plague; free communications may facilitate, lack of communications may hinder, the spread of the infection. But it may safely be affirmed that there is practically no part of the country where the known conditions are definitely and permanently against the establishment of an epidemic. The escape of large and populous tracts throughout a long period of years cannot be explained on the facts as at present known.

The Plague Research Commission paid special attention to this problem of the inequality of distribution. In the case of Eastern Bengal and Assam they expressed the opinion that the physical features of the country, which is bounded on the north and east by mountain chains, and is subject to annual floods, would tend to protect it from the importation of the disease, and to limit its spread if introduced; they found that rats are much less abundant in the houses of the people than in other parts, the houses being less fitted to afford shelter to these
vermin, and food supply for them being less abundant on account of the tidier habits of the people. In the case of the United Provinces, contrasting the heavily infected city of Cawnpore with Lucknow, only forty-five miles away and presenting apparently similar conditions, but showing a much lighter infection, they lay stress on the fact that Cawnpore is a large and important trade centre, with a large trade in grain. Contrasting the comparatively plague-free district of Bundelkhand with the severely infected Ballia district, they found that the climatic conditions of Ballia were the more favourable, and considered that the wandering habits of the people rendered the introduction of the disease more likely. Their conclusions with regard to the irregularity of the distribution of plague in the Madras Presidency are less definite. They lay stress on the importance of the physical features of the country and of the climate, and on the proximity of the infected areas of the Presidency to heavily infected areas in other administrative divisions; they also point out that the more humid and cooler coastal regions, which have generally escaped, are separated from the infected parts by a low-lying and comparatively hot and dry plain, across which infection would not so easily pass.

It may be noted that this irregularity of distribution is to be observed within smaller tracts as well as in the country as a whole. It has been pointed out that the villages within any district of the Punjab are not all equally infected, and that the inequality is not necessarily related to the freedom or paucity of communications.

It is not proposed to enter into a discussion or criticism of the findings or conclusions of the Plague Commission. The writers themselves were clearly not satisfied that they had come to the root of the matter, and do not hesitate to express their doubts. In discussing the distribution of the disease in the United Provinces, after stating in general terms the conditions, or combinations of conditions, affecting the distribution of the disease, they go on to say 'It may well be, however, that other unexplained factors are at work, without knowledge of which a completely satisfactory explanation of the geographical distribution of plague cannot be arrived at.' They came to the conclusion that in Madras City, which has remained practically free from plague, the conditions as regards house construction, etc., are not unfavourable, and that suitable climatic conditions prevail during the winter months. Their later work on the distribution of the disease in the Madras Presidency as a whole did not disclose any very definite explanation as
to why the city should have escaped. It is admitted that plague has been introduced there many times.

We may, therefore, not unreasonably presume that there is an important factor, not yet recognized, in the combination of factors which govern the epidemiology of plague. It is the purpose of this communication to record preliminary observations which indicate that this missing factor is the geographical distribution of the species of *Xenopsylla*.

Throughout their work the Plague Research Commission assumed that there was only one species of this genus on rats in India, *viz.*, *cheopis*. According to their reports on the examination of very large numbers of rat fleas in different parts of the country, this species made up 98 per cent or more of the total, and was therefore the only species of any importance. This, however, is not the case. There are three species, *astia*, *brasiliensis*, and *cheopis*; of these, as will be shown presently, *astia* and *cheopis* are equally common and widespread.

It was pointed out by L. F. Hirst in 1913 that *X. astia* is the common rat flea of Colombo. He recorded also that the whole of a collection of rat fleas which he obtained from Madras, sent by him to Rothschild, were identified as *astia*. Hirst drew attention to the connection between these observations and the relative immunity of Colombo and Madras from Plague, and suggested that further investigations into the geographical distribution of the two species might throw light on the epidemiology of the disease in Madras and Burma. In a further communication in which an account of the plague outbreak in Colombo was given by Phillips and Hirst, certain suggestive peculiarities in the habits of *astia* were noted. From the entomological point of view the suggestion that the several species of the genus, or of other genera, should not be equally efficient as vectors of the disease, is a very reasonable one. We know that parasitic insects differ widely in their host preferences, feeding habits, in their reactions to temperature and humidity conditions and in their powers of resistance to an unfavourable environment. There may easily be some factor or factors in the bionomics of one or other of these species which may render it practically ineffective as a vector of plague. If that is so, the geographical distribution of the several species is a factor of prime importance in the epidemiology of the disease.
378 Geographical Distribution of the Indian Rat Fleas.

Material and Technique.

At the writer's request arrangements were made by the Public Health Commissioner with the Government of India, and by the Sanitary Commissioners of Provinces, for the collection of rat fleas in the Punjab, the Madras and Bombay Presidencies, Central India, and in Burma. Altogether 107 collections were received, representing heavily infected, lightly infected, and plague-free areas. The first collections were received in November, 1919, and the work was continued during the following year. The total number of fleas examined was 17,358.

It is practically impossible to make out the distinguishing features of these species unless the specimens are suitably prepared. The females can, on account of the characteristic shape of the spermatheca, be recognized with a hand lens after the soft parts have been dissolved out by caustic potash or rendered transparent by a clearing agent, but for the certain identification of the males the use of the compound microscope is necessary. The fleas were therefore mounted in the following way. After a short preliminary treatment with caustic potash solution they are brought through alcohol to xylol, and allowed to remain overnight in a thin solution of balsam in xyloé. The slides are prepared by coating them with a thin layer of balsam, and allowing them to dry overnight in the incubator. The fleas are then picked up with forceps and laid, similarly orientated throughout, on the slide, in regular rows of ten, five such rows to a slide of ordinary size. The coverslip is moistened with xylol and lowered gently on to the slide. With a little practice the fleas can be arranged as regularly as a series of sections. This method of mounting, which can be done by a trained assistant, saves a great deal of time in the subsequent examination, for the rows can be passed rapidly across the field of the microscope by the mechanical stage; it has the obvious advantage that specimens can be easily marked for future reference.

Statement of the Results.

Detailed statements of the results, arranged according to Provinces are given in the accompanying tables. The following summary shows that astia and cheopis are both common fleas in the parts of India covered by this investigation.
TABLE I.

Summary of the Results of the Examination of 17,339 Rat Fleas.

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<tr>
<th></th>
<th>Xenopsylla.</th>
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<th>Leptopsylla muscoli</th>
<th>Total</th>
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<td>cheopis</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>liensis</td>
<td></td>
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<td>Bombay</td>
<td>547</td>
<td>849</td>
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<tr>
<td>Central India</td>
<td>6</td>
<td>202</td>
<td>72</td>
<td>..</td>
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<tr>
<td>Madras</td>
<td>2,122</td>
<td>280</td>
<td>1,938</td>
<td>84</td>
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<tr>
<td>Burma</td>
<td>1,549</td>
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<td><strong>Total</strong></td>
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<td><strong>53.9</strong></td>
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</table>

It will be noted that the 19 specimens of Ctenocephalus felis and Echidnophaga sp. are not included in the above table, and have not been taken into account in calculating the percentages in the detailed statements.

The majority of the collections were obtained from towns of considerable size, a few from villages. It will be noted that the differences between the several collections from one district are often considerable.
TABLE II.

Punjab.

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<th>Ceratophyllus punjabensis</th>
<th>Actual Total</th>
<th>Ctenocephalus felis</th>
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<td></td>
<td></td>
<td></td>
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* When the year is not stated it is understood to be 1920.

† The inclusion of a figure in brackets signifies that it stands for an actual number, not a percentage.
### Table II—contd.

<table>
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<th>Clenocephalus felis</th>
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<td></td>
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### Table III.

**Bombay.**

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<thead>
<tr>
<th>District</th>
<th>Locality</th>
<th>Date</th>
<th>Percentages</th>
<th>Actual Total</th>
<th>Clenocephalus felis</th>
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</thead>
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<td>Nov. 1919</td>
<td></td>
<td>100</td>
<td>111</td>
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<tr>
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<td>82.5</td>
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<td>H mowar</td>
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<td><strong>Do.</strong></td>
<td>Do.</td>
<td></td>
<td>30.4</td>
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</tr>
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<td></td>
<td></td>
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<td>439</td>
</tr>
<tr>
<td><strong>Sholapur</strong></td>
<td>Wai</td>
<td>July</td>
<td>3.0</td>
<td>97.0</td>
<td>100</td>
</tr>
<tr>
<td><strong>Sholapur</strong></td>
<td>Mar.</td>
<td></td>
<td>3.0</td>
<td>97.0</td>
<td>100</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>547</td>
<td>849</td>
<td>5183</td>
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</tbody>
</table>
**Table IV.**

Central India.

<table>
<thead>
<tr>
<th>Province or State</th>
<th>Locality</th>
<th>Date</th>
<th>Xenopsylla</th>
<th>Actual Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Provinces</td>
<td>Nagpur</td>
<td>Mar.</td>
<td>astia brasiliensis cheopis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Narsingar</td>
<td>Mar.</td>
<td>(1) 5 95.0</td>
<td>20</td>
</tr>
<tr>
<td>Narsingar State</td>
<td>Narsingar</td>
<td>Feb.</td>
<td>27.8 72.2</td>
<td>18</td>
</tr>
<tr>
<td>Rewa State</td>
<td>Rewa</td>
<td>Jan.</td>
<td>98.6 1.4</td>
<td>202</td>
</tr>
<tr>
<td>Do.</td>
<td>Umaria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>6 202 72</td>
<td>280</td>
</tr>
</tbody>
</table>

**Table V.**

Madras.

<table>
<thead>
<tr>
<th>District</th>
<th>Locality</th>
<th>Date</th>
<th>Xenopsylla</th>
<th>Ctenocephalus felis</th>
<th>Actual Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anantapur</td>
<td>Urvakonda</td>
<td>Jan.</td>
<td>(1) 82.5</td>
<td>...</td>
<td>(6)</td>
</tr>
<tr>
<td>Bangalore</td>
<td>Mar.</td>
<td>41.2</td>
<td>96.0 95.0</td>
<td>...</td>
<td>293</td>
</tr>
<tr>
<td>Bellary</td>
<td>Jan.</td>
<td>62.5</td>
<td>84.2 84.2</td>
<td>...</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>60.3</td>
<td>84.2 84.2</td>
<td>...</td>
<td>1,000</td>
</tr>
<tr>
<td></td>
<td>May</td>
<td>33.6</td>
<td>84.2 84.2</td>
<td>...</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Feb.</td>
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<td>84.2 84.2</td>
<td>...</td>
<td>50</td>
</tr>
<tr>
<td>Hospet</td>
<td>Aug.</td>
<td>84.5</td>
<td>84.2 84.2</td>
<td>...</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42.4 57.6</td>
<td>...</td>
<td>1,610</td>
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<tr>
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<td>Bhawani</td>
<td>Aug.</td>
<td>54.3 39.2</td>
<td>...</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Feb.</td>
<td>39.2 39.2</td>
<td>...</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Dhapurm</td>
<td>May</td>
<td>84.2 84.2</td>
<td>...</td>
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</tr>
<tr>
<td></td>
<td>Do.</td>
<td>...</td>
<td>84.2 84.2</td>
<td>...</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Kokagal</td>
<td>May</td>
<td>39.2 39.2</td>
<td>...</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Cuddapah</td>
<td>Mar.</td>
<td>39.2 39.2</td>
<td>...</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Oct.</td>
<td>39.2 39.2</td>
<td>...</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>...</td>
<td>40</td>
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|        |          |      | 54.3 39.2  | ...                 | 40           |
### Geographical Distribution of the Indian Rat Fleas.

#### Table V—contd.

<table>
<thead>
<tr>
<th>District</th>
<th>Locality</th>
<th>Date</th>
<th>PERCENTAGE OF EACH SPECIES</th>
<th>Actual Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Xenopsylla</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>astia</td>
<td>brasiliensis</td>
</tr>
<tr>
<td>Godaveri</td>
<td>Tuni</td>
<td>Jan. 1919</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Cocanada</td>
<td>Feb. 1919</td>
<td>77.3</td>
<td>..</td>
</tr>
<tr>
<td>Guntur</td>
<td>Guntur</td>
<td>Jan. 1919</td>
<td>90.6</td>
<td>..</td>
</tr>
<tr>
<td>Madras</td>
<td>Madras</td>
<td>Feb. 1919</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td>Nellore</td>
<td>Nellore</td>
<td>Mar. 1919</td>
<td>80.5</td>
<td>46.9</td>
</tr>
<tr>
<td></td>
<td>Ootacamund</td>
<td>Dec. 1919</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Do.</td>
<td>22.7</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Jan. 1919</td>
<td>..</td>
<td>39.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>28.8</td>
<td>37.2</td>
</tr>
<tr>
<td>North Arcot</td>
<td>Chittoor</td>
<td>Feb. 1919</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td>Salem</td>
<td>Salem</td>
<td>Feb. 1919</td>
<td>10.0</td>
<td>90.0</td>
</tr>
<tr>
<td>South Arcot</td>
<td>Tirukkoilur</td>
<td>Sept. 1919</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Kallakurichi</td>
<td>June 1919</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Do.</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td>South Canara</td>
<td>Mangalore</td>
<td>Nov. 1919</td>
<td>16.9</td>
<td>80.1</td>
</tr>
<tr>
<td>Tanjore</td>
<td>Negapatam</td>
<td>Dec. 1919</td>
<td>100</td>
<td>..</td>
</tr>
<tr>
<td>Trichinopoly</td>
<td>Trichinopoly</td>
<td>Jan. 1919</td>
<td>95.5</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Feb. 1919</td>
<td>51.3</td>
<td>48.7</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Do.</td>
<td>70.0</td>
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<tr>
<td></td>
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<td>Do.</td>
<td>90.0</td>
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<td>Total</td>
<td></td>
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<td>74.7</td>
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</tr>
<tr>
<td>Vizagapatam</td>
<td>Vizagapatam</td>
<td>Jan. 1919</td>
<td>58.3</td>
<td>41.7</td>
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<td>Apr. 1919</td>
<td>Apr. 1919</td>
<td>40.0</td>
<td>60.0</td>
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<tr>
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<td></td>
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<td>48.2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>2,122</td>
<td>280</td>
</tr>
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</table>
MAJOR F. W. CRAGG.—The Geographical Distribution of the Indian Rat Fleas.
EXPLANATION OF PLATE XXVIII.

Figs. 1, 2, and 3. *X. astia* Rothschild, terminal segments of male, female, and the spermatheca.


,, 7, 8, and 9. *X. cheopis* Rothschild.
### Table VI.

**Burma.**

<table>
<thead>
<tr>
<th>District</th>
<th>Locality</th>
<th>Date</th>
<th>X. astia</th>
<th>X. brasiliensis</th>
<th>X. cheopis</th>
<th>Actual Number</th>
<th>Ctenocephalus felis</th>
</tr>
</thead>
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<tr>
<td>Akyab</td>
<td>Akyab</td>
<td>Nov. 1919</td>
<td>96.0</td>
<td>..</td>
<td>4.0</td>
<td>50</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Dec. 1919</td>
<td>100</td>
<td>..</td>
<td>..</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Jan. 1919</td>
<td>84.4</td>
<td>..</td>
<td>15.6</td>
<td>141</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>April 1919</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>July 1919</td>
<td>99.5</td>
<td>..</td>
<td>0.5</td>
<td>599</td>
<td>..</td>
</tr>
<tr>
<td></td>
<td>Do.</td>
<td>Oct. 1919</td>
<td>100</td>
<td>..</td>
<td>..</td>
<td>163</td>
<td>..</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>97.8</td>
<td>2.2</td>
<td>1,421</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Moulmein</td>
<td>Moulmein</td>
<td>June 1919</td>
<td>70.6</td>
<td>..</td>
<td>29.4</td>
<td>95</td>
<td>..</td>
</tr>
<tr>
<td>Rangoon</td>
<td>Rangoon</td>
<td>Dec. 1919</td>
<td>54.4</td>
<td>..</td>
<td>45.6</td>
<td>136</td>
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</tr>
<tr>
<td></td>
<td>Do.</td>
<td>July 1919</td>
<td>40.0</td>
<td>..</td>
<td>60.0</td>
<td>45</td>
<td>..</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>50.8</td>
<td>49.2</td>
<td>181</td>
<td>..</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>1,549</td>
<td>148</td>
<td>1,697</td>
<td>2</td>
<td></td>
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</tbody>
</table>

**The Three Species of Xenopsylla.**

Descriptions of these three species were given by Rothschild in the Bulletin of Entomological Research in 1914. References to the original more detailed descriptions are given at the end of this paper. *X. cheopis* is the only one of the three which was included in the 'Revision' published in 1906.

The diagnosis of these species depends mainly on the form of the terminal segments of the abdomen. These are illustrated on Plate XXVIII. The writer has seen no deviations from the described types which were sufficient to lead to doubt regarding the identification. The spermatheca of the female is remarkably constant in shape in each of the three species, and furnishes a ready means of distinguishing them.
Geographical Distribution of the Indian Rat Fleas.

Of the large number examined, only two variations were found, both in cheopis; in one of these the tail of the spermatheca was very short, giving the organ an L-shape; in the other there were two spermathecae, each of the normal size and shape.

There is one point not mentioned by Rothschild which serves to distinguish the male of astia from the males of the other two species. The median sagittal incrassation of the head has an even contour in cheopis and in brasiliensis, but in astia it is produced ventrally in its posterior portion, so as to have a ‘keel’ like appearance. This character may not, of course, be peculiar to astia, but it is not present in the other two Indian species.

It should be noted that Rothschild’s synopsis refers only to these three Indian species. There are others of this genus in which the antepygidial bristle is placed on a raised tubercle, as in brasiliensis, and others which have a ribbon-like ninth sternite, as in astia.

The Geographical Distribution of the Three Species of Xenopsylla.

An examination of the tabular statements given, and of the accompanying map, shows at once that the distribution of these species, so far as it is indicated by the scattered and far too scanty observations here recorded, is most irregular, and, in the case of astia and cheopis, is not clearly correlated with any of the factors which ordinarily govern the geographical distribution of animals. The case of brasiliensis is less obscure, and it will be convenient to consider this species first.

Speaking very generally, India consists geographically of two distinct parts: Peninsular India, an elevated table-land, geologically ancient, occupying the central and southern portions; and Extra-Peninsular India, which is mainly composed of the great Indo-Gangetic Plain, a geologically recent tract of country which, though it extends from the coast to the foot of the Himalayas on either side of the Peninsula, is not more than a few hundred feet above sea-level. The habitat of X. brasiliensis is clearly Peninsular India. Of the seventeen stations from which it is recorded, only three are in the Punjab, and from each of these only a single specimen was obtained. In the remaining fourteen stations the percentage of this species varied from less than one to nearly 100, with an average of about 29. Only three of these stations are on the coast line, and these, it will be noted, are all on the west coast, where the range of the Western Ghats runs almost down to the sea. The
numerous stations on the east coast of Madras from which batches have been obtained are all far distant from any high land, and have yielded no specimens of *brasiliensis*.

The special characters of the stations which show a considerable percentage of *brasiliensis*, in contrast to the Indo-Gangetic Plain and to the coast line on the east, are the absence of extreme ranges of temperature such as are met with in the Punjab, and a moderate degree of humidity. All these stations are in the tropics.

The nature of the conditions favourable to this species is well illustrated by the three batches obtained from the Coimbatore district in Madras. No specimens of *brasiliensis* were present in the 349 fleas obtained from Bhawani and Bharapurem, which are between 600 and 1,000 feet above sea-level, while at Kollegal, in the same district, but at the edge of the Mysore plateau and at the 1,500--3,000 level, more than half were of this species. It will be noted that *Ceratophyllus punjabensis* and *X. brasiliensis* do not occur together.

*X. astia* and *cheopis* occur all over India, equally in the Peninsula and in the Extra-Peninsular portions; their relative prevalence cannot be correlated with climatic conditions, nor does it appear to follow the divisions into which zoologists have mapped out the country from a study of the fauna as a whole. Each species is present in considerable numbers in the sub-tropical regions of the Punjab, where the climate is subject to wide seasonal variations, and on the east coast, where the climate is tropical throughout the year and the humidity always high. Of the forty-two stations from which substantial batches of fleas were obtained, *cheopis* was present in all but five, *astia* in all but eight; it may be noted that in three of the batches from which *cheopis* was absent the total number of fleas examined was small. On the whole, however, it is evident that *cheopis* is the commoner flea in the Punjab, while *astia* is the more common on the Madras coast, taking the two extremes of climate. In some of the stations on the Peninsula *astia* appears to be replaced by *brasiliensis*; all batches which contain *brasiliensis* also contain *cheopis*.

The bearing of climate on the relative prevalence of these two species is indicated in the accompanying map, on which the means of temperature and humidity are shown. The group of Punjab stations having a mean annual temperature of less than 77.5° F. show a high percentage of *cheopis*, but in two of them more *astia* are recorded than *cheopis*; the proportions here appear to be more nearly related to the mean annual
humidity. The group of stations having a mean annual temperature between 77.5° and 79°F. show a distinctly higher proportion of *astia*. The stations on the Madras coast show generally a low percentage of *cheopis*, but at Vizagapatam the proportions of *astia* and *cheopis* are nearly equal. Perhaps the most striking contrast is shown by the three stations in Burma from which collections were obtained. Out of the 1,421 fleas sent from Akyab, only 2.2 per cent were *cheopis*, while at Rangoon, not far distant and with a similar climate, the 181 fleas collected were divided equally between *astia* and *cheopis*. It is clearly not possible, therefore, to correlate the constitution of the flea population with climatic and geographical conditions.

A critical examination of the figures given in the tables shows that the irregularity of the distribution of these two species holds for the smaller areas as well as for the country as a whole. In the Gurgaon district, for instance, a batch from the village of Sakris showed 100 per cent *astia*, while at Jacob Pura, in the same district, the percentage of this species was 18.1, the remainder being *cheopis*. In some instances, two batches collected from the same town during the same month show considerable differences; for example, of the two batches collected at Lyallpur during December, one contained 55.3 per cent *astia*, the other only 18.3. This is probably to be accounted for by the collections having been made in different parts of a large town. The figures suggest, as one would indeed expect to be the case, that, in an area in which both species occur, they are not uniformly distributed over all the rats, but sometimes the one, sometimes the other, will predominate on the rats of particular localities within the area; it is to be expected that batches from adjacent villages will differ within wide limits, when the total collections are small. It is obvious that large collections are essential if statistical methods are to be applied to this problem.

*Seasonal Prevalence of these Species.*

The number of large collections obtained at different seasons from the same station is unfortunately too small to allow of conclusions being drawn regarding the seasonal prevalence of these species. The best series is that from Poona. As is shown in the accompanying chart, the proportion of *cheopis* rises from May to September, probably in association with the marked fall in temperature and the increase in humidity which accompany the breaking of the monsoon in June. This suggests that *cheopis* is better adapted to a cool and damp climate than is *brasiliensis*,...
a suggestion which is borne out by other considerations already indicated. The figures for Belgaum point in the same direction, though

here it is to be noted that the fleas obtained in November were collected at the town of Athni, not in Belgaum itself. The only station from which successive large batches containing astia and cheopis were obtained

CHART I.—Showing the relative seasonal prevalence of *cheopis* and *brasiliensis* in Poona.

CHART II.—Showing the relative seasonal prevalence of *cheopis* and *brasiliensis* in Belgaum and Athni.

CHART III.—Showing the relative seasonal prevalence of *cheopis* and *astia* in Bellary and Hospet.

The numbers shown below the months are the totals of the collections from which the graphs are constructed.
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is Bellary. Combining the figures for Bellary and Hospet, which is about forty miles distant and in the same kind of country, we find a similar increase in the proportion of cheopis during the colder months of the year. Further observations on this point, which is clearly of importance, are required.

Proportions according to the Species of Host.

In few cases has it been possible to obtain precise information regarding the species of rat from which the fleas were obtained. The batches sent from Akyab and from Rangoon were labelled with the name of the host, and the figures are given below. They are sufficient to show that either astia or cheopis may parasitize any one of several rodents. This is what one would expect from a general knowledge of the relations of fleas to their hosts, and is in agreement with the data given by Jordan and Rothschild.

**TABLE VII**

*Showing the Host Species of 1,232 Fleas from Akyab and of 181 Fleas from Rangoon.*

<table>
<thead>
<tr>
<th></th>
<th>Xenopsylla.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>astia</td>
<td>cheopis</td>
</tr>
<tr>
<td>Rangoon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. bengaliensis</td>
<td>42</td>
<td>20</td>
</tr>
<tr>
<td>M. concolor</td>
<td>28</td>
<td>57</td>
</tr>
<tr>
<td>M. norwegicus</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>M. rattus</td>
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<td>1</td>
</tr>
<tr>
<td>&quot; Bandicoot &quot;</td>
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<td></td>
</tr>
<tr>
<td>&quot; Shrew &quot;</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&quot; Mouse &quot;</td>
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<td>8</td>
</tr>
<tr>
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<td><strong>89</strong></td>
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</table>

<table>
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<th>Xenopsylla.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<td>cheopis</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>N. bengaliensis</td>
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<td>5</td>
</tr>
<tr>
<td>M. rattus</td>
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<td>23</td>
</tr>
<tr>
<td>M. concolor</td>
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<td>3</td>
</tr>
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<td><strong>31</strong></td>
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Until quite recently it was supposed that the Ceratophyllus found on rats in India was the same as that commonly found on rats in Europe, fasciatus Bosc, and it is thus referred to in plague literature. There are, however, not one but several species, and of these fasciatus is so rare that there is a doubt as to whether it is a true Indian flea. Jordan and Rothschild, in a paper published in January of this year, give eight species of Ceratophyllus from rats and squirrels in India, and of these seven are new. They give two records only of C. fasciatus in India.

The results of the present investigation confirm the findings of the Plague Research Commission with regard to the distribution of Ceratophyllus. With the exception of a single specimen among the 1,572 fleas from Poona, the genus was not represented in the Bombay collections. In Madras C. nilgiriensis was found in considerable numbers at Ootacamund, in the Nilgiri Hills and some 6,000 feet above sea-level. In the Punjab, on the other hand, C. punjabensis was present in the collections from twelve out of the fifteen stations, and in some instances occurred in considerable numbers; three batches from Jullundur contained 14·7, 16·1, and 25·4 per cent.

The marked seasonal prevalence of this species, which was commented on by the Plague Commission, is strongly brought out by the figures from the Punjab. None of the seven batches of fleas collected during the hot weather contained Ceratophyllus. It will be noted in Table II that of the seven batches from Gurgaon, collected during August and September, none contained this flea, while the batch collected in December contained 8·1 per cent. If we omit the fleas collected during the hot weather the average percentage of 0 batches is 6·5. The observations of Bacot on the prolongation of the cocoon stage of C. fasciatus are particularly interesting in this connection.

Fleas of other Genera.

The only other true rat flea found is Leptopsylla musculi, which was present in the collections from Ootacamund. Specimens from these batches were sent to Mr Rothschild, who pronounced them to be true musculi. The occurrence of this species in the Nilgiri Hills, and not elsewhere, is particularly interesting.

The absence of fleas other than those proper to rats is rather striking in view of the large numbers dealt with. It will be noted that there
were only ten specimens of *Ctenocephalus felis*, the common flea of dogs and cats in India, and not a single *Pulex*.

**The Geographical Distribution of the Indian Rat Fleas in Relation to the Epidemiology of Plague.**

The nature of the problem towards the solution of which the present investigation has been directed has already been indicated at the beginning of this paper. We have now to consider how far the observations here recorded bear out the hypothesis that the several species are not equally efficient as vectors of plague, with the corollary that there is a direct relation between the geographical distribution and relative numbers of these species and the incidence of plague.

The observations so far made are neither sufficiently numerous, nor are they sufficiently evenly distributed. The collections vary greatly in size, and at the best represent only a portion of the country; they have not been made in a manner calculated to eliminate the error due to the uneven distribution of the species in the particular locality which they represent; the factor of a difference in the seasonal prevalence of the species has not been altogether eliminated. On the other hand, the figures for the plague mortality of the area represented by each collection or series of collections are averages, and it is known that the mortality is not very evenly spread over the areas to which the figures refer. Nevertheless, the results are striking, and leave little doubt regarding the importance of the difference in species of the predominant flea in heavily infected and in lightly infected parts of the country.

The only two species of which large numbers have been obtained are *X. astia* and *X. cheopis*, which together make up 90 per cent of the total. The present problem is therefore a comparison of those stations in which *astia* is the common flea with those in which *cheopis* is predominant.

The source of information regarding the incidence of plague in the numerous scattered stations from which collections have been received is the map, already referred to, which accompanied the Annual Report of the Sanitary Commissioner with the Government of India for 1917. In this the distribution of plague in India is shown graphically by different kinds of shading; the variations within small units such as districts are not of course shown. The information obtained from this
MAP OF INDIA AND BURMA.
Showing the places mentioned in the text.

The mean annual humidity (black lines), and the mean annual temperature (red lines) are copied from the Climatological Atlas of India. The interrupted red line is the mean isotherm.

MAJOR F. W. CROGH.—The Geographical Distribution of the Indian Rat Fleas.
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**TABLE VIII.**

Forty-two stations from which fleas were obtained classified according to the incidence of Plague and the percentage of *X. cheopis.*

**Plague Mortality per 1,000, 20-year period, 1896-1917.**

- Free.
- 1-5
- 10-20
- 50-100
- 100-150
- 150-200
- 200-250
- 250-300

- Salem
- Rawalpindi
- Sholapur
- Poona
- Jhelum
- Sialkot
- Ludhiana
- Belgaum
- Muzaffargh
- Karwar
- Lyalpur
- Bellary
- Rangoon
- Bombay
- Coenada
- Trichinopoly
- Moulmein
- Bangalore
- Gurgaon
- Akyab
- Guntur
- Multan
- Umaria
- South Arcot
- Chittoor, Cuddapah, Madras, Nagaapatam.
map has been supplemented, through the courtesy of Civil Surgeons and Health Officers of Municipalities, by detailed records from some of the localities from which the fleas were collected.

Omitting places from which small collections were obtained, the localities are arranged in Table VIII according to the percentage of *X. cheopis* and the plague mortality as shown in the above map I. This table should be read in conjunction with Tables II to VI, in which the details of the constitution of each batch of fleas are given. It will be seen that, though the nature of the records is not well adapted for the application of the statistical method by which such a problem should be approached, the observations point very strongly to a close relation between the predominance of *X. cheopis* and high plague mortality. *X. cheopis*, in fact, appears to be truly the 'plague flea,' while *astia* is the predominant species in those areas which have remained free from the disease or have suffered only lightly.

The figures at the two extremes of the table are naturally of the most interest and importance. At Akyab, from which only 31 specimens of *cheopis* were obtained out of a total of 1,421 fleas, there has never been a case of indigenous plague; the collections represent all seasons of the year. At Chinea Salem, in the Kallakurchi Taluk of the South Arcot district, from which 124 specimens of *astia* and none of *cheopis* were obtained, there were two cases of plague in 1911, none before or since. At Guntur, where the proportion of *astia* is 90.6 per cent, there was an outbreak of plague in 1918-1919, with a total of 232 deaths. Special interest attaches to the figures for Madras Municipality, since the conditions there were examined by the Plague Research Commission, who came to the conclusion that they were generally such as would permit of the establishment of the disease. The whole of a batch of 186 fleas were *X. astia*. The plague mortality from 1897 to 1917 has ranged from nil in 1915 to 0.1 in 1906 with an average of 0.013; although the disease has been present in twenty years out of the twenty-one, in thirteen of these years the mortality has been less than 0.01; in other words, though the infection has been repeatedly introduced, it has each time failed to set up an epidemic. At Negapatam, in the Tanjore district, plague was imported in 1918 and in 1919, one fatal case only occurring in each of these years; all of the 84 fleas obtained there were *astia*. At the other extreme we have the very heavily infected districts in the Punjab and in the Bombay Presidency. Unfortunately, no figures for these areas, more exact than
those given in the map, are available at the time of writing. The Punjab has throughout been the most heavily infected part of India, and the incidence has been spread fairly evenly over the province, with the exception of the south-western part, including Multan, Muzaffargarh, and Mianwali, which have not suffered severely. The districts named, it will be noted, show a considerable percentage of astia. Rohtak and Gurgaon are south-east of the most heavily infected parts. The heavily infected districts in the Bombay Presidency, Wai, Satara and Poona, are all situated at a considerable altitude, and in the two latter stations the collections have included a large proportion of brasiliensis.

The liability to severe epidemics of plague in those regions in which cheopis is the predominant rat flea, and the absence of such epidemics in regions where astia is predominant, seems fairly clear. The observations with regard to brasiliensis and Ceratophyllus are not sufficient to justify any inference, and their presence in considerable numbers rather complicates the question as between astia and cheopis. The case of brasiliensis can only be dealt with by the examination of a series of collections from Peninsular India, where this species appears to be a common rat flea, and partly to replace astia. Ceratophyllus is unlikely to be important, as there are few stations in which it makes up a substantial proportion of the whole.

To obtain the more precise data which will justify the full application of statistical methods, it will be necessary to make a series of observations under definitely chosen conditions. Large batches of fleas, collected at the same time of year and preferably during the plague season, are required, and it will be of advantage if only two species are present, either astia and cheopis or brasiliensis and cheopis. The stations chosen should be within fairly narrow geographical limits, and the range of plague mortality a wide one. The writer hopes to be able to record such a series of observations shortly.

The great practical importance of the matter is obvious. If it is really the case that cheopis is the 'plague flea,' while astia is not, it will be possible, by an examination of the fleas of a locality, to estimate precisely its liability to plague; in fact, to map out the 'cheopis-belts' just as the 'fly-belts' of Africa have been mapped out. It would clearly be unnecessary to take elaborate and expensive measures against plague in a district in which the rat fleas were of a species which is not a vector of plague. The significance of an imported case of plague will depend in a large measure on the local species of flea.
Acknowledgments.

The writer is glad to have this opportunity of expressing his most sincere thanks to those who have provided him with the material from which these observations have been made. He is fully conscious of the fact that without the assistance so freely given him nothing could have been done, that, indeed, the present contribution represents to a large extent the work of others. Where so many have been so helpful individual acknowledgments would be rather out of place, but the writer would like to be permitted to express his particular thanks to Chitre, Assistant Surgeon of the Plague Prevention Enquiry, and to his successor, Dr. Strickland, for the large batches, sent regularly at three-monthly intervals, which illustrate the seasonal prevalence of the two species occurring in Poona; and to Major MacWatters, I.M.S., for the large collections from Akyab.

The assistance given by Dr. Jordan and by Mr. Rothschild, who have named the species of *Ceratophyllum* and confirmed the identification of *L. musculi*, is gratefully acknowledged.

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REFERENCE LETTERING.

a.s., anal style of the female.
ap.b., antepygidal bristle.
pg., pygidium.
P, P2. Processes of the clasper.
S VIII, IX, X. The eighth, ninth, and tenth sternites.
sp., spiracle.
T VII, VIII, IX, X. The seventh to tenth tergites.
ON THE SO-CALLED 'PENIS' OF THE BED-BUG (CIMEX LECTULARIUS L.) AND ON THE HOMOLOGIES GENERALLY OF THE MALE AND FEMALE GENITALIA OF THIS INSECT.

BY

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AND

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The male and female external genital apparatus in the bed-bug has been described by several authors, notably Landois(1) Patton and Cragg,(2) Rothschild,(3) Hay Murray(4) and Hase(5). The most complete description is that of Rothschild, who studied the parts with a view to utilising them in the differentiation of species. None of these authors, however, have given a satisfactory account of the homology of the different structures, and their descriptions are consequently somewhat lacking in enlightenment.

The terminal segments of the male in Cimex* are modified in connection with the altogether extraordinary manner of copulation. The female has two sexual apertures, distinct and widely separate from one another, a copulatory orifice situated on the right side of the ventral aspect of the apparent fourth sternite just posterior to

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* Note.—We have dealt throughout with C. lectularius, but there is no reason to believe that the other species differ materially as regards the structures we have described.
the mid-point of the abdomen, and an orifice for oviposition in the normal ventro-posterior position. Moreover, the copulatory orifice of the female is of a special kind; there is a short longitudinal slit in the sternite and beneath this a solid structure, Berlese's organ, recently described in detail by Cragg, which has no aperture which could possibly admit the obvious male part. At the most the so-called penis of the male can be passed into the slit in the sternite. The so-called penis is claw-like, carried laterally and bent to the left, in correlation to the position of the copulatory orifice of the female and to the position taken up by the pair during the act of copulation. But there are obvious anatomical difficulties in the way of its use for the introduction of the spermatozoa into the organ of Berlese. A closer examination of this interesting structure reveals the fact that it is not the penis at all, but merely a grooved director along which the true intromittent organ glides during the act of copulation. By a comparison of the parts with those of other Rhynchota it has been possible to determine the homology of the organ. The sexually modified segments of the female with their appendages are also described and given their homologous denotation.

THE ABDOMEN IN CIMEX.

Before proceeding to discuss the sexually modified parts, it is necessary to define the taxonomy of the abdominal segments as a whole.

The abdomen in Cimex is composed of the following segments. There is an apparent first segment, consisting of both tergite and sternite, and carrying ventrally on each side well up towards its anterior edge a pair of spiracles. There follow five large unmodified, or but slightly modified, segments each carrying on the lateral portions of the sternite a pair of spiracles. Following the last of these and considerably smaller is an apparent seventh segment also bearing spiracles, but modified ventrally in the female in connection with the genital opening. The end of the abdomen is formed by the apparent eighth segment, which is without spiracles and is modified both in the male and in the female for sexual purposes. Particularly in the artificially distended bug can be seen a further minute ring-shaped segment, and beyond this at the anal orifice still another possible segment consisting of tiny hair-bearing plates resembling in appearance a minute tergite and sternite. (Plate XXX fig. 1).
It is certain that the first of the segments described above is not homologous with the true first abdominal segment in insects. Though this seems to us fairly obvious we have not seen it anywhere definitely stated in the description of *Cimex* that this is so. Later in this paper we give evidence for considering this segment to be in reality the second abdominal segment, and in the meantime, to avoid confusion, we shall in description give the segments their true homologous numbers, the apparent first being the true second and the minute ring-shaped segment the tenth with a possible eleventh segment represented by the two minute crescentic anal plates.

There is apparently no trace of the true first sternite, but what is almost certainly the remains of the tergite is to be seen as a crescentic portion of the large second tergite separated off by a distinct incrassation. In the nymph the existence of a first tergite is still more demonstrable, there being a distinct separate chitinisation representing this structure (Plate XXIX, fig. 7). In the nymph, as in the adult, no sternite is demonstrable. Comparative studies confirm the view that the crescentically marked off portion referred to is in reality the first tergite. In some bugs it exists as a distinct piece, but most frequently it is more or less fused with the second tergite, the junction, however, being almost invariably clearly indicated by a deep sulcus or incrassation.

The second true segment in *Cimex* is very large and at the sides extends forwards in two blunt cornuate processes. Dorsally the chitinisation extends without a break across the body, there being no indication of separation into tergite and connexivium. Ventrally also the segment is continuously chitinised for its full width, but it is deeply impressed centrally, where the chitinisation is less intense, by the extension backwards of the metasternum and posterior coxae. Close up to the metasternum, almost at the anterior edge of the segment, are the spiracles.

Abdominal segments 3-7 are very similar to one another in shape and size. Each consists dorsally of a broad tergal plate, chitinised uninterruptedly from margin to margin, and a ventral area similarly chitinised across the whole width but less intensely in the middle. As in segment 2 there is no indication of separate connexivial plates or of lateral pleural membrane. Each of these segments carries ventrally near its outer edge a pair of spiracles. The fifth segment has on the right side, in the female, a longitudinal slit extending the whole depth.
of the segment, and leading to the structure known as the 'organ of Ribaga' which is the entrance to the 'organ of Berlese.' The so-called *penis* of the male is inserted into this slit during copulation. Dorsally between segments 3-4, 4-5 and 5-6 in the median line are indications of the invaginations usually termed stink glands.

The eighth segment consists dorsally of a continuously chitinised tergal plate differing only from those of the preceding segments in being smaller and more contracted owing to its approach to the termination of the abdomen. Ventrally in the female the segment is considerably modified, as described later in connection with the female genital opening; in the male there is a slight modification only which will be described when dealing with the male genital apparatus. On each side of the ventral surface of the eighth segment is a large and conspicuous spiracle. No trace of a spiracle is visible even on the closest examination on the succeeding segments. The presence of a spiracle on this segment and on no succeeding one is of importance. In almost all insects the last pair of spiracles are on the eighth segment; only in the Lepidoptera and Diptera are the last pair generally on the seventh segment. Exceptions in other orders than those mentioned are practically only seen when the eighth segment is obsolescent, and even in this case careful search may frequently show some mark or indication of the spiracle.* The position of the spiracles is therefore additional evidence of the correctness of our interpretation of the segmental notation in *Cimex.*

The ninth segment is profoundly modified in both male and female. In both it forms the end of the abdominal box proper, the succeeding segments being loosely articulated and in life more or less telescoped within the body.

The tenth segment in the male is in the form of an almost complete chitinous ring without differentiation into tergite and sternite; in the female it is deficiently chitinised beneath. The small anal plates referred to previously are extremely characteristic of the Heteroptera; whether these are traces of an eleventh, or of the twelfth segment, or do not represent a true segment at all, we are unable to say. The tenth segment and the extension beyond it clearly form in *Cimex* the structure so characteristic of the Rhynchota generally and known as the *cauda.*

* A curious exception is the apterygote *Japyx.*
Fig. 1. Partially dissected potash preparation of *C. lectularius* adult, showing the small ring-like tenth segment and the appendage. To the left of the base of the appendage is seen the chitinous framework of the phallosome. (Dorsal view.)

2. Potash preparation of *C. lectularius* adult, showing the ninth segment with the appendage, ring-like tenth segment, groove, etc. (Ventral view).

3. Potash preparation showing the phallosome dissected out. The heavily chitinised basal folds are conspicuous and around the organ is seen the delicate membranous capsule (membrane of genital cavity). Below is seen the delicate chitinous lining of the ejaculatory duct entering the organ and above the continuation of the phallosome as a delicate chitinous extension which in this specimen reached the full length of the groove in the appendage.

4. Ventral view of potash preparation of a *Tingid Bug* to show bilateral symmetry with two appendages and a large median phallosome as is usual in the Heteroptera.

5. Potash preparation of nymph showing ventral chitinous plates on abdominal segments 7, 8 and 9. The chitinous rudiment of the genital apparatus is seen behind the ninth segment which is undivided.

6. Potash preparation of nymph showing chitinous plates on abdominal segments 7, 8 and 9, the latter divided into two lateral portions, etc.

7. Dorsal view of abdomen of nymph showing presence of a first abdominal tergite. The last segment seen is the ninth as the small ring-like tenth segment is not shown. Vide, however, figs. 5 and 6.

8. Partially dissected potash preparation of adult showing the eighth and ninth segment with appendages. On the left side the lateral connexival piece of the eighth segment is seen with spine. Below are the two portions of the ninth sternite with their boss-like inner projections. Posterior to the hairy bosses lies the tenth segment. Anteriorly to the ninth sternal pieces are the narrow chitinous strips of the posterior gonapophyses.
In the nymph the presence of a distinct first tergite has already been referred to. The second tergite consists of a median transversely elongated chitinisation (tergite) and two lateral chitinised areas. The tergites 3-7 are much as in the adult; the third tergite is, however, unchitinised in its anterior portion. Ventrally the sternites are almost completely unchitinised, but there are median chitinous plates on the seventh, eighth and ninth segments and at the extreme lateral edge of each segment a small chitinous 'island.' In the fully grown nymph small chitinised spots also appear internal to the stigmata. In the young nymph, though the differentiation of the first five segments is well marked, the indications of the remaining segments dorsally are somewhat indistinct. In the last nymphal stage, however, the whole series of eleven abdominal segments composing the abdomen can be clearly made out. (Plate XXIX, figs. 5, 6 and 7).

THE GENITAL ARMATURE IN THE FEMALE.

The female opening is posterior to the eighth sternite. In connection with it the sternites of the eighth and of the ninth segments are modified.

The eighth sternite, instead of being continuously chitinised, as are the preceding segments, is subdivided into four separate plates, two lateral and two median. (Plate XXIX, fig. 8).

The lateral portions of the sternite carry, as already noted, well marked spiracles. Externally these portions are closely applied to the tergal plate without the intervention of a pleural membrane and form part of the sharp abdominal margin. There seems no doubt that these plates are homologous with the eighth sternal connexivial piece to be seen in most Heteroptera, where they usually carry the eighth pair of spiracles.

The median portions of the sternite have been termed by Rothschild( ) the admedian plates. These are entirely separated by a deep sulcus and soft membrane from the lateral pieces and from each other. Each plate is roughly quadrangular in shape, the posterior border, which is thin and somewhat rounded covering, in a resting condition, the lateral portions of the succeeding segment. From the internal border of the admedian plates spring a pair of processes shaped something like two quarters of an orange, the ventral surfaces, corresponding to the rind, being rather thickly chitinised and hairy. These processes lie almost directly over the opening of the common
oviduct and in dissections they often come away attached to this. The homology of the admedian plates and their processes is fairly simple. Throughout most of the Heteroptera a somewhat similar condition is seen, enabling their identity to be determined respectively as the halves of the eighth sternite and the processes commonly springing from these (*anterior gonapophyses, valvulae inferiores* of authors). As will be seen later, this view is confirmed by a study of the development. In *Cimex*, however, these processes are much reduced from their ancestral condition and lack characters which are to be found in those bugs where they are more developed and help to form an ovipositor.

The ninth segment completes the apex of the abdomen. In shape it is more or less of a triangular pyramid. Forming one side of the pyramid and completing the whole dorsal surface is the tergal chitinisation. The other two sides of the pyramid are ventral, and are formed respectively by the two separated portions of what for the time we may take to be the ninth sternite. These lateral sternal pieces are in most of their outer extent smooth and hairless, but their inner edges are raised to form conspicuous blunt hairy elevations. (Plate XXIX, fig. 8). Between these elevations lies a deep groove in which, at the extreme apex of the body, lies the minute tenth segment and the *cauda*. Anteriorly the groove is continuous with a hollow normally covered in and protected by the anterior gonapophyses, into which the oviduct opens.

Lying along the inner and anterior edges of the lateral sterna pieces of the ninth segment are the two peculiar lobes described by Rothschild. Each springs from a broad base and terminates in a narrow pointed extremity directed backwards and lying in the median genital groove. There seems little doubt that these are processes (*gonapophyses*) of the ninth segment or the *valvulae intermediae* of authors. The most striking peculiarity of these lobes is a long narrow line of chitinisation along their inner margin, which in a cleared preparation appears, as remarked by Rothschild, to be a long narrow free rod.

Chitinised strips are present on the gonapophyseal processes of many Heteroptera, both the anterior and posterior pair, and in less retrograde types form a groove and socket mechanism for linking these processes together. The strip in *Cimex* is therefore apparently a relic from an ovipositor bearing ancestor.
In *C. lectularius* these strips, it will be noted from the figure given, arise at the base close to, but not in direct continuation with, the lateral ninth sternal pieces. The explanation of this appears to be that normally in the Heteroptera the gonapophyseal chitinisation which they represent is a forward continuation of the outer angles of the median portion of the ninth sternite. The sternite in such cases is often divided into two lateral halves, widely separated, the central parts between these are depressed and, whilst wholly membranous in some forms, still exist as a chitinised plate or plates in others. In *C. lectularius* there is no central chitinisation and we may suppose that by disappearance of the median portion of the sternite the chitinisations of the gonapophyses have been left in a floating condition.

The third component part of the typical ovipositor *i.e.* the gonapods, styles or valvulae superiores of authors are unrepresented in *Cimex*.

**The Genital Armature in the Male.**

The eighth segment of the male in general characters resembles the preceding segments and shows no sub-division of the ventral surface into separate plates. The only modification in connection with the sexual function is a slight asymmetry and the presence of a depression on the left side at the apex, where the groove traversing the ninth segment ends and the tip of the so-called penis approaches it.

The ninth segment forms a somewhat flattened cap-like termination to the body. There is no separation into tergite and sternite, the dorsal and ventral surfaces being connected by continuous chitinisation so that the whole segment forms an almost completely closed box. The line of apparent junction of tergite and sternite is, however, depressed and forms a broad groove. This groove commences about midway on the right lateral border of the segment and, passing transversely around the apex of the body, continues along the whole length of the left side of the segment to terminate in the depression already referred to on the eighth segment. (Plate XXIX, fig. 2.)

Emerging from an invaginated hollow in the floor of this groove a little to the right of the middle line of the body, is the so-called penis. This, at rest, is directed along the groove to the left passing under the tenth segment and cauda. Opening into the groove beneath the small tenth segment is, however, a second chitinous invagination in which lies
a structure so far not referred to by any author; this is the true intromittent organ or Phallosome.*

The so-called penis is a claw-like structure formed of a continuous piece of chitin; it has been sufficiently described by Hay Murray ands others. The most important feature is that it does not form a true canal, but is merely deeply grooved for a portion of its convex or posterior border. The phallosome is a complicated organ resembling in its structure the phallosome of other insects. In order to understand the nature of both these structures in Cimex, it is necessary to say a few words regarding the normal condition of the male parts in the order Heteroptera to which the bed-bug belongs.

In the Heteroptera, in accordance with the general plan of the male armature of insects, the ninth segment is specially modified. The modification most characteristic of the order is a tendency, often very pronounced, for the fusion of the tergite and sternite of this segment and the crumpling up of the posterior lip of the latter so that the whole segment forms a cup-shaped box. This chitinous box encloses a deep hollow, often opening externally only by a comparatively restricted opening, the terminal chamber of Sharp, or as we prefer to call it the genital cavity. Springing from the base of the genital cavity is a comparatively large non-segmental phallosome. On either side of the phallosome projects a structure composed of a single chitinous piece and usually more or less antler shaped. These antler-like structures arise from the inner aspect of the ninth sternite. They are termed by Sharp lateral appendages. In the Cryptocerata and some other forms they are seen as appendages arising from the edge of the sternite in the neighbourhood of the junction of the sternite with the tergite and lie externally. In some form or another, with a few exceptions, they are present throughout the Heteroptera and higher Homoptera. We have provisionally retained for them the name lateral appendages. The so-called penis of Cimex is one of the two lateral appendages normally

* No satisfactory term to indicate the complicated non-segmental intromittent organ as a whole appears to exist. Sharp(7) dealing with the male organs in the Pentatomidae, speaks of the aedoeagus, but apparently does not include in this term the theca, an essential and important part of the non-segmental mass. Edwards(8) uses aedoeagus for the parts in the mosquito, but the same term is used in a restricted sense in the Lepidoptera. As it is often convenient to signify the whole non-segmental mass without implying homologies other than in the organ as a whole we have used the term phallosome, which is in keeping with the nomenclature now coming into general use and is devoid of ambiguity.
present in the order, the other appendage having disappeared. Owing to the distension of the parts in Cimex it is not possible by inspection to be quite certain which of the appendages has developed and which has disappeared, but a study of the development (vide next section) shows that it is the left appendage which has become the body hitherto called the penis.

The phallosome springs in the middle line from the membrane between the ninth and tenth sternites. It is usually a comparatively large organ and extremely complex in structure. Essentially it consists of (1) a basal fold very heavily chitinised and most often shaped remarkably like a stirrup; (2) the portion called by Sharp, the theca; (3) a median organ carrying the opening of the ejaculatory duct.

The massive stirrup-shaped piece is highly characteristic of the order and occurs in more or less recognisable form in practically all bugs. It may be regarded as a circumphallic chitinisation of the membrane of the base of the genital cavity, encircling the base of the phallosome and usually forming a bed for its reception. By it the whole organ is slung and it is to this body that the powerful muscles of the part are attached. For the time we may call it the basal fold.

The theca is also present throughout the order. An important part of the theca is the inversely reflexed fold of membrane lining its cavity. This is frequently eversible and may be furnished with most complicated saccular dilatations with chitinous thickenings and processes.

The third portion of the phallosome may probably be correctly homologised as the mesosome. Sharp refers to it along with certain lateral processes as the aedeagus. This is essentially a papilla carrying the opening of the ejaculatory duct, the outer layer being continuous below with the lining membrane of the theca. When the parts are fully extended the mesosome sometimes carries with it this lining membrane with all its accessory parts (if present) and there may thus be formed a large compound eversible sac.

In Cimex the phallosome has the appearance of this organ in bugs generally, though it is much reduced in relative size and complexity. It lies in a sac-like envelope, the membrane of which is continuous with the external chitin of the body, the cavity opening by a narrow neck, under the tenth segment. This sac appears to be the greatly reduced genital cavity or hollow of the ninth sternite, here reduced out of all resemblance
to its original condition. On the sides of this saccular envelope, closely embracing the phallosome, are two peculiar thick chitinous folds (Plate I, figs. 1 and 3), very conspicuous both in potash preparations and in sections. The appearance of these suggests the basal fold of other Heteroptera. Of these folds one lies to the right and somewhat dorsally and the other, which is somewhat larger, to the left and more ventrally. Partly enveloped by these folds is a thick basal portion of the phallosome springing from the base of the cavity and receiving the common ejaculatory duct. This we take to be the theca. Finally there is a soft terminal portion, capable of extension and covered in part with minute chitinous teeth; this we take to be the mesosome. In sections the ejaculatory duct after entering the base of the phallosome is seen to form a dilatation. This appears to be followed by a complicated folding within the thecal portion and then by a straighter portion lying in the papillary projection we have called the mesosome.

As ordinarily seen in dissections or in sections of the parts, the mesosome is a flaccid papilla, which at the neck of the phallosomic capsule is directed somewhat to the left and comes practically to lie upon (dorsal to) the basal portion of the appendage and just about reaches the commencement of the groove in this structure. In some cases it is found extending the whole length of the groove and can be pulled out from this. There can be little doubt that in the act of copulation, the appendage having been inserted into the cleft in the fifth sternite, the mesosome passes along the groove until it comes into relation with the so-called organ of 'Ribaga,' through which the spermatozoa enter the body of the female.

**Development of the Genital Structures in Cimex.**

*External indications of sex in the nymph.*

From the earliest stage in the nymph it is possible, in a suitable preparation, to distinguish the male from the female.

In the nymph the ventral surface of the abdomen is mainly unchitinised. There are, however, on the seventh, eighth, and ninth segments rather conspicuous median chitinous plates. These plates, especially that on the ninth segment, differ in shape and size in the two sexes. In addition, especially in the last instar, there are certain indications of the future genital structures of the adult,
In the female the plate on the eighth segment shews posteriorly in the middle line a notch, on either side of which is a differentiation of the chitin suggesting a fore indication of the anterior gonapophyses. The ninth plate abuts closely upon the base of the tenth segment. In the last instar this plate consists of two lateral triangular portions separated by a median area of smooth chitin. The lateral portions have the appearance of separated halves of the sternite, the central portion being interpolated between these. The central portion is more or less deficiently chitinised and there is usually to be made out on either side, close to the middle line, a circular or oval pale spot. (Plate XXXI, fig. 9.) These spots as will be seen are indications of the gonapophyses of the ninth segment.

In the male the eighth plate is unmarked by any notch. The ninth plate is also entire, but at its posterior border and separating this from the tenth segment is a chitinous complex of peculiar character. This is indicated even in early instars, but is most distinctly developed in the last instar where it has the appearance shown in Plate XXXI, fig. 8 (vide also Plate XXIX, fig. 5).

Both in the male and in the female, these chitinous appearances are, as will be seen, the cuticular expression of early developmental changes, the nature and significance of which will be seen later.

**Development of the parts in the female.**

A complete description of the development of the sexual organs in the female is reserved for a future communication by one of us. For the present it is only necessary to refer to such portions of the development as will throw light on the nature of the parts we have so far described in the adult and in the cuticle of the nymph.

Indications of the future parts are clearly seen at the commencement of the last instar, as shown in Plate XXXI, fig. 6. Beneath the eighth sternal plate of the nymphal cuticle is the epidermal layer which will form the eighth sternite of the adult. From the posterior edge of this epidermal forecast of the future eighth segment there projects backwards on either side of the middle line, a flattish projection. As development proceeds these projections gradually assume the appearance and character of the processes we have termed the anterior gonapophyses.

Posteriorly to the eighth sternite the epidermal layer that will form the ninth sternite becomes differentiated into a central depressed portion and two lateral raised areas. These latter lie under
Penis' of the Bed-Bug (Cimex Lectularius L.).

(and at the penultimate ecdysis are coterminous with) the divided chitinous sternal plate of the nymph. The lateral areas become eventually the inner raised and hairy portions of the plates in the adult which we have termed the lateral sternal pieces. The depressed central portion of the ninth sternite (if it is not more correctly considered as intersegmental membrane exposed by the drawing apart of the halves of the sternite) is sharply demarcated laterally by the abrupt and even overhung edges of the lateral sternal areas and is anteriorly continued deeply under the overhanging eighth sternite with its processes. Arising from this depressed portion, on either side of the middle line, are early seen two flat projections, the rudiments of the gonapophyses of the ninth segment. This is the stage shown by the cuticle of the last instar nymph, as will be seen by comparing fig. 6 with fig. 9 in Plate XXXI. At this time the structures lying posterior to the ninth segment are exposed but later the hinder edge of the eighth sternite with its appendages comes more and more to project over and roof them in, forming at the same time a large intersegmental cavity.

Following the penultimate ecdysis there appears between the posterior pair of gonapophyseal processes a median longitudinal groove, the rudiment of the invagination which will form the common oviduct. This groove appears first as a narrow furrow. As development proceeds the furrow becomes a shallow flat invaginated cavity, which burrows its way forwards whilst its opening at the same time widens out. Eventually, whilst the invaginated undermining portion reaches the oviducts, the opening has become so extended that it practically occupies the whole of the dorsal aspect of the original intersegmental cavity formed by the continued growth of the eighth sternite and its appendages. The edges of the furrow have now been carried so far laterally that they reach the position which in the adult is marked by the bases of the chitinous strips on the gonapophyses. These processes, which we have seen are at first situated near the middle line, are carried by the widening of the oviductal margins far to the side; it is this which causes them to appear in the adult as appendages from the lateral portions of the ninth sternite. We have already seen that they originate as projections from the median depressed area.

As regards the common oviduct it is evident that there is considerable complexity in the composition of this apparently simple structure as seen in the adult. There are represented three separate cavities: (a) the original cavity of the intersegmental space; (b) the cavity formed by the
growth of the oviductal furrow; (c) an upper pouch arising from (b) and forming a Y-shaped extension reaching to the oviducts. (Plate XXXI, figs. 7, 0°, 0' and 0, respectively.)

Development of the parts in the male.

Briefly the generative organs in the male are as follows. Passing backwards from the testes are the vasa deferentia. These are swollen towards their lower ends and act as receptacula seminales. Lying external to the lower ends of the vasa deferentia are the accessory glands with their ducts. At a level with the hinder end of the eighth segment the vasa deferentia along with the ducts of the accessory glands enter a muscular organ of conspicuous nature, the sacculus. Immediately posterior to the sacculus is the wide short ductus ejaculatorius. The arrangement of parts at the junction of the sacculus and ductus is peculiar, since from the former a kind of papilla or velum projects into the lumen of the latter. On the surface of this papilla the four ducts of the vasa and accessory glands, respectively, open simultaneously. The ductus ejaculatorius, which has an obvious chitinous lining and is of large lumen, enters, after a short course, the base of the phallosome and comes in relation with the parts we have already described.

At an early stage of development there are present at the posterior border of the ninth sternite two bilaterally arranged blunt epidermal outgrowths (primitive projections) which spring from the dorso-anterior wall of a shallow invagination behind the sternite (genital cavity). The outgrowths fully occupy this cavity and project beyond its rim (Plate XXXI, fig. 1). On the anterior wall of the genital cavity between the bases of the primitive projections is a small pocket-shaped invagination, the first rudiment of the ejaculatory invagination. This is the stage represented by the cuticular parts of the last nymphal instar. The cuticular parts of the last nymphal instar are shewn in Plate XXXI, fig. 8. It will be seen by a comparison of this figure with that giving the early epidermal condition (Plate XXXI, fig. 1) that the chitinous structure situated posteriorly to the ninth sternite in the nymph, at first rather puzzling, really consists of (a) the edge of the genital cavity; (b) the primitive epidermal processes; (c) the early indications of the ejaculatory invagination, all as they exist at the end of the penultimate instar. At the time of the penultimate ecdysis, however, there is considerable lateral expansion and some flattening of the parts as
will be seen from the figures which are drawn to the same scale. Already at this stage the chitin shews some differentiation of the inner portions of the primitive projections.

At a later stage there appears a fissure which starts posteriorly and dorsally and finally divides off on each side an inner portion of the primitive projection from an outer. Thus a fold is developed on either side of the rudimentary ejaculatory depression. These folds ultimately develop into the phallosome, the outer portions of the primitive projections becoming the appendages. The phallosomic folds at a later stage become a conspicuous median structure projecting from the ventral surface of the body anterior to the tenth segment. This structure is at first perfectly symmetrical, somewhat spout-shaped and with a deep groove along its whole length ventrally. This groove anteriorly is continued into a comparatively large globular sac, the now much enlarged ejaculatory invagination. The epidermal projections forming the rudiments of the future appendages lie symmetrically on either side of the rudiment of the phallosome and are at first similar in size and shape (Plate XXX, fig. 4, and Plate XXXI, fig. 3).

As development proceeds and the phallosomic rudiment increases in size it comes to lie somewhat asymmetrically. The left appendage also becomes larger than the right and exhibits at its outer angle a sharpish projection, the first indication of the blade portion of the penis (Plate XXXI, figs. 4 and 5). A fold is now present on either side of the phallosome lying between this and the rudiments of the appendages. This becomes the structure we have termed the basal fold. Later stages of development show the originally grooved phallosomic rudiment as a tubular organ. It appeared to us that this transformation from a grooved to a tubular structure takes place by the blending of the lips of the groove, and in some sections there appears to remain after closure of most of the groove into a canal, a still patent opening at the base where a depression is shown in Plate XXXI, fig. 5. This, however, requires confirmation. The phallosome has now very much the characters of this structure in the adult. It is of considerable relative size, however, and in this respect resembles the phallosome as usually seen in the Heteroptera.

By the time the phallosome has reached this stage, the appendages have become markedly different in character. The right appendage is of its original size and shape; but the left appendage has greatly increased in size and has extended beyond the border of the genital cavity as a
PLATE XXX.

Fig. 1. Dissection showing, above and in the centre, the vasa deferentia and their entrance into the sacculus. Laterally are the dilatations and glandular portions of the accessory glands. Below on the right is the tenth segment with the extruded cauda. On the left is the appendage. Between the tenth segment and the appendage is the minute phallosome with its chitinous basal folds. Between the phallosome and the sacculus can be seen the transparent ductus ejaculatorius. The four ducts in the sacculus can be made out.

2. Horizontal section through caudal extremity of male nymph near penultimate ecdysis. This shows in the centre the primitive ejaculatory invagination and above this, on either side, the rudiments of the accessory glands, etc. Below on the right one of the two primitive projections is cut through the section being somewhat oblique has missed that of the opposite side.

3. As in fig. 2, but shortly after penultimate ecdysis. In the centre is seen the ejaculatory invagination and on either side of this is the fold which will eventually form the phallosome.

4. Transverse section of a later stage showing formation of the scoop-like rudiment of the phallosome and on either side in transverse section the appendages. The cavity in which they lie is the genital cavity.

5. Section at about the same stage, but cut horizontally to show globular ejaculatory invagination and plaque. The phallosome is cut longitudinally through its lateral lips. A section or two further on the opening to the ejaculatory invagination is reached.

6. Section through the U-shaped loops forming the rudiments of the vasa deferens and accessory glands. The ejaculatory invagination is just missed, but a few cells of its wall lie just below and between the bends of the loops. On the right the continuation of the inner arm of the loop into the delicate genital cord can be seen.
long blade-like organ, on which can be clearly seen the commencement of the formation of the groove characteristic of this structure in the adult. The extra-genital portion of the left appendage lies at this stage upon the outer surface of the epidermal layer of the ninth segment of the future adult in a position external to the chitinous plate of the nymph. Its presence causes a sulcus to be formed in this situation, which eventually becomes the groove in which the so-called penis lies. This groove has not therefore, in its early stages at least, any very direct relation to the line of junction of tergite and sternite. Where the appendage leaves the genital cavity its presence causes the edge of this cavity to be somewhat flattened out, and at this point (the left angle of the genital cavity rim) very early chitinisation occurs so that in the newly emerged adult there is seen in this situation a dark V-shaped chitinisation even when the remaining parts are mostly still soft and uncoloured.

The further development of the ejaculatory invagination and of the rudiments of the vasa deferentia and accessory glands which become associated with it may be briefly mentioned, especially as this is of considerable interest and demonstrates very clearly the origin of most of the male genital structures we have mentioned at the commencement of this section. At the stage when the primitive projections are still undifferentiated there are seen, one on either side of the quite small ejaculatory invagination, the early common rudiments of the vasa deferentia and accessory glands. At this stage these are hollow, almost spherical dilatations at the terminations of a tube on either side passing backwards from the developing testes. These bodies are quite independent of the ejaculatory invagination of the epidermal layer and appear not to be epidermal in origin. At a later stage the original more or less globular vesicles become elongated and bent upon themselves forming on either side a U-shaped tube with the convexity of the loop directed posteriorly. These tubes lie in the eighth segment. The inner limb of each is continued as the delicate tubule we have already referred to (genital cord), and is evidently the rudiment of the vas deferens. The outer limb forms a thick walled sac ending blindly. This sac early begins to take on the form and characters of the accessory gland complex. At this stage the vas deferens and accessory gland of each side form parts of a single tube, their cavities being continuous through the bend of the loop.
The rudiments lie at a little distance from the ejaculatory invagination, which, by the time the vas deferens and accessory gland portions have begun to be differentiated, has become a large and conspicuous globular sac on the floor of which has been developed a peculiar thickened cellular plaque (Plate XXX, fig. 5). The lower ends of the U-shaped loops as development proceeds grow towards and finally enter this plaque. We have not actually seen the loops reach the surface of the plaque, but this no doubt occurs with the eventual opening of their lumen and the formation of four separate ducts. Muscular elements appear around the loops as they grow towards the plaque and in this way is formed the muscular sacculus. The short wide ejaculatory duct is the original globular epidermal invagination; the velum is the cellular plaque on the floor of this cavity.

We have already stated that the rudiments of the vasa deferentia and accessory glands have no connection with the epidermal invagination forming the ejaculatory duct, and that they arise in connection with the testes. That they are not of epidermal origin is further shown by the fact that in the adult a chitinous lining is demonstrable after treatment with caustic potash only so far as the end of the ductus ejaculatorius. The observation that in Cimex the ducts are formed entirely from the loops themselves without the aid of any special outgrowth is supported by the condition in the adult, there being in this case no common duct as in some insects.

As regards the development of the external genitalia of the male in Cimex our observations therefore show that the conclusions originally based upon comparative studies are correct and that the asymmetrical arrangement of the parts is a special adaptation of the ordinary condition in the Heteroptera. It is of interest to observe in this case the appearance during development, of the right appendage, a structure which, if present at all in the adult, is not easily demonstrated.

The earlier stages in the development of the male organs appears to be in close accord with the findings of Zander(9), (10) in the Hymenoptera and Trichoptera; and though the bug, on account of the small size of the organs, is not perhaps the most suitable form in which to study these early changes, yet our observations distinctly suggest that the lateral appendages in the Heteroptera are homologous with the valvae of Zander.
PLATE XXXI.

Fig. 1. Construction from sections of epidermal layer of male nymph at end of penultimate instar. Showing genital cavity, primitive projections, early rudiment of ejaculatory invagination and position of rudiments of the vasa deferentia and accessory glands. The globular body below is the epidermal tenth segment. (Ventral view.)

" 2. Construction from sections giving posterior view of a later stage. The parts have expanded following ecdysis and a deep groove has commenced to separate off the median portions of primitive projections as the phallosome. Above is the tenth segment, in the centre the ejaculatory invagination, or rather a groove which finally is continued into this. On either side of the invagination are the halves of the phallosome in process of formation and outside these the portions of the primitive projections which will become the appendages. Below is the genital cavity and the ninth sternite.

" 3. Construction from sections of epidermal layer of male nymph. Last instar. Showing further formation of phallosome and of symmetrical lateral appendages, also the position of the ejaculatory invagination and the rudiments of the vasa deferentia and accessory glands. This is the stage shown in the photograph. (Plate II, fig. 4.)

" 4. Construction from sections of epidermal layer of male nymph. Last instar. Later stage than shown in fig. 3. Showing commencing asymmetrical development, enlargement of left appendage, the growth of the ejaculatory invagination and of the rudiments of the vasa deferentia and accessory glands.

" 5. Construction from sections of epidermal layer of male nymph approaching end of last instar. The position of the loops approaching the ejaculatory invagination is indicated.

" 6. Construction from sections of epidermal layer of female nymph at beginning of last instar. Showing the eighth sternite with processes, the lateral sternal pieces and median depression. In the centre of the figure are the posterior gonapophyses and between them the furrow which eventually becomes the oviduct. Below is the tenth segment with a ridge separating this from the median depressed area.

" 7. Construction, from sections, of epidermal layer of female nymph approaching final ecdysis.

0' intersegmental cavity.
0' lower pouch of oviductal invagination.
0 upper pouch of ditto.

" 8. Cuticular appearances in the male nymph at last instar. The shaded plate lying above the chitinous complex is the ninth sternal plate of the nymph.

" 9. Cuticular appearances of female nymph at last instar. The shaded triangular plates are the divided halves of the ninth sternal plate of the nymph.
CONCLUSIONS.

1. The abdominal segments in the bed-bug have not so far been correctly noted. The condition in the nymph and other considerations, including the presence of a relic of the first tergite, the position of the last pair of spiracles and the relations of the segments to the genital passages, etc., show that the large apparent first segment is the true second abdominal segment.

2. The female opening is behind the eighth sternite. The processes arising from this sternite are the gonapophyses of the eighth segment or valvulae inferiores of some authors. The peculiar lobes described by Rothschild are the gonapophyses of the ninth segment, or valvulae intermédiae of some authors. The valvulae supérieures (gonapods, styloids) though seen in some Heteroptera are absent in Cimex. The prominences on either side of the genital furrow are divided halves of the ninth sternite corresponding to the lateral chitinous portions of the sternal plate in the last instar nymph.

3. The male opening is behind the ninth sternite and between this and the tenth segment. The hollow in the ninth sternite (genital cavity) in which the male organs usually lie in the Heteroptera is reduced in Cimex to a tiny oval pouch enclosing the phallosome. The phallosome is a structure that has so far been overlooked. It lies at the root of the so-called penis and has all the chief characters of the phallosome in the order generally, though greatly reduced in size and complexity. The grooved false penis is one of the pair of processes (lateral appendages of Sharp) present with a few exceptions throughout the whole of the Heteroptera and higher Homoptera. The groove in the appendage does not function as a duct, but as a sheath for the mesosomal portion of the phallosome which can pass along it.

4. The sex can be distinguished exteriorly in the nymph, even when first hatched from the egg. The female parts are clearly indicated in the last instar of the female nymph and an asymmetrical chitinous complex is seen in the male nymph in the last instar behind the ninth sternal chitinisation, which represents the stage of development reached by the epidermal layer at the end of the penultimate nymphal instar.

5. The so-called penis is formed by the development of one of the bilaterally arranged processes which arise from division of the primitive projections. The outgrowth becoming the penis (the left appendage) continues to develop whilst that on the right eventually disappears.
The phallosome is formed from folds on either side of the early rudiment of the ejaculatory invagination and appears to be, as described by Zander for the Hymenoptera and Trichoptera, formed from the inner portions of the two primitive projections springing from the primitive genital cavity. It is at first an asymmetrical organ with a ventral groove. The stages by which it seemingly becomes a tubular organ require further study.

The vasa deferentia and accessory glands develop from a pair of small globular rudiments, one on either side, which arise independently of the ejaculatory invagination of the epidermal layer and are terminal dilatations of tubes which pass down, one on either side from the testes. The rudiments elongate and form on either side a U-shaped tube. The inner limb of each loop becomes the vas deferens, the outer the accessory gland complex. The bights of the loops grow downwards to meet the epidermal ejaculatory invagination, so that when eventually these open into this, two ducts and two separate organs, the vas deferens and the accessory gland, are formed on each side. The muscular fibres of the sacculus form round the bights of the loops as they grow to meet the epidermal invagination.

The ejaculatory duct is formed from an invagination of the epidermal layer which forms a spherical cavity with a button-like cellular plaque on its antero-ventral wall. The spherical cavity becomes the ejaculatory duct of the adult. The plaque becomes the velum, which projects into the ejaculatory duct and carries the openings of the four ducts derived from the bights of the loops described above.

6. The lateral appendages of the Heteroptera, so far as can be judged from our observations on Cimex, develop in the same manner as the valvae of Zander.

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