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On the Anatomy of Conus tulipa, Linn., and Conus textile, Linn.

By

H. O. N. Shaw, B.Sc., F.Z.S.

With Plates 1 to 6, and 12 Text-figures.

Since 1895, few workers on the anatomy of mollusca have devoted their attention to the genus Conus. In that year Dr. Bergh (**3**) published an extensive memoir on a large number of species in this genus, and his work may be considered as the most complete, and embracing the greatest number of species examined, though his description of each species was not exhaustive. Troschel (**20**) devoted most of his attention to the radulæ of the different genera and species of which his excellent work is composed, and although he gives a certain number of figures with descriptions of various anatomical points, these latter are for the most part of rather a crude and diagrammatic kind.

While malacologists have done a certain amount towards working out and elucidating the anatomy of various members of this genus, the conchologists, as is generally the case, have produced many excellent monographs, and such names as Reeve, Sowerby, Tryon, Weinkauff and others will always be remembered for the general excellence of their figures and descriptions of the numerous species which are contained in this genus. Various writers have essayed different forms of classification, but for the most part on purely conchological grounds, and when more is known about the inhabitants of these shells, and their different points of resemblance to one

another, whether they vary as much or more than their shells, and if the conchological grouping also holds good from an anatomical point of view, we shall be on the high road to founding a solid and logical form of classification.

The two species with which I shall deal in this paper are Conus tulipa, Linn., and Conus textile, Linn. The specimens of both species were females. The Conidæ belong to the order Prosobranchiata of the class Gastropoda, are diœcious, and according to the most widely accepted form of classification, Conus tulipa is included in that sub-genus, or, as some would have it, section, of the genus Conus, called Rollus by Montfort, while Conus textile forms the type of the sub-genus or section Cylinder, of the same author.

Rollus was first described by Montfort in 1810 (**15**, p. 395), with Conus geographus as the type, and the sub-genus was characterised as follows: "Shell light, sub-cylindrical, spire short but pointed at the summit, whorls slightly coronated, aperture effuse, emarginate in front, columella smooth, outer lip with a wide but not deep notch at the suture."

This group corresponds to Nubecula of Klein, 1753; but owing to his being pre-Linnean and non-binomial, this designation cannot be accepted. Utriculus, of Schumacher, 1817, and Tuliparia, Swainson, 1840, are synonymous.

Cylinder was described on p. 391 of the same work.

"Shell sub-conic, smooth, spire elevated, pointed, whorls numerous, body whorl ventricose, notched at the suture, aperture effuse at the fore part."

Textilia, Swainson, 1840, is a synonym.

One of the difficulties to be contended with in working on the anatomy of tropical molluscs is the trouble in getting sufficient material, and in a good state of preservation. The two specimens here described are from the British Museum (Natural History), and had been there in spirit for a good many years, and I had started working on these before some recently collected specimens came to hand.

My thanks are due to J. Hornell, Esq., Pearl and Chank Fisheries, Tuticorin, for sending me specimens which I have

not yet worked out, also to E. A. Smith, Esq., I.S.O., of the Natural History Museum, for kindly allowing me to use some of the Museum specimens. My especial thanks are due to Prof. G. C. Bourne, Merton College, Oxford, for his kindly help and advice on many points connected with this paper, for which I am much indebted.

With the exception of Conus mediterraneus, which is found all round the Mediterranean and west coast of Spain, no other species inhabit European waters, though this large genus is plentifully represented in the tropical seas, and round the coasts of Australia, Japan and America. It generally lives in fairly shallow water, and is found on reefs and in pools under stones, corals, etc., and is supposed to be able to inflict a poisonous bite. I have made inquires from those who have collected and handled them alive. They tell me that they have never had this experience.[1] The animal is extremely timid; on the slightest touch it withdraws itself into its shell, and will remain in this retracted condition for a considerable time.

The operculum is generally elongate or unguiform, and so small that it is useless for closing the mouth of the shell when the animal has withdrawn itself inside.

The shells are covered with yellowish periostracum, which in some species is only a thin, smooth, transparent, but tough coating. In others, as in C. tulipa, the periostracum is exceedingly thick and of a dark-brown colour. It is rough, furrowed longitudinally, and of a leather-like texture, and has tufts or outgrowths disposed in even rows along its surface. When dry, this thick periostracum becomes very brittle and peels off the shell.

No doubt this shell covering, which, as I have said, in some species is tufted and of a leathery formation, is both a protection to the shell and also a form of protective coloration for the animal. C. tulipa has one of the lightest and

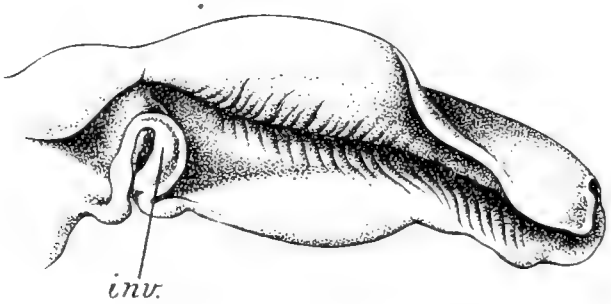
[1] While this paper was in the press, a note appeared in 'The Nautilus,' vol xxvii, pt. 10, pp. 117–120, 1914, "Poisoning by the Bite of Conus geographus."

thinnest of shells, and at the same time one of the thickest and most tufted periostraca. As the animal grows, and outer whorls are formed surrounding the inner ones, the walls of the internal convolutions of the shell are so reduced in thickness as to be hardly as thick as a sheet of paper, and semi-transparent.

Description of Conus Textile.

Maximum length of shell $2\frac{1}{4}$ in., maximum breath $1\frac{1}{8}$ in. Operculum long and narrow, and slightly reflected outwards

Text-fig. 1.



Conus textile. The siphon, with one side reflected to show ridges and furrows, and invagination (*inv.*).

at its anterior end. The foot, which is large and muscular, when withdrawn completely fills the opening of the shell. Along the internal edge of the foot (next the columella) is a row or ridge of large tubercles or nodules of a reddish-brown colour. The opening of the branchial cavity extends along the right-hand side of the animal, and the cavity is enclosed on its dorsal surface by a thin covering of membrane which along its outer or free edge is considerably thickened and muscular for a space of about $\frac{1}{8}$ in. This covering is attached at its posterior end to the body-wall, and has no attachment forward till it reaches the siphon, round which it is fused. It extends forward as a kind of flap beyond its point of attachment round the siphon, and overhangs the latter about $\frac{1}{8}$ in.

The siphon (Text-fig. 1) is large, and has thick muscular

walls which are folded over and form a funnel, open in front and beneath. The funnel is in the shape of a triangle, with its base above, and apex ventral, the apex being formed by the two free edges of the siphon, which run parallel and slightly to the right of the foot. The interior and dorsal surface of the siphon has a number of deep grooves and ridges running across and at right angles to its longitudinal axis, and also extending down its sides. They start near the anterior and free end, and continue about half its length backwards. At its junction with the body, the ventral edge of the left-hand fold of the siphon is sharply reflected upwards and at right angles into the funnel, forming a V-shaped invagination or elbow (*inv.*), which closes up more than half the passage, and fits into a depression in the foot.

The eyes are situated on the tentacles, about the middle, and on the outer side, this position being due to the tubercles on which they were borne having fused with the tentacles. The anterior end of the foot has a glandular groove running at right angles and across it, with a reflex fold above the groove. About a quarter of an inch behind this groove, and on the ventral surface, and equi-distant from both sides of the foot, is the orifice of the pedal gland, or pedal sinus.

When, as in Pl. 1, fig. 1, the branchial cavity (*br.c.*) is opened from above, with the siphon and buccal openings turned away from the observer, by cutting through the mantle (*ml.*) longitudinally and about three quarters of an inch to the left of the branchial opening, the ctenidium (*ct.*) is seen starting just behind the opening of the siphon (*si.*) into the branchial cavity, running backwards and across the latter in a curve to its dorsal attachment to the mantle, with the "fausse-branchie" on the right, and inside the ctenidium. The "fausse-branchie," or osphradium, is trifid, the result of specialisation from the more archaic form, where the osphradium is merely a filiform epithelial ridge. This specialisation is common to most of the Gastropod Toxoglossa.

Below the large branchial cavity, and separating it from the body-cavity, is a thin membrane or covering, which

gradually becomes thicker on each side, where it is attached to the body-wall. Immediately beneath this membrane, and at the posterior end of the body-cavity, lies a large yellowish mass, the poison gland (*p. g.*). Anterior to this latter are the numerous coils of the duct (*p. g. d.*) leading from the gland into the œsophagus (*œ.*); and anterior again to these and over-lapping them is the radula-sac (*r. s.*). The œsophagus is continued past the radula-sac and so to the mouth (*m. h.*).

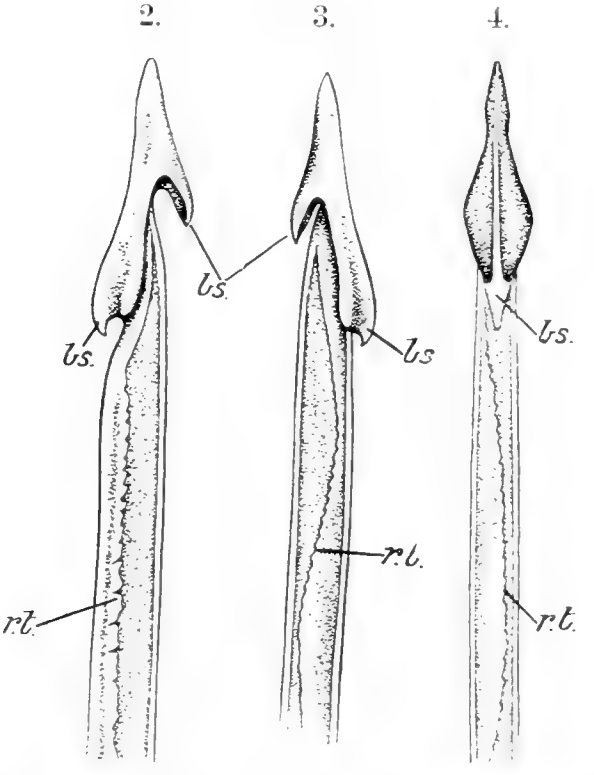
The duct of the poison-gland enters the œsophagus close behind the opening of the radula-sac, and passes backwards and to the right of the body-cavity, where it is twisted into a large coil. After leaving this coil it runs forwards and downwards parallel to the œsophagus, both it and the latter organ being surrounded by the nerve collars. Having passed backwards through the nerve collar, the duct is composed of very numerous and tightly twisted coils and knots, which are situated in front of and under the poison gland. The duct then straightens, passes to the right across the body-cavity, and enters the gland at its right-hand extremity. For about half an inch before the opening into the gland the duct is much constricted (Pl. 1, fig. 2). The whole of the poison duct is firmly bound together by connective tissue, which also surrounds the nerve collars and nerves given off from it, and binds all these organs tightly to the œsophagus. The poison gland (Pl. 1, fig. 2, *p. g.*) is a long narrow mass, nearly circular in section, pointed at each end, and slightly curved. It lies directly across the body-cavity with its two curved ends pointing downwards.

It is impossible to completely straighten out the numerous kinks and twists of the poison duct (Pl. 1, fig. 2) and so measure its length accurately, 270 mm. being as near as possible correct. The length of gland is 17 mm. and maximum width 5·5 mm. The length of the duct is about five times the total length of the animal, and the duct and gland together occupy the greater part of the body-cavity.

The salivary gland (Pl. 1, figs. 1, 2, *s. g.*) is a small yellowish, rather oval-shaped body, situated to the left of the body-

cavity, and in front of the poison ducts. It is provided with a pair of extremely fine thread-like ducts (*s. d.*), which open into the right side of the gland, one above and the other below. The lower one passes under the œsophagus, and the other above, and both enter the base of the V of the radula-

TEXT-FIGS.



The tops of teeth of Conus textile. *bs.* Barbs. *r.t.* Row of denticulations.

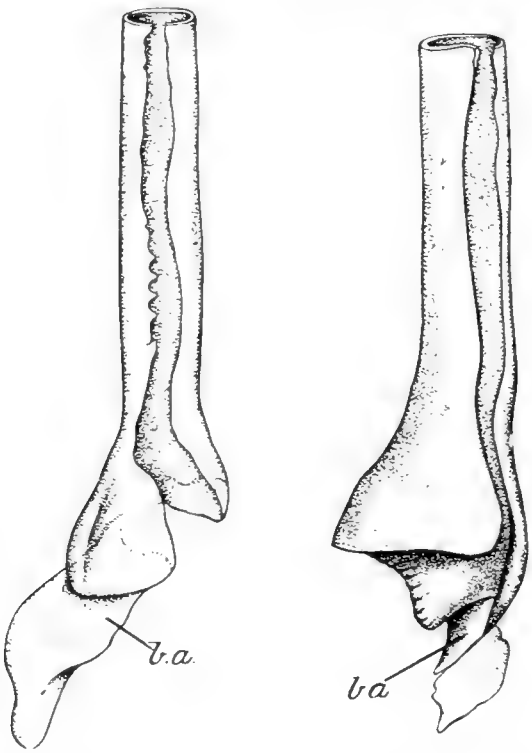
sac (*r. s.*), one above the other, as in the case of their gland-openings. These two ducts lie at right angles to the body axis.

The radula-sac is in the shape of a V; the right arm is elongated and about twice the length of the left, and ends in a cul-de-sac; the base is produced downwards and forms a knob, the left arm being the opening into the œsophagus. The long or right arm lies across the body-cavity and over the œsophagus, and behind the nerve collars. It is joined on the right by the left arm, which runs forward and downwards

where it joins the œsophagus. The long arm is slightly curved, and its length is 20 mm. The radula-sac is thick-walled and muscular.

The radular teeth (Pl. 1, fig. 4) are long, thin-walled tubes composed of chitin, and are very brittle, transparent, generally

TEXT-FIGS. 5 and 6.

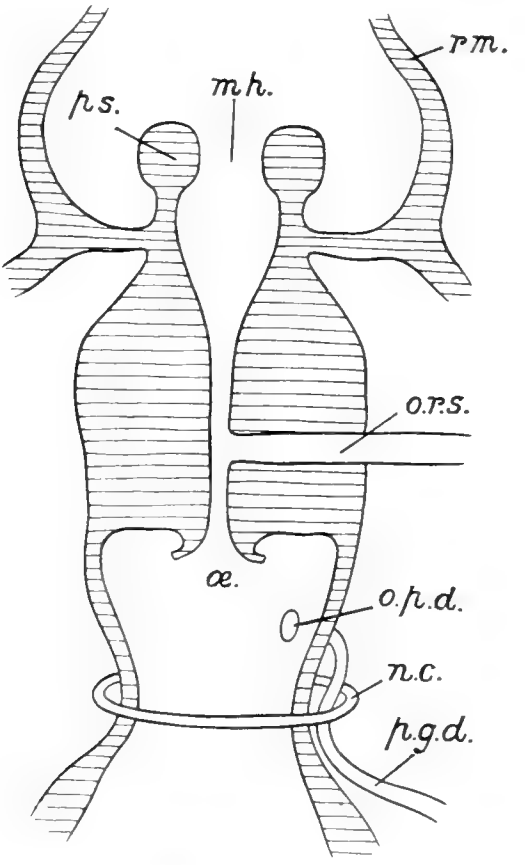


A view of the base of two teeth of Conus textile. *b.a.* Showing basal attachments.

light yellow, but sometimes quite dark. When placed in xylol to clear them for mounting purposes, they immediately underwent various contortions, tying themselves in knots and twisting into circles, etc. They are provided at their anterior ends with a flat lance-like point (Text-figs. 2, 3, 4), and on each side have a large and hooked barb (*bs.*), pointing backwards. The teeth are slightly curved and the barbs are placed one on each side of the curve, the one on the outside of the curve being about equi-distant between the one on the

inside and the lance-point. The average total length is 8–10 mm., and the diameter at the base, above the attachment, ·2 mm. The attachment varies a good deal in shape, but

TEXT-FIG. 7.



Conus textile. The base of rostrum, proboscis, mouth and œsophagus. *mh.* Mouth, *rm.* rostrum, *ps.* proboscis (with thick reflected lips), *o. r. s.* opening of radula-sac into œsophagus, *œ.*, *o. p. d.* opening of poison duct (*p. g. d.*) into œsophagus, *n. c.* nerve collars (only one represented). The shortness of the proboscis, and thick wall through which the radula-sac opens, will be noticed. (Diagrammatic.)

is generally a swelling of the base (Text-figs. 5, 6, *b. a.*), and thickened in parts. The points and barbs hardly vary at all. The lance-points in the radulæ I have seen, are not serrated as shown by Bergh (**3**), tab. vi, figs. 143, 144, 145. The radular teeth are placed in two groups in the radula-sac with their bases in the angle formed by the union of the two arms.

They are so arranged that one group have their barbs in the cul-de-sac of the long arm, while the points and barbs of the other protrude beyond the radula-sac opening, and into the œsophagus.

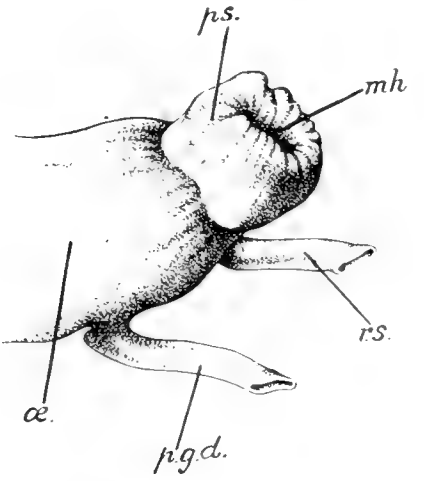
The teeth are fixed or anchored by means of an attachment or ligament which is firmly connected to their bases, and also to the wall of the radula-sac, but is of sufficient length to allow each tooth to move backwards and forwards. The teeth are placed in rows one behind the other for a short way up each side of the radula-sac but are all of nearly equal length, and are surrounded and connected with one another by a stout layer of connective tissue.

The rostrum (Text-fig. 7, and Pl. 1, fig. 1, *rm.*) is non-retractile, and forms what Gray (**10**) calls a veil ; it is thick-walled, muscular, and longitudinally ridged. The open or free end of the veil is provided with a single row of tentacles (*ts.*), by means of which the animal can attach itself to its prey. The posterior and internal wall of the veil is reflected forward, and, with the mouth (*mh.*), forms a retractile proboscis (Text-fig. 7, and Pl. 1, fig. 1, *ps.*). The edges of the mouth or lips are thick, ridged and corrugated and reflected internally (Text-figs. 7, 8). The retractile proboscis is in this species short, and after passing through the posterior wall of the veil, opens out into the œsophagus. The opening of the mouth is in the shape of a funnel (Text-fig. 7), the outer edge being formed by the thick lips, and as the cavity of the funnel narrows down internally, so the walls get thicker.

The œsophagus commences behind the posterior wall of the veil, where it immediately swells out, and as it increases in size, the funnel opening inside becomes smaller and the walls exceedingly thick and muscular, until at the base of the funnel only a very small passage to the mouth is left. These thick œsophageal walls end suddenly as though cut across at right angles just in front of where the œsophagus is slightly constricted owing to its being surrounded by the nerve-collars. The walls now become quite thin, with the result that the much constricted opening at the end of the funnel suddenly

opens out into a large cavity. The edge of this thick wall forms a ring or lip round the opening which hangs down into the cavity. The outside edge of the lip is reflected backwards, so forming a minute funnel. The radula-sac opens on the right (*o. r. s.*) in the thick-walled part, and the poison duct (*o. p. d.*) opens immediately behind it on the same side into the thin-walled cavity. Behind this last the œsophagus

TEXT-FIG. 8.



Conus textile. The proboscis and mouth showing thick reflected lips. The rostrum has been removed. *p. s.* Proboscis, *mh.* mouth, *r. s.* radula-sac, *p. g. d.* poison gland duct, *œ.* œsophagus (see diagrammatic, fig. 7).

is slightly constricted and the poison duct runs parallel and close to it, and both are surrounded by the nerve collars (*n. c.*). The œsophagus now expands into a large and thick-walled dilatation which corresponds to the stomach; it is nearly circular and passes backwards and dorsad of the liver (Pl. 1, fig. 1, *l. l.*). Here it narrows down, descends over the edge of the liver and turns with a sharp bend to the left, and so to the anus, situated at the posterior and right end of the recto-genital mass (*r. g. m.*). Where the stomach passes over the liver there is a large depression on the dorsal surface of the former, in which lies the left side of the poison gland.

The whole of the stomach, œsophagus and duct are pleated or corrugated, with the plications running parallel to their length, and the thick and deep ridges of the mouth are simply a continuation of these ridges in the œsophagus (Pl. 2, fig. 15).

Histology.

On account of the age and state of its preservation this specimen was not satisfactory for histological purposes. The sections were very hard to cut on account of the preparation being macerated, and were very difficult to stain. Various stains were tried, and those which gave the best results were Ehrlich's hæmatoxylin and eosin, but even with this method the nuclei were very badly defined, and in some cases were unstainable.

Poison Gland, or Glande de Leiblein (Pl. 2, fig. 9).

Down the centre of this gland runs a nearly circular canal (*ca.*). The poison duct opens into this canal at the right-hand extremity of the gland. The duct joins the gland in front and at right angles, so that the opening into the canal (Pl. 2, fig. 14, *o. p. d.*) is in reality to the side of one of its extremities and not at the end. A considerable portion of the coils of duct lies under and so ventral to the gland.

The canal in the gland is lined internally with non-ciliated epithelium (Pl. 2, fig. 9, *l. ep.*); this is surrounded by a thin ring of circular muscular-fibres (*c. m. s.*), external to which is a layer of longitudinal muscle-fibres and connective tissue (*l. m. s.*). This is again surrounded by another layer of circular muscular tissue (*c. m. s.*), of about a third the thickness of the preceding one. The last or external layer (*l. m. s.*) is about three times as thick as the other four layers taken together, and is composed of longitudinal muscular fibres and connective tissue. In the two layers of longitudinal muscles, i. e. the external and the middle layers, the muscle-fibres, though all running longitudinally and parallel to the length of

the gland, are disposed in bands of different depth and width so that they converge and overlap one another (Pl. 2, fig. 14).

These thick muscular layers forming the walls of the poison gland serve to eject the secretion along the duct to its opening in the œsophagus whenever its use is required.

Poison Duct (Pl. 2, fig. 10).

The wall of this duct is built up of two layers: an external layer (*l. l. m.*) of longitudinal muscular fibres and connective tissue, and an internal layer (*l. c. m.*) of connective tissue and circular muscular fibres. The internal layer is about half the thickness of the external one. The edges of these layers are well defined and do not merge into one another. This internal layer is again lined with a thick epithelium (*ep. cs.*), composed of very elongated club-shaped cells having basal nuclei which vary slightly in their position. The cells are filled with a fine granular substance. Interspersed among the cells of the epithelium are a number of granular vesicles. The club cells, which are extremely attenuated, are as much as ·2 m. in length. The average external diameter of the duct is ·65 m. An invagination of epithelium (*inv. ca.*) hangs down into the free passage of the duct. This passage or channel (*ca.*) along the centre of the duct is of a very irregular shape, having deep grooves or arms running into the epithelium.

The numerous coils of the poison duct are firmly bound together by connective tissue.

Stomach and Œsophagus (Pl. 2, fig. 15).

The stomach is nearly circular in section and the wall is of considerable thickness. The exterior layer (*l. l. m.*) is composed of longitudinal intermingled with a few transverse muscle-fibres and connective tissue. Beneath this layer and running into it is a complex network of transverse and circular muscular fibres (*l. c. l. m.*), intermingled with connective tissue and a few longitudinal fibres. Between this last layer and the thin coat of epithelium (*l. ep.*) lining the internal

surface of the stomach and œsophagus is a thick layer (*l. l. m*) of, for the most part, connective tissue and longitudinal muscle-fibres. In this there are a certain number of circular and transverse fibres disposed about the whole wall of the œsophagus and stomach, forming a complex mass of muscular fibres and connective tissue. These run to a certain extent in layers, one outside the other, and in some places are more distinct than in others, but have no definite margins, and merge one into the other.

The epithelium is in a very macerated state, but is not detached from the wall.

The projections or invaginations on the inner surface take the form of rounded lobes or ridges and have no very deep recesses between them.

The average external diameter of a transverse section is 2·75 mm., length 4 mm. Internal diameter of passage 1 mm., length 2·5 mm.

The sections were by far the worst of all, the stain hardly taking, nuclei being invisible.

Salivary Gland (Pl. 2, figs. 16, 17).

This gland is a small yellowish-white looking body which lies to the left of the œsophagus, and is connected with the latter by two very fine and twisted ducts, which enter at the base of the radula-sac. The maximum length of the gland is approximately 4 mm.

The two ducts (*s. d.*) opening into it are lined with cubical ciliated epithelium, and some little way after their entry into the gland branch off into two smaller ducts. These ducts are again split up into smaller ones, which in turn are divided up into still smaller ducts, so that the whole of the gland has a sponge-like appearance, and is composed of an extremely fine network of glands and ducts. The two main ducts enter some distance into the gland before receiving any branches.

In the ordinary way one would expect to find these minute glands grouped together in little bunches which empty into

one duct, and on account of their resemblance to a bunch of grapes are known as "acinous glands."

In the case of Conus textile, however, this is not so; the glands are unicellular, of various irregular shapes and sizes, placed side by side, and each cell has a separate duct (*dt. op.*), which empties into a larger one.

The cells are filled with a very fine granular substance (*g. c. c.*), which is secreted by their lining, and discharged through each individual duct (*dt. op.*) into larger ones, and so eventually finds its way through one or other of the main salivary ducts to the œsophagus.

This system of grouped unicellular glands is extremely rare, the acinous arrangement being much more common. Owing to the inferior state of the preservation of this material I have been unable to work out this point as minutely as I could wish.

Circulatory System of Conus Textile (Pl. 5, fig. 23, and Pl. 6, fig. 24).

Owing to my researches on the nervous system, and one specimen only being available, I have been unable to do much with regard to the blood circulation and supply. There is one artery (*g.*), the only one I have been able to follow out, which passes from the heart under the œsophagus in an oblique manner to the right. The artery is of considerable size, is oval in section, attached to the œsophagus by connective tissue, and passes forward with it through the nerve collars. The artery now leaves the under-surface of the œsophagus, passes to the right and across, anterior to the left pedal ganglion, to divide slightly to the right of the latter into three large branches, forming a cross, with its arms at right angles. The left branch (*x.*) passes to the base of the siphon, the central one (*y.*) plunges down to the anterior and central portion of the foot, while that on the right (*p. ft.*) proceeds to the extreme posterior and dorsal surface of the foot, along the right side of the latter, and only a short

distance below the external surface. About one quarter of its length from its posterior extremity a branch emerges from this last artery and passes downwards into the foot. There are no branches given off from the other two arteries, and in all cases they end abruptly. Just before the main artery passes through the nerve collar formed by the pleuro-pedal connectives, a right-angled branch is given off which is directed upwards. After a short space this branch is again divided at right angles, the one (*j.*) passing backwards. This latter runs parallel to the main artery (*g.*), and both are connected to the œsophagus, and, together with it, are surrounded by the pleuro-subintestinal collar. The branch artery, having passed backward for a short distance beyond this last nerve collar, is sharply reflected to the left and then turns forward again, forming a **U**, which in section is flat, and closely attached to the œsophagus. From its base and extremity three small branches pass over the surface of the œsophagus. The artery (*w.*) forming the right arm of the **T**, bifurcates, and its two branches are attached to the radula-sac on its ventral surface. Anterior to the nerve collars a large artery (*c. ft.*) proceeds from the main artery at the point where it leaves the œsophagus, passes to the right, across and dorsal to the pedal ganglia, and plunges into the centre of the foot, giving it the appearance of a pedal nerve. The arteries, as I have already stated, are in section oval or flat externally (Pl. 5, fig. 23), and are composed of an external sheath of areolar and circular muscular tissue, called "tunica adventitia" (*l.c.m.*), with an internal lining (tunica media) (*l.c.l.m.*) of circular and longitudinal muscular tissue. This last layer is internally coated with a thin lining of endothelium (*end.*)

Description of Conus Tulipa (Pl. 1, fig. 1).

Maximum length of shell 2 in., maximum breadth $1\frac{1}{8}$ in. Operculum oblong, small, and thinner than in C. textile. The foot is larger and wider, and the row of tubercles are not present; body-walls thick and muscular.

The siphon is the same as in C. textile, but has not the invagination or elbow, and is not so deeply ridged.

The rostrum is the same as in C. textile, having thick muscular walls which are corrugated and ridged throughout internally. There is also a single row of tentacles at the open end of the rostrum. This latter is in the form of a funnel with the constricted open end in front. The walls forming the base of the funnel are curved inwards to the centre, where they form a free and retractile proboscis (*ps.*) which is about two thirds the length of the rostrum, the mouth (*mh.*) being situated in the centre of the free end. The rostrum, which is annulate, has grooves and ridges of muscle running circularly round it on the outside, while internally these are lined with strong muscle bands attached to its anterior end and to its base, by means of which it can be exserted or contracted. The œsophagus (*œ.*) runs from the mouth back towards the stomach in the centre of the ring of muscle bands. The muscular bands are bound firmly to the walls and to the œsophagus by connective tissue.

The œsophagus, on its emergence from the posterior end of the proboscis, bends at right-angles to the right side of the body-cavity; it is then sharply reflected on itself (Pl. 1, fig. 3), becomes slightly larger, and passes back to the centre of the body-cavity, where it is constricted, and again takes a sharp turn to the right and back to the left. It now rapidly becomes larger, and the radula-sac opens on the right side, and the poison duct on the same side and slightly behind. Here it is again constricted, and, together with the poison duct, is surrounded by the nerve collars. The œsophagus now opens out into the stomach, which has the form of a flattened tube running from the centre backwards to the left, lying under the poison duct and gland on the floor of the body-cavity. The canal is again constricted, and passes over the dorsal surface of the liver (Pl. 1, fig. 1, *l. l.*), where it expands, and passes downwards and backwards over the back of the liver to the anus. The liver, a large brownish mass, lies on the left of the body and under the ctenidium (*ct.*). Where the stomach

passes over the liver, it has the same depression as C. textile on its dorsal surface; in this depression lies the left end of the poison gland (*pg.*).

The ctenidium is situated on the inside and dorsal surface of the mantle (*ml.*); starting in front and on the left side, it runs across the branchial cavity (*br. c.*) and then backwards, forming a semi-circle, lying above the liver.

The osphradium, or "fausse branchie," is, as in C. textile, trifid, being parallel to and on the right of the ctenidium, and runs backward about half its length.

The eyes are placed on tubercles which are fused with the tentacles in the same way as in C. textile, one on each side of the rostrum on its dorsal surface and on the external edges. These tentacles are situated about $\frac{5}{16}$ in. behind the opening of the rostrum.

The radula-sac (Pl. 1, fig. 3, *r. s.*) differs from that in C. textile in the fact that the left-hand arm of the V, which opens into the œsophagus (*œ.*) through a very constricted passage, is much shorter than in the latter species. Moreover, the right arm is curved in two places in the shape of an S, is shorter and thicker, and the base of the V, instead of being expanded so as to form a kind of bulb, in C. tulipa is very much larger and forms a sort of triangular hood (*h.*). The walls of the radula-sac are of about the same thickness and texture in both species, with this exception, that the hood-like process in C. tulipa is much thinner than the rest of the sac. The right-hand arm of the radula-sac lies above and across the œsophagus.

Immediately behind and above the radula-sac, and running across the body-cavity, are the coils of the poison duct (*p. g. d.*). These coils are much shorter and less twisted than in C. textile (Pl. 1, fig. 3). Behind them the poison gland (*p. g.*) lies athwart the body. The poison gland is about twice the diameter but shorter in length than that of C. textile. The poison duct enters the right extremity of the gland at right-angles in the same way in both species (Pl. 2, fig. 14). In C. textile it is constricted for about half an inch before its

junction with the gland, while in C. tulipa it is only constricted just at its entry (Pl. 1, fig. 3). The poison duct posteriorly to its opening into the œsophagus passes backwards through the nerve collars, and forms a large coil on the left of the body-cavity; it then passes across and in front of the gland to the right side, where there is another coil. From thence it passes under the gland and enters on the right. The length of duct after unravelling the coils is about 115 mm., or $2\frac{3}{10}$ times the total length of the animal. In C. textile the length of duct is 270 mm., or considerably more than twice as long. The length of the gland is 15·5 mm., diameter 8·3 mm., as compared to 17 mm. and 5·5 mm. in C. textile.

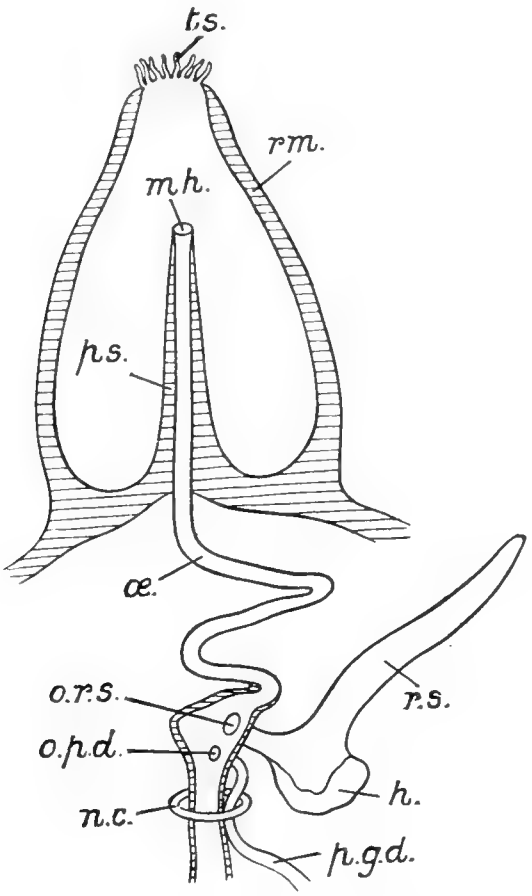
The salivary gland (Pl. 1, fig. 3, *s. g.*) lies to the left of the œsophagus and opposite the radula-sac. Its two fine and crenulated ducts (*s. d.*) pass in the same way, one above and one below the œsophagus, and enter the hood of the radula-sac one on each side, and above the nerve collars. The gland itself is larger than in C. textile.

This gland, called by Bouvier (**5**) the "glande impaire," is in this species in such a macerated condition that I have been unable to obtain any sections sufficiently good to warrant description.

There is considerable difference between the œsophagus and proboscis in C. textile and those in C. tulipa, as can be seen from the descriptions and figures of each. In C. tulipa the proboscis, which is conical and annulate, is two thirds the length of the rostrum ; the œsophagus runs down its centre, and the mouth is simply an opening at its anterior end. In C. textile the proboscis is quite short (Text-figs. 7, 8), and the mouth is provided with thick reflected muscular lips. The œsophagus is here simply a canal surrounded by thick muscular walls; while in C. tulipa it is a free duct surrounded by muscle-bands, which in turn are enclosed by the wall of the proboscis (Pl. 1, fig. 1), the ducts and muscle-bands only being held together and to the internal wall of the proboscis by connective tissue. Again, the distance in C. textile between the mouth and the opening of the radula-sac into the œsophagus

is short (Text-fig. 7), about a quarter of an inch, and the passage straight. In C. tulipa (Text-fig. 9) it is about four

TEXT-FIG. 9.



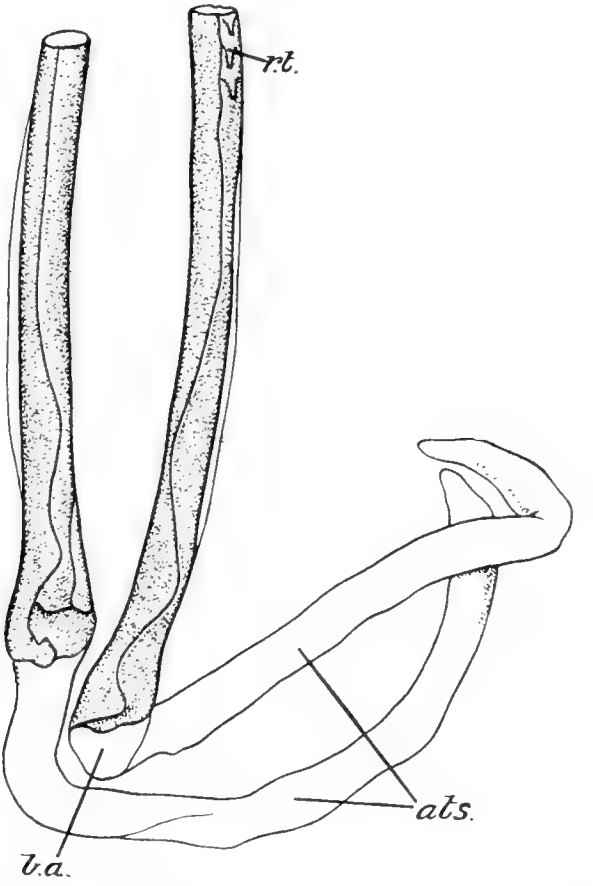
Conus tulipa. The rostrum, proboscis, œsophagus and radula-sac. *mh.* Mouth, *ts.* tentacles, *rm.* rostrum, *ps.* proboscis, *œ.* œsophagus, *r. s.* radula-sac, *o. r. s.* opening of radula-sac, *o. p. d.* opening of poison duct (*p. g. d.*), *n. c.* nerve collars, *h.* hood. The difference will be seen between this species and C. textile, in the length of proboscis and length of œsophagus between base of rostrum and opening of radula-sac into the œsophagus, which is thin-walled. (Diagrammatic).

times as long, or about one inch. The duct is also thin walled, and twice sharply reflected on itself. Lastly, the constricted funnel opening with its very thick walls suddenly emerging into a large cavity, is, in C. tulipa entirely absent. The radula-sac (*o.r.s.*) and poison duct (*o. p. d.*) in the latter species

simply open into the straight-walled œsophagus, which is here rather dilated.

Troschel (**20**, pl. vi, fig. 142) gives a drawing of the radula-sac, thick and short proboscis, and part of the œsophagus of

TEXT-FIG. 10.



The base of two teeth of Conus tulipa in natural position showing (*ats.*) attachments to wall of radula-sac. *b. a.* basal attachments, *r. t.* row of denticulations on side of teeth, *ats.* tooth attachment to sac-wall.

C. textile, which resembles my dissection of this species, though the œsophagus as shown by him is of greater size than I found to be the case in my specimen of the same species.

Whereas in C. textile (Pl. 1, fig. 2) the œsophagus and stomach for the greater part of their length are large, thick-walled and almost circular canals, being only constricted

where surrounded by the nerve collars, in C. tulipa (Pl. 1, fig. 3) the walls are thin, the duct is three times constricted, and never more than a third of the width of the former. In C. tulipa the canal is flat instead of round, and barely one tenth of the diameter of that of C. textile.

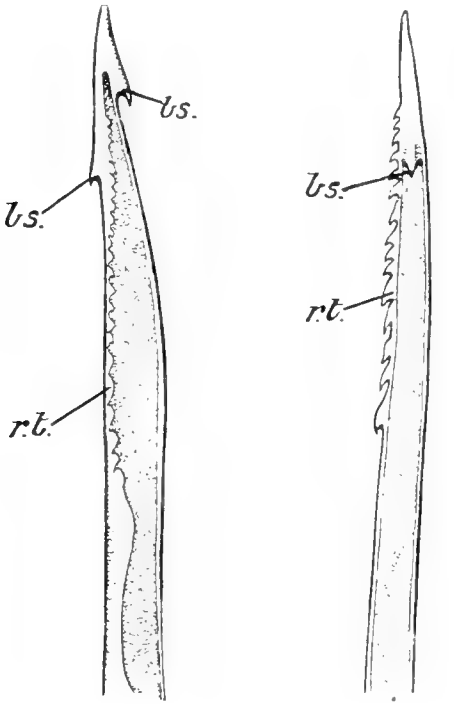
The radula-sac (*r. s.*) is, as in C. textile, thick-walled and muscular, but the length of the right arm is only 11 mm. as compared to 20 mm. in that species, and I have already given the difference in the shapes of the two sacs.

The teeth are placed in two groups, with the barbs or free ends of one group in the cul-de-sac of the right arm, those of the other group protruding through the œsophageal opening as in C. textile. At the base of each tooth, and firmly connected to it, is a roundish muscular attachment (Text-fig. 10) fixing the tooth to the wall of the radula-sac. Each attachment is generally about half the length of a tooth, and curves forward from its base in such a way that the other end is fixed to the sac wall on the side, and anterior to the base of the tooth. As in C. textile, the teeth are surrounded and connected together by connective tissue, and are generally hollow, and of a dark yellow colour. The anterior end of each tooth (Text-figs. 11, 12) is provided with a lance point, while on each edge behind this point is a hooked barb (*bs.*) pointing backwards, the barbs, as in C. textile, being so placed that the distance between the lance point and the first barb is the same as between the latter and the barb behind it on the other side. The point and barbs, though resembling those in C. textile, are, however, not nearly as large or stout, and the whole tooth is much straighter. The base (*b. a.*) is generally slightly swollen, and the chitinous walls are thicker than the rest of the tooth.

The teeth seem very constant in size and shape. On one side of each, and on the flat surface of the lance-point, and therefore at right angles to each of the two barbs, is a row of stout hooked denticulations (*r. t.*) with their points curved backwards and directed to the base of the tooth. These denticulations commence anteriorly about midway between the

two large barbs, and extend backwards in a row for about one third of the total length of the tooth. The denticulations vary in number from 15 to 25, and also in size in nearly every tooth, though 20 to 22 seems about the average number. In this respect the teeth of C. tulipa differ from those in C. textile,

TEXT-FIGS. 11 and 12.



11 12

The tops of teeth of Conus tulipa. *bs.* Barbs, *rt.* row of denticulations.

as the latter have not got this row of lateral denticulations. The average length of the teeth is 4 mm., or less than half the length of those in C. textile (8 to 10 mm.); while their diameter at the base above the attachment is the same in both species, viz. ·2 mm.

HISTOLOGY.

The state of this material, though better than that of C. textile, was not of the best, the specimen having been in

spirit for some considerable time. The same stains were employed as in C. textile, but again the nuclei were only partially defined.

Poison Gland.

As I have already stated, this gland occupies the greater part of the body-cavity and lies across it, with its right end slightly in advance. The canal runs down the centre of the gland, and the poison duct enters it from in front and at right angles at its right extremity in the same way as in C. textile (Pl. 2, fig. 14).

The canal (Pl. 2, fig. 7) (*c. a.*) is oviform, and lined throughout its interior with a thin layer of epithelium (*l. ep.*).

The wall of the gland is very thick and muscular (Pl. 2, figs. 6, 7, 8). It is composed of a deep external layer of longitudinal muscular fibres (*l. m. s.*), which run parallel with the length of the gland and form a thick sheath. Beneath this layer is a very much thinner layer (*c. m. s.*) (about one-sixteenth the thickness of the former) of muscular fibres running round the gland. A third layer (*l. m. s.*), in thickness about one third of the outer sheath, has the same composition as the outer sheath of longitudinal muscular fibres, and again runs longitudinally. Between this layer and the lining epithelium of the canal is a very narrow ring of muscle-fibres (*c. m. s.*) which run circularly round the gland. When compared to C. textile the poison gland is twice the diameter; the external sheath of longitudinal fibres is also twice as thick, the second layer is the same thickness, the third layer slightly deeper. The fourth or internal sheath of fibres running round the gland is about half the thickness, the epithelium is the same; the canal is one quarter the diameter, and oval instead of round. The muscle-fibres are disposed in the same irregular layers (Pl. 2, fig. 6) as in C. textile.

Poison Duct (Pl. 2, fig. 11).

The condition of these sections is not at all good, as the lining epithelium is very much macerated. The outer wall is

formed of a sheath of muscle-fibres (*l. l. m.*) which run parallel to the length of the duct; this is lined by a layer of fibres (*l. c. m.*) which run round the duct and are half the thickness of the external sheath. This last layer has an internal lining of epithelium (*ep. cs.*), which, as in C. textile, is composed of elongated club-shaped cells with basal nuclei. The cells, which are not as long or as fine as in the last species, are also filled with the same fine granular substance. The epithelium varies in length in different parts of the duct. The external surface of the poison duct in C. textile is circular, while in C. tulipa it is of a more irregular shape.

The whole of the centre of the duct is occupied by an irregular invagination of the epithelium (*inv. ca.*), which hangs down into the duct, and is connected by a restricted attachment to the lining epithelium. There is thus formed, with the exception of the place of attachment, an irregular circular canal (*ca.*) between the lining epithelium and the invagination. In the right side of this latter is a deep groove which runs in a curve upwards and then down to the centre. The average external diameter of the duct is ·65 mm., the thickness of the wall ·05 mm. and the epithelial lining ·02 mm.

In C. textile there is only a slight invagination hanging down into the centre of the duct, but owing to the epithelial lining being much thicker in this species (·2 mm.), the area of the canal is about one sixth of that in C. tulipa in spite of the invagination in the latter.

Stomach and Œsophagus (Pl. 2, fig. 13).

The œsophagus and stomach are in section oval externally with thin walls. Their internal surfaces are very deeply crenulated and pleated, forming large folds and furrows. This internal ridged surface is lined with columnar epithelium (*l. ep.*) of considerable thickness, which, as can be seen in the figure, has become detached from the muscle and connective tissue of the wall that surrounds it, this effect being due to its contraction in spirit, and where they are still

attached, the epithelium is distorted owing to its greater contraction and tendency to pull away from the outer layer. As far as I can ascertain from the sections (and, as I have previously said, the material is not of the best) the epithelium is non-ciliated, but appears to have the striated border which is characteristic of columnar epithelial cells. Embedded in this epithelial layer are a considerable number of what appear to be sporozoon parasites (*sp. pa.*). These are large, generally round or oval bodies, scattered all over the epithelium and buried in it at different depths. Some are so large as to extend through the whole depth of the layer, but never beyond into the muscle or connective tissue. In Ehrlich's hæmatoxylin they stain a deep purple colour, and in Heidenhain's hæmatoxylin a greenish-brown. It is impossible to say much about them with any accuracy, as they are extremely difficult if not impossible to define. From their appearance and the fact that they are clearly foreign bodies introduced into the epithelium, there is little doubt that they are parasites such as occur not uncommonly in Molluscs. The external layer of the œsophagus (*l. c. m.*) is composed of circular muscle-fibres which run round and ensheath it, intermingled with connective tissue and a certain number of longitudinal muscle-fibres. Beneath this layer, but very much confused and mixed up with it, is an imperfect sheath of longitudinal muscular tissue. Between this last and the epithelial lining of the œsophagus is a thick but irregular layer of longitudinal granular muscular tissue (*l. l. m.*), intermixed with transverse muscles and connective tissue which bind the epithelium to the œsophageal wall. In some places the epithelium has an internal lining of a granular appearance, while in other places it is wanting. No doubt this is a glandular secretion or mucus derived from the epithelial cells. The average external length of a transverse section is 1·6 mm. with width ·75 mm., the length of the œsophageal canal being 1·15 mm. and width ·15.

In C. textile the external length is 4 mm. and width 2·75 mm., length of canal 2·5 mm., width 1·0 mm. It will thus

be seen that all the measurements are far greater in C. textile than in C. tulipa.

In C. textile the wall of the œsophagus and stomach are thick, while in C. tulipa they are comparatively thin. In the former the canal is nearly circular, while in the latter it is oval.

In C. textile the lining epithelium is much thinner, and the internal surface, instead of being deeply crenulated and ridged, has large curved projections of the wall into the canal.

The eyes, as I have already mentioned, are situated on the internal edge of the tentacles, at the extremity of a small outgrowth produced by the fusion of the tentacle and tubercle. In both species they are identical, so I shall only describe that of C. tulipa, which is in the best state of preservation. The eyes are enclosed by a thin transparent membrane or outer corneal layer (Pl. 2, fig. 12, *co. o.*), which on each side of the eye is continuous with the coat of epithelium (*ep.*) covering the tentacle. Beneath the outer membrane is an inner corneal layer (*co. i.*), which is considerably thinner in front than at the back of the eye and composed of epithelium. This inner layer forms a hollow vesicle or eyeball, with a transparent cuticular lens (*le.*) occupying its interior. The optic nerve (*o.*), which is of considerable thickness, does not enter the retina, but expands out over the back of the eyeball and chiefly on the right side. As I have said, the inner layer of cornea is much thicker at the back of the eye, and is so modified that retinal cells (*ret.*) are formed in the epithelium, directed inwards to the hollow vesicle. These retinal cells run across the back of the eye, and round each side for a considerable distance, extending forward most on the right. These cells, which are embedded in pigment, are longest at the back of the eye, and gradually get smaller as they advance forward on each side, till they disappear posterior to the junction of the inner and outer corneal layers. The eye, which is highly developed as is the case in most molluscs, contains no points of special interest. The eye is easily discernible with the naked eye, appearing as a black spot on each tentacle.

Nervous System of Conus Tulipa.

I found that of the two specimens under discussion C. tulipa offered the most advantageous dissection for the nervous system, and I therefore described and illustrated this species first. As a complete description of the same system in C. textile would be for the most part simply a repetition of the former, though both have been equally carefully dissected, I shall content myself in C. textile with pointing out the differences which exist between the two species.

Description of the Nerve Centres of Conus Tulipa (Pl. 5, fig. 21).

There are thirteen nerve centres or ganglia, a right and left cerebral (*C.*), pleural (*Pl.*), buccal (*B.*), and pedal (*P.*, *P.* 1), a right (*V.* 1), left (*V.* 3), and median (*V.* 2) visceral, one sub-intestinal (*Si.*) and one supra-intestinal ganglion (*S.*). The ganglia with their nerves and connectives are of a whitish-yellow colour, and may be brought into prominence by treatment with osmic acid. The right and left cerebral ganglia (*C.*) are round in shape and fused together on their adjacent sides, and each is posteriorly united to the left and right pleural ganglia (*Pl.*) respectively, the right pleural ganglion being again attached on its posterior side to the supra-intestinal ganglion (*S.*). All these ganglia are extremely hard to define by reason of their junctions being indicated only by very slight constrictions and the whole being bound to the œsophagus by connective tissue. These ganglia lie on the œsophagus, where it is slightly constricted behind the opening of the radula-sac and poison duct. This position is seen in Text-fig. 9 (*n. c.*), but as this figure is purely diagrammatic, only one collar in the form of a ring, and without any ganglia, is shown. To the right, and slightly posterior, are situated the pedal ganglia (*P.*, *P.* 1).

These ganglia lie acoss the right side of the body-cavity under the radula-sac with their anterior extremities pointing

slightly forward. Both ganglia are closely connected together along their internal sides, being only distinguishable at both extremities, and more so at their anterior ends. The ganglia are slightly pyriform or attenuated anteriorly.

A cerebro-pedal connective (*ce. pl.*) passes from the left side of the left cerebral ganglion under the œsophagus, and enters the left pedal ganglia (*P.*) on its dorsal and posterior surface. The left pleuro-pedal connective (*pa. pl.*) runs under the œsophagus and parallel to and behind the cerebro-pedal connective, and joins the left pedal ganglion behind the latter. In like manner a right pleuro-pedal and cerebro-pedal connective passes over the œsophagus, connecting the right pedal ganglion (*P.* 1) to the right cerebral and pleural ganglia. The cerebro-pedal connective is smaller than the pleuro-pedal. Posterior to this last connective, and having their origins one in the left and one in the right pleural ganglia, are the pleuro-subintestinal connective (*d.'*) and the zygoneurous conective (*z.*) respectively of the visceral commissure (*d.*). The pleuro-subintestinal connective (*d.'*) issues, as I have stated, from the left pleural ganglion behind the left pleuro-pedal connective, passes in an oblique direction backwards and under the œsophagus, and enters the anterior and left extremity of the subintestinal ganglion (*Si.*). The zygoneurous connective (*z.*) issues from the same place in the right pleural ganglion, passes backwards and over the œsophagus, and enters the right anterior extremity of the subintestinal ganglion. This ganglion is pyriform, its attenuated posterior end forming the origin of the right loop of the visceral commissure (Pl. 4, fig. 19). The left pleural ganglion terminates posteriorly rather bluntly, being little attenuated, while the right ganglion, lying between the right cerebral and the supra-intestinal ganglia, is hardly distinguishable. This last ganglion, which is pyriform, is continued backwards, forming the left loop of the visceral commissure. This latter (*d.*), after leaving the ganglion, passes over to the left of the body-cavity, and through the wall where is situated the left visceral ganglion (*V.* 3). This ganglion ends abruptly

posteriorly, the commissure passing backwards till it reaches the median visceral ganglion (*V.* 2), which is placed across the body, anterior to the recto-genital mass, and is attenuated at both ends. The visceral commissure leaves the right extremity, passing forward and to the right, where it joins the right visceral ganglion (*V.* 1). This latter is pyriform anteriorly, while posteriorly it is produced where connected to a large nerve (*k.*) and the visceral commissure, the latter, after passing forward for some considerable distance, joining the anterior of the right visceral ganglion, with the posterior extremity of the subintestinal ganglion. The commissure is shortest between the median and right visceral ganglia.

From the anterior edges of both cerebral ganglia (Pl. 3, fig. 18, and Pl. 5, fig. 21) issue the cerebro-buccal connectives (*c. b.*). Both pass round the œsophagus, one on each side, and join the external edges of the right and left buccal ganglia (*B.*) respectively. These ganglia are small, circular, flat bodies lying under the œsophagus. They are united by two commissures, but are so close together that they appear almost to touch. The anterior commissure is of extreme fineness, while the posterior, which is of considerable stoutness, is the origin of the large buccal nerve (*s.* 5) which leaves the commissure close to the left buccal ganglion and passes backwards.

The œsophagus and the poison gland duct (Pl. 5, fig. 21) are completely surrounded by four nerve collars, with ganglia at each extremity of the collars, the most anterior being formed by the two cerebral and two buccal ganglia with their connectives. This collar is the smallest and lies close round the œsophagus, to which it is attached by connective tissue. Behind this comes the cerebro-pedal collar, and posterior again, the pleuro-pedal, these last two being larger and not so closely attached to the œsophagus, round which they lie obliquely, owing to the pedal ganglia being slightly behind the cerebrals and buccals on the right. The fourth and last nerve collar is the pleuro-subintestinal, and owing to the latter ganglion being situated still further backwards, this last collar is more oblique with regard to the œsophagus than the second and

third, but is smaller in circumference. The left half of this collar, which is the stoutest, is formed by the pleuro-sub-intestinal connective of the visceral loop (*d.'*), while the right half is the zygoneurous connective (*z.*).

The nerves issuing from the various ganglia, with the description of their functions and the parts they innervate, etc., will be dealt with under the various headings hereafter mentioned.

Tentacular and Optic Nerves (Pl. 3, fig. 18).

The tentacular nerves (*t.*), or, as they may be called, cephalo-tentacular, are the largest nerves given off from the cerebral ganglia (*C.*). They issue from the dorsal and anterior surface of the ganglia, that from the right supplying the right half of the rostrum, while the left side is supplied from the left ganglion. This left nerve, after leaving its origin in the ganglion, passes to the right, and is then sharply reflected back to the left, where, after proceeding a short way, a stout nerve (*t.* 1) is given off from its anterior side, which passes over to the left and is ramified, splitting up into numerous fine branches. Slightly anterior to the base of this nerve the main tentacular nerve is bifurcated, both nerves passing underneath it, the left branch (*t.* 3) being ramified in the same way as the preceding one. The right branch (*t.* 2) is subdivided into three, of which the median is the optic nerve (*o.*), while those on either side of it are divided up into numerous fine nerves like the other tentacular nerves.

The optic nerve, which, as has been shown, is only a branch of the tentacular nerve, is stouter than the branch on each side of it. When it reaches the place in the rostrum immediately below where the eye is situated, it turns at right angles through the rostrum wall and so to the eye.

The right tentacular nerve on leaving its ganglion passes to the right and slightly forward, where, after a short course, it is bent sharply backwards and then forward again, forming a kink. In the left nerve this kink is close to the left

ganglion, no doubt the duty of these two kinks being to allow a slight contraction or extension of the thick muscular walls of the rostrum. After its flexion or kink a fine nerve is given off on the inside of the right tentacular nerve, which supplies the rostrum base, and anterior to this latter the main nerve is bifurcated, the left branch again dividing into two, the left of these two (*t.* 1) being similarly split up. Both these last give off fine and very numerous branches on their course towards the tentacles, as is also the case with the right branch (*t.* 3) at the first bifurcation of the main nerve. The right branch of the second division of the main nerve (*t.* 2), after proceeding a short way, gives off a fine nerve, while the main nerve of these two, the left, is the optic nerve (*o.*), which proceeds to the eye in the same manner as the left optic nerve, and has two or three small branches issuing from it.

In his description of Conus virgo, Bouvier (**5**) says that the left optic nerve is given off from the tentacular nerve of the same side sooner than is the case on the right side. In my dissection of C. tulipa the reverse is the case, but, like Bouvier, I have been unable to ascertain whether the optic nerve is simply a branch of the tentacular, or whether, though both are in the same nerve-sheath, they really issue as two distinct nerves from the ganglia. The latter seems improbable, as in the case of the left optic nerve there are stout branches on each side supplying the tentacles, the optic nerve issuing from between them. All the tentacular nerves are extraordinarily ramified, splitting up and giving off very numerous fine branches. The ends of the nerves which supply the tentacles themselves, before entering the latter, are generally divided into at least three branches. Both the main tentacular nerves proceed from the ganglia under the base of the proboscis.

A most remarkable fact in connection with the nerve system of this species is the following: From the posterior surface of the base of the kink in the right tentacular nerve a stout branch (*t. p.*) issues; it runs backwards, and is much crenulated. This latter joins at right-angles a nerve (*r.*) running across the floor of the body-cavity, thus forming an inverted

T. The right branch is ramified, and supplies the base of the rostrum on the right, while the left branch proceeds to the dorsal surface of the right pedal ganglion, thus forming a connective between the tentacular nerve and the right pedal ganglion. This nerve, as far as I have been able to ascertain, has not been noticed before, and its presence is hard to explain.

There is no nerve or connective of any sort given off from the left tentacular nerve until the latter is bifurcated, as I have shown, the right branch passing forward to the rostrum and cephalic teguments.

Acoustic Nerves (Pl. 5, fig. 21).

These nerves are extremely fine and very hard to follow, on account of the great number of pedal nerves through which they pass. The origin of the left nerve (*ac. l.*) is in the left cerebral ganglion, immediately below the left cerebro-pedal connective, and between the latter and the left pleuro-pedal connective. This nerve runs between these two connectives to the left pedal ganglion (*P.*) along the anterior side of the latter, passing between numerous pedal nerves, then slightly backwards, and reaches the left otocyst (*ot. l.*) situated some little distance to the right and slightly posterior to the pedal ganglia. The right acoustic nerve (*ac. r.*) issues from the right cerebral ganglion at about the same place as does the left, but descends to the right pleuro-pedal connective, and runs along its anterior edge till both reach the right pedal ganglion (*P.* 1). The acoustic nerve, after following the posterior surface of the latter, passes along the pedal nerves backwards and to the right, till it reaches the right otocyst (*ot. r.*), which is situated in the muscles of the foot, and at a considerable distance from the pedal ganglia and behind them.

Proboscidean Nerves (Pl. 4, fig. 19).

The nerves to the proboscis, or labio-proboscidean nerves, are three in number on each side, and issue from the anterior

edges of the cerebral ganglia. The nerves are stout, of a white colour, not deeply imbedded in the muscular wall of the proboscis, and run parallel to the œsophagus and at about equal intervals round it, the central one (*l.* 1) being on the ventral surface. The two central nerves (*l.* 1) are the longest, and have the fewest nerves given off along their length, and are the finest and most crenulated of the three sets. They run to the anterior end of the proboscis, and are here broken up into a number of fine nerves. The second pair (*l.* 2) are straighter, and the left member of the pair gives off a large branch about one third of its length from the ganglion, while in the right nerve the branch is half-way. The left nerve of the pair (*l.* 3) is trifurcated, the right one bifurcated, all branches being much ramified. In section these nerves are nearly flat and much crenulated.

The walls of the rostrum and cephalic tegument are supplied by three pairs of nerves (*l.* 4–*l.* 6) as well as by branches given off from the tentacular nerves. As was the case with labio-proboscidean nerves, these nerves to the rostrum are in three pairs, and have their origin in the anterior part of the cerebral ganglia (*C.*). The three pairs are nearly the same size, but are very much finer and shorter than the three pairs of labio-proboscideans. As was the case in Bouvier's dissection of C. virgo, so in this species, the first pair of nerves (*l.* 4) go to the base of the rostrum wall, where they are ramified and run forward, while the other two pairs on the right (*l.* 5, *l.* 6), which are slightly finer, pass from the cerebral ganglion across the cerebro-pedal connective, and so enter the muscular wall of the rostrum, where they run anterior to the first pair (*l.* 4). Before they enter the muscular rostrum wall, and between here and their ganglia, the nerves (*l.* 5, *l.* 6) are very sinuous, as is the case more or less with all the nerves to the rostrum and also the labio-proboscideans. This is due to the fact that nerves, being non-extensile or retractile, or nearly so, when they enter a wall which may be exserted or contracted, as in the case of the proboscis, and slightly so in the rostrum, are supplied with enough slack nerve between the ganglia and

their entry to allow the wall to be fully extended without pulling on the nerve. When fully contracted or abnormally so, as in spirit, the nerves have a very sinuous appearance.

Buccal Ganglia and their Nerves (Pl. 3, fig. 18).

The buccal ganglia (*B.*) are small, circular, flat bodies which lie under the œsophagus, and their external edges are joined by a stout connective to the anterio-dorsal surface of the cerebral ganglia (*C.*). The two internal and adjacent edges of the buccal ganglia are connected by two sub-œsophageal commissures, but the ganglia are so close together that they almost touch, the commissures being about a sixteenth of an inch long. The anterior of the two is extremely fine, while the posterior one is as stout as the cerebro-buccal connectives (*c. b.*). These two ganglia, therefore, with their connectives, form a complete nerve collar round the œsophagus, the buccal ganglia being slightly posterior to the cerebrals. The openings of the radula-sac and poison duct into the œsophagus are anterior to the nerve collar.

For the sake of comparison I have used the same letters as employed by Bouvier in his figures. From the posterior surface of the right buccal ganglion a nerve (*s.* 1) is given off, which innervates the anterior portion of the poison duct, till a branch is given off to the latter from the main poison-gland nerve. There is no corresponding nerve given off from the left ganglion. The buccal proboscidean nerve (*s.* 2), which is anteriorly ramified, supplies the wall of the œsophagus in the proboscis, the wall of the proboscis being innervated from the cerebral ganglia by the labio-proboscidean nerves. Here, in like manner, I have been unable to find any such nerve issuing from the left ganglion, nor was I able to find the nerve mentioned by Bouvier as being given off by the side and proceeding to the œsophagus.

Two pairs of nerves (*s.* 3, *s.* 4), one pair issuing from each ganglion, supply the wall of the radula-sac, those given off from the right ganglion supplying the dorsal surface of the

sac, while the two from the left ganglion supply the ventral surface. The main nerve (*s.* 5) which innervates the poison gland and duct, issues from the posterior commissure, and runs backwards under the œsophagus and through the nerve collars. This nerve, which is of considerable size, after passing backwards for some distance curves to the right, where a branch is given off which supplies the poison duct and takes the place of the first fine nerve (*s.* 1). The main nerve now doubles back to the left and then again to the right, three or four slender nerves being thrown off to the coils of duct among which the main nerve runs. This nerve, after passing to the right, is trifurcated, the three branches being ramified and supplying the walls of the poison gland, the branch to the right entering the right extremity of the gland by the duct opening. After the main poison gland nerve has passed backwards from the buccal commissure, a fine nerve (*s.* 6) is given off on the left, which runs backwards along the wall of the œsophagus and is much ramified. The existence of this nerve was indicated by Bouvier (**5**) p. 341, where he says: "Dans un individu femelle, il me sembla qu'un filet grêle se rendait de ce nerf a l'œsophage, en arrière des colliers nerveux." About this point he was not certain, and mentions the difficulty experienced in removing the connective tissue which completely surrounds and binds firmly together the nerves, the œsophagus, and coils of poison duct; this I also found most troublesome, but managed to free the nerve, and ascertained that this nerve (*s.* 6) does run from the main nerve to the œsophagus. As stated by Bouvier, the main nerve (*s.* 5), on account of both its position and origin, would correspond to the œsophageal-aortic nerve in Buccinum, if a branch was given off to the œsophagus, and this I have shown to be the case.

Left Pleural Ganglion and Nerves Issuing (Pl. 4, fig. 19).

This ganglion is attached to the posterior end of the left cerebral ganglion, the end of the one and the beginning of

the other being only distinguished by a slight constriction between them. The posterior extremity of this pleural ganglion (*Pl.*) is directed slightly to the left, while the ganglion is very slightly pyriform and attenuated posteriorly. On the left, and from the external and ventral surface, a stout connective (*d.'*), the pleuro-subintestinal connective of the visceral commissure, issues, turns to the right, across the floor of the body-cavity, runs slightly backwards and under the œsophagus, and enters the ventral and anterior end of the sub-intestinal ganglion (*Si.*).

Two columellar nerves (*i.* 1, *i.* 2) are given off from the centre of the ventral surface of this pleural ganglion, while none are present in the corresponding right ganglion. The nerve on the right (*i.* 2) passes backwards and slightly to the right, being much crenulated and unattached to the body-cavity till it splits into four fine nerves, which diverge in the columellar muscle. The left of this pair (*i.* 1) is twice the size of the former, being sinuous or crenulated in the same way, and runs directly backwards, being unattached to the wall for some considerable distance. Shortly after its entry in the wall it is bifurcated and descends almost vertically into the base of the columellar muscle on the right, and about level with the left visceral ganglion (*V.* 2). Both nerves pass under the œsophagus, the left one running parallel with it, and supply the anterior and left half of the columellar muscle and base of the body-cavity. These two nerves differ from those in Conus virgo, described by Bouvier (**5**), in the following respects; they are shorter, supply the columellar muscle anteriorly and to the left, instead of posteriorly and on the right, do not pass through the nerve collars or go anywhere near the right pleural ganglion or the sub-intestinal, but are absolutely distinct and run directly backwards from their origin.

Four nerves are given off from the postero-dorsal edge of the left pleural ganglion, two being parietal, the other two being the main and lesser pleuro-siphonal nerves.

The most anterior of these four (*f.* 1) is the lesser pleuro-

siphonal nerve, the second (*f.*), the main pleuro-siphon, the third and fourth (*c.* and *c.* 1) being parietal. All these four nerves, after leaving their origins in the ganglion, pass directly to the left, being suspended and quite unattached for some considerable distance between the ganglion and their entries into the base of the body-wall. The lesser pleuro-siphonal nerve passes through the base of the rostrum and at once runs downwards over the attachment of the base of the siphon to the base of the rostrum. When it reaches the siphon proper it gives off two or three small branches and passes forward along the left siphonal wall. The main siphonal nerve (*f.*), which is very much larger, runs parallel but posterior to the former till it reaches the base of the siphon, where it proceeds along the centre of the channel and gives off fine nerves which run into the walls on both sides. When one third of the length from the anterior extremity of the siphon, the main nerve is trifurcated, each branch ramifying in the sides and extremity. At its entry into the base of the siphon, a stout anastomosis (*a.*) connects it with the anterior branchial nerve (*b.*).

The parietal nerve (*c.*) enters the floor of the body-cavity on the left, just behind the main pleuro-siphonal nerve, giving off fine branches to the floor and wall. The other nerve of this pair (*c.* 1) issues from the ganglion behind the former, and after a short distance passes forward under it, and also beneath both the pleuro-siphonal nerves, entering the body-wall anterior to the foregoing. Both parietal nerves are by far the finest of the four nerves given off from this ganglion.

Supra-intestinal Ganglion and Nerves (Pl. 4. fig. 19).

This ganglion (*S.*), which is closely connected to the posterior extremity of the right pleural ganglion, serves as origin for six nerves. There is hardly any constriction at the junction of the two ganglia, which are, therefore, hard to distinguish in a dissection. The supra-intestinal ganglion is pyriform in shape with its posterior extremity directed slightly

to the left. Bouvier (**5**) mentions only four nerves as coming from this ganglion; of the two additional nerves that I have recognised, one is only a parietal nerve and of little or no importance; the other will be mentioned in due course.

There are, then, as I have said, six nerves from this ganglion, two being parietal, two branchial, one, the largest, the supra-intestinal branch of the visceral commissure, and the last supplying the inner edge of the branchial cavity. All these six nerves lie above the two columellar nerves (*i.* 1, *i.* 2).

All the nerves issue from the posterior extremity and dorsal surface of the ganglion, the most anterior being the main branchial nerve (*b.*), which is the stoutest of the six with the exception of the supra-intestinal branch of the visceral commissure.

The main branchial nerve (*b.*), after leaving the ganglion, passes slightly backwards and to the left of the body-cavity where it enters the wall running forward and parallel to the pleuro-siphonal nerve (*f.*). In front of and above the anterior end of the liver these two nerves come closer together, and, as I have already stated, a stout anastomosis (*a*) connects them before the branchial nerve reaches the mantle. The branchial nerve is now reflected sharply to the left and passes under the extreme anterior edge of the osphradium and ctenidium, supplying fine nerves to each; the main nerve ramifies in front and to the left of the ctenidium, and forms a fine network, with numerous anastomoses, in the mantle. Slightly to the left of where the branchial nerve lies above the left columellar nerve (*i.* 1), a stout branch (*b.* 1) is given off which runs parallel to and almost touches the main nerve for some considerable distance. After passing down through the body-wall this branch lies across the anterior lobe of the liver, whence it proceeds backwards and under the edge of the osphradium, supplying this and the central portion of the ctenidium.

The posterior branchial nerve (*b.* 2) is much finer than the anterior, and crosses over to the mantle behind the branch of the main branchial nerve which I have just described, being

practically parallel to it throughout its length. Having passed under the posterior end of the osphradium and given off two or three fine nerves, the main portion of the posterior branchial nerve innervates the internal portion of the mantle and the posterior parts of the ctenidium. According to Bouvier the nerve with its fine branches and anastomoses supplying the anterior edge of the mantle is given off after the anastomosis (*a.*) between the anterior branchial nerve (*b.*) and the main pleuro-siphonal nerve (*f.*), and is thus a product of the pleuro-siphonal nerve. In my dissection this is not so, for the branchial nerve bifurcates, the right branch, which is the finer of the two, forms the anastomosis (*a.*), while the left branch supplies the anterior portions of the mantle in addition to the ctenidium and osphradium, so that this nerve, which in both cases supplies the anterior mantle edge, is in my dissection not pleuro-siphonal but branchial. The branchial nerve, therefore, is of much greater length than that figured by Bouvier.

Another curious point and one worthy of note is the presence of the large branch (*b.* 1) issuing from the main branchial nerve close to its origin. At first sight I was under the impression that this was the posterior branchial nerve, but closer inspection soon proved that this was not the case, for it is undoubtedly simply a branch of the anterior nerve, the posterior branchial nerve (*b.* 2) being quite distinct and originating to the right of the former. The typical position for the posterior banchial nerve would be slightly behind this branch nerve, though not as far back as it is in this specimen.

There are, therefore, according to the parts they innervate, three, and not two branchial nerves. The main and anterior nerve is normal; its branch forms a median branchial nerve; the posterior nerve proper is displaced backwards, and with the aforesaid branch, innervates rather more than the area covered by a normal posterior branchial nerve.

There is nothing of much interest about the two parietal nerves (*c.* 2, *c*, 3). Both cross over to the left and supply the body-wall. The anterior (*c.* 2) leaves its ganglion between

the anterior and posterior branchial nerves, while the posterior (*c.* 3) has its origin between the visceral commissure (*d.*) and the posterior branchial nerve. Soon after its entry in the wall, this posterior parietal nerve expands out into a small, ganglionic-looking mass, from which two fine nerves run forward and three backwards into the body-wall.

The four parietal nerves, two from the left pleural ganglion (*c.*, *c.* 1) and two from the supra-intestinal ganglion (*c.* 2, *c.* 3) innervate the body-wall and side, anterior to the left visceral ganglion (*V.* 2).

The sixth nerve (*c.* 4), which I have mentioned, leaves the supra-intestinal ganglion on its right and postero-ventral edge, runs backwards and to the left, and plunges into the body-wall just in front of the left visceral ganglion, and after running down through the wall, emerges above and passes across the liver to the mantle base, where it ramifies and supplies the mantle wall between the hinder portion of the ctenidium and the base of the mantle. This nerve is not noted by Bouvier in C. virgo, but in the specimen under discussion it is as stout as the posterior branchial nerve, and from its position is of some interest.

The remaining nerve, issuing from the supra-intestinal ganglion, is the left or supra-intestinal branch of the visceral commissure; this I shall discuss with the visceral ganglia.

The Visceral Commissure and Ganglia (Pl. 4, fig. 19).

There are three visceral ganglia. The left one (*V.* 3) is situated in the body-wall near the anterior portion of the liver and above it on the right side. The right ganglion (*V.* 1) is found in the wall enclosing the posterior part of the body-cavity and near its left extremity, while the median visceral ganglion (*V.* 2) lies to the right of the posterior part of the liver, and in front of the recto-genital mass (*r. g. m.*)

The left branch of the visceral commissure (*d.*) has its origin in the posterior and dorsal surface of the supra-intestinal ganglion, passes obliquely backwards and to the left, and so

into the body-wall, and enters the left visceral ganglion close to the right edge of the liver. From the ventral surface of this ganglion, two nerves issue, which might equally well be called parietal or columellar nerves, since they supply the walls and columellar muscle to the right and below their ganglion and almost touch the left columellar nerve (*i.* 1) which issues from the left pleural ganglion. The commissure, after leaving the left ganglion, runs backwards and downwards and slightly to the right, where it meets the median visceral ganglion. Between these two last ganglia, and about one third of the distance from the left visceral, a nerve (*n.*) issues from the left side of the commissure, and runs through the base of the mantle to the dorsal surface of the liver. This hepatic nerve turns to the right and passes back through the dorsal and right edge of the liver and emerges on the right side, where it is trifurcated. The commissure itself does not run out in a loop over the dorsal surface of the liver, but is more normal, being situated in the thick tissue formed by the junction of the mantle to the body-wall, and is thus on the extreme right edge of the liver. The hepatic nerve (*n.*), which I have just described, is peculiar both from its origin and from the fact that it is of considerable size, and the only nerve I have been able to trace which supplies the liver from the commissure. Bouvier mentions the existence of a fine hepatic nerve in C. virgo.

The median visceral ganglion is not so large as the left visceral. Three nerves issue from it; that to the left (*m.*) supplies the posterior lobe of the liver, while the central nerve (*m.* 1), which is the largest of the three, is the visceral nerve, innervating the heart as well as the genital organs and kidney. The nerve on the right (*m.* 2) is the genito-rectal nerve. Between the median and right visceral ganglia, two parietal nerves are given off to the body-wall from the commissure, which latter, after leaving the median ganglion, passes forward and to the right, and so to the right visceral ganglion. One nerve (*k.*) arising from this last ganglion (*V.* 1) is of considerable size. As stated by Bouvier, this nerve is pleural,

and issues from the posterior and right side of the ganglion, passes backwards and into the mantle, beneath the recto-genital mass and up over the back without entering it, where it again reaches the mantle, turns sharply to the right and is ramified.

After leaving the anterior end of the right visceral ganglion, the visceral commissure runs forward and to the right through the body-wall till it reaches the posterior portion of the body-cavity. Here it emerges from the body-wall under the poison gland and duct, where it enters the hindmost part of the sub-intestinal ganglion. I have been unable to find any nerves issuing from the left visceral ganglion and passing to the liver as indicated by Bouvier.

Sub-intestinal Ganglion (Pl. 4, fig. 19).

This ganglion gives off five nerves, four of which innervate the right part of the body, while the fifth is the visceral commissure. All these five nerves are unattached, and lie on the floor of the body-cavity till they enter the posterior wall of the latter.

The ganglion (*Si.*) is pyriform and attenuated posteriorly and slightly to the right, the visceral commissure (*d.*) entering the posterior extremity. One nerve (*e.*) issues from the left and ventral surface of the ganglion, passes backwards and slightly to the left, and enters the body-wall under the visceral commissure, where, after its entry, it divides up into numerous fine branches. The branches supply the body-wall as well as the posterior muscles of the latter, which eventually unite with the columellar muscle, so that this nerve, though really parietal, is in part also a columellar nerve.

There are two true parietal nerves given off from the ventral and right side of the sub-intestinal ganglion, both being finer than the nerve just described, and lying to the right of it. Both these nerves (*e.* 1, *e.* 2) supply the hinder portions of the body on the right, as also part of the right side, but do not extend as far back as the parietal-columellar

nerve. The right of these two nerves (*e.* 1) issues from its ganglion more anteriorly than does the left. Between these two and starting more from the right side of the ganglion is the largest of the four nerves, the right pleural nerve (*e.* 3). This nerve passes backwards and slightly to the right, running at no great depth through the body-wall till it reaches the thick muscular ridge formed by the posterior edge of the body-wall and bounding the anterior side of the anal channel. On reaching this ridge, the nerve plunges straight down and under the channel and to the right, where the nerve divides into two, the left branch running back and ramifying in the edge of the mantle to the right, while the right branch, curving forward again, supplies the under-surface of the body by its junction with the mantle. The zygoneurous connective (*z.*) unites the right pleural ganglion (*Pl.*) with the sub-intestinal ganglion (*Si.*). It is of considerable stoutness, and issues from the ventral and right side of the former to pass backwards and to the right, and is then bent abruptly posteriorly and enters the sub-intestinal ganglion anteriorly and on its dorsal and right side. This connective passes over the œsophagus, while the pleuro-subintestinal connective (*d.'*) of the visceral commissure is sub-œsophageal.

There are no nerves given off from the right pleural ganglion, the right side of the body being innervated from the sub-intestinal ganglion. Bouvier, in his figures and text, has confused these two connectives, since he calls that between the left pleural ganglion and the sub-intestinal ganglion the zygoneurous connective, while he describes the one between the latter and the right pleural ganglion as the connective of the visceral commissure, whereas it is exactly the reverse.

Pedal Ganglia (Pl. 4, fig. 20, and Pl. 5, fig. 21).

These two ganglia are so closely connected together that they look like one irregular ganglion. On closer inspection it will be noticed that at both anterior and posterior extremities there is a slight cleft or constriction between them, which is

most distinct at their anterior end. The ganglia lie under the radula-sac on the floor of the body-cavity and are inclined so that their anterior ends are slightly lower than the posterior. The ganglia are so displaced that they lie across the body-cavity instead of their longitudinal axes running parallel to the foot, the anterior extremities being slightly in advance of the posterior, the ganglia thus lying at a tangent to the longitudinal body-axis and considerably to the right. From this it will be noticed that owing to torsion, the symmetry of the anterior part of the body has been entirely displaced. Of this not only the pedal ganglia but also the otocysts bear witness, for the latter are not really right and left, but anterior and posterior, the right (*ot. r.*) being practically directly behind the left (*ot. l.*), and both are situated at the base of the foot and on the right side. Each of the two pedal ganglia is connected with the cerebral and pleural ganglia of its own side by the cerebro-pedal and pleuro-pedal connectives. The cerebro-pedal connectives join the pedal ganglia anteriorly to the pleuro-pedals, the latter being the stoutest of the two pairs. These connectives with their ganglia form two complete and wide nerve collars round the œsophagus and lie obliquely round the latter, since the pedal ganglia are to the right, and slightly posterior to the cerebral and pleural ganglia.

The nerves given off from the pedal ganglia are extraordinarily numerous, and for the most part of considerable size. They issue from the sides and anterior extremities of the ganglia. The posterior part of the foot is innervated from the right ganglion, while the anterior half is supplied from both right and left ganglia, the right ganglion sending nerves to the anterior and right half, the left ganglion to the left anterior portion.

There are many more nerves issuing from the right ganglion than from the left, the former giving off thirty, while only eleven proceed from the left ganglion. As I have already stated, most of these nerves are of considerable size, some—8, 9, 14, 29—equal in stoutness the tentacular nerves;

thus the foot and right side of the body are very highly innervated.

I do not propose to describe all these forty-one pedal nerves, but they will be seen in Pl. 4, fig. 20, and the parts they supply are described in the explanation of that figure.

The following nerves are worthy of a passing note. The posterior portion of the foot is supplied from the right ganglion by the nerves 29, 31 and 32, of which the first is the stoutest while the latter pair run side by side for most of their length. The right ganglion also innervates the central and ventral parts of the foot, with the exception of the one bifurcated nerve 23, which proceeds backwards from the left ganglion, while the chief nerves from the right ganglion are 25, 27 and 28.

There are three main nerves to the anterior region of the foot, of which 8 and 9 issue from the ventral and anterior side of the left ganglion, and supply the left side, while 14 passes to the right side from the anterior edge of the right pedal ganglion. An anastomosis exists between this nerve and the smaller nerve 13.

One very sinuous nerve (1) after leaving the anterior and ventral surface of the left ganglion, passes under the œsophagus and cerebro-pedal and pleuro-pedal connectives, directly over to the left side, and enters the floor of the body-cavity immediately in front of the left columellar nerve (*i.* 1), where it is trifurcated and innervates the floor under the left pleural ganglion. A fine nerve, 41, issues from the ventral surface of the right ganglion, and supplies the cavity floor directly beneath the pedal ganglia.

From the foregoing account of the nervous system of this species, several points of interest will be noticed, some new, and others confirming the descriptions of previous writers on this genus. Among the latter is evidence of the existence of an œsophageal nerve given off from the poison gland nerve, and indicated by Bouvier. Both my dissections have been of females, as was Bouvier's, and I have not yet been able to dissect a male of either species owing to lack of material.

Perhaps the most interesting feature about the nervous system of the above species is the connection between the right tentacular nerve with the right pedal ganglion, by means of a branch uniting the former to a nerve passing to the body-wall from the right ganglion.

Differences between Nervous Systems of Conus textile and Conus tulipa.

The nerve centres are situated in similar positions in both species; in C. textile both the centres and their nerves are of a reddish-yellow colour. The cerebral, pleural and supra-intestinal ganglia are more closely connected, and even harder to differentiate. The internal edges of both pleural ganglia almost meet, and the constriction is hardly noticeable between the cerebral ganglia, while the whole mass is covered by a thick sheath of connective tissue. The supra-intestinal ganglion is connected, as in C. tulipa, to the right pleural ganglion, their junction being hardly determinable, while the posterior extremity of the former is directed sharply to the left, from whence the left-hand loop of the visceral commissure issues. This flexion of the ganglion is so abrupt as to bring the posterior portion immediately behind the left pleural ganglion, with the result that, at first sight, there appear to be two supra-intestinal ganglia.

The visceral commissure and nerves issuing from the supra-intestinal ganglion, owing to its peculiar shape, proceed across the body-cavity to the left, and at right angles to its axis, instead of obliquely backwards as in the former species. The left pleural ganglion is more pyriform, but in other respects is the same. The positions of the ganglia lying around the œsophagus and also of the sub-intestinal ganglion are similar, but the pedal ganglia lie further back than in C. tulipa, and are not so attenuated anteriorly. These last ganglia are attached with the cerebral and pleural ganglia by the usual pairs of connectives, the anterior, the cerebro-pedal, being very much finer than the pleuro-pedal con-

nectives. Both run parallel and close alongside of each other entering the sides of the pedal ganglia, and issuing close together from the posterior and external edges of the cerebral ganglia and the anterior edges of the pleural ganglia. The pleuro-subintestinal and the zygoneurous connectives are of about equal size. The right visceral ganglion, to which the former proceeds, is not pyriform, but of an oblong shape, and the connectives enter at the opposite extremities on the anterior surface. The three visceral ganglia are situated in about the same positions, but the commissure connecting them is shorter. This latter has its origin in the posterior and left side of the sub-intestinal ganglion behind the entry of the pleuro-subintestinal connective.

The buccal ganglia are attached to the cerebral ganglia in the same manner, but their connectives are almost twice as long. The former ganglia are smaller, but globular in shape instead of flat, while their commissures are much longer, the anterior being almost as stout as the posterior. The most anterior nerve collar, the cerebro-buccal, is not closely attached to the walls of the œsophagus, but is considerably larger than the latter, being very nearly as large as the cerebro-pedal nerve collar. This latter with the pleuro-pedal are the same as in C. tulipa, with the exception that they both lie more obliquely across the œsophagus. The fourth collar, connecting the pleural and the sub-intestinal ganglia, like the second and third, is much more oblique.

The tentacular and optic nerves are the same, with the exception that the former are not nearly as stout, and have not the kink or elbow bend, the optic nerves issuing from them as already described.

The connective between the right tentacular nerve and the nerve issuing from the right pedal ganglion, and which, in the former species, was so noticeable, is absent in C. textile, there being no connection of any sort between the tentacular nerve and the pedal ganglia.

The left or anterior otocyst is placed closer to the pedal ganglia, while the right one is in about the same position.

The left acoustic nerve issues above the left cerebro-pedal connective instead of below it and follows much the same course, but runs along the floor of the body-cavity under the anterior pedal nerves instead of through them. The right acoustic nerve runs along the dorsal surface of the right cerebro-pedal connective.

There is no difference of note in the proboscidean nerves.

I have already mentioned that the nerve collar round the œsophagus formed by the two cerebral and two buccal ganglia is of much greater diameter. Two nerves (*s.* 3, *s.* 4) issue from the left of these latter ganglia and proceed to the radula-sac in like manner, but are stouter, the fine nerve (*s.* 2) running forward; the buccal-proboscidean nerve is present, but only one nerve from the right ganglion to the radula-sac, and the small nerve (*s.* 1) from the same ganglion to the anterior part of the poison duct is also absent. The main poison gland nerve (*s.* 5) is slightly modified, as shortly after leaving the posterior commissure it is bifurcated, the right branch innervating most of the poison duct. The left branch, after proceeding backwards for some distance, is in turn divided into two, these two innervating the poison gland itself.

Lastly, the fine nerve (*s.* 6) from the main nerve (*s.* 5) to the œsophagus, branches off from the former further back, and on reaching the surface of the œsophagus sends one nerve forward and one back over the surface of the latter. Though somewhat modified, the function of this nerve is the same, and its existence is the chief point in question.

The two columellar nerves having their origin in the left pleural ganglion are of equal size, and slightly stouter, and not so sinuous. Both lie under the œsophagus, and innervate the columellar muscle in much the same way. The four nerves issuing from this ganglion—viz. the main and lesser pleuro-siphonal and the two parietal nerves—differ but little, the anastomosis between the anterior branchial nerve and the main pleuro-siphonal being slightly longer.

I have already mentioned that the posterior portion of the

supra-intestinal ganglion is reflected over to the left. There are only three nerves besides the left loop of the visceral commissure which proceed from this ganglion, namely, the anterior and posterior branchial nerves and one parietal nerve. With the exception that they pass straight across the body-cavity to the left they are of no particular interest. The branch given off from the main or anterior branchial nerve is again present, but much smaller, with the result that the anterior and posterior branchial nerves are closer together, and, therefore, in more normal positions.

The sixth nerve (*c.* 4) in C. tulipa is entirely absent in this species.

The three visceral ganglia and their commissures require no special comment, being much the same in both species, the commissure being slightly shorter.

The difference in shape of the sub-intestinal ganglion has already been noticed. There are only three nerves which issue from the ganglion besides the right loop of the visceral commissure. The nerve (*e.*) performing the functions of a parietal and columellar nerve is absent.

The pedal ganglia are even more closely connected and ensheathed by connective tissue. The nerves leave the right ganglion, with one exception, from its anterior extremity only, while they issue from the left side as well as from the anterior of the left ganglion; thus, more nerves are given off from the left than from the right ganglion—the reverse of what occurs in C. tulipa. In the latter there were 41 nerves altogether from both ganglia; in this species there are 39—21 from the left, 18 from the right ganglia, as compared to 30 and 11. None of these 39 pedal nerves attain so great a size as the chief ones in C. tulipa, and all enter the floor of the body-cavity sooner. The right ganglion supplies the posterior portion of the foot, while the central and anterior parts are innervated from the left ganglion. It will thus be noticed that the functions of the ganglia in the different species have been reversed, since in C. textile it is the left one that is the most important. The ganglia themselves do not lie at so

great a tangent to the longitudinal body-axis, but more at right-angles to it.

There are no points of any great difference, or of special interest, between the nervous systems of both species beyond those already mentioned.

Circulatory System of Conus Tulipa (Pl. 5, fig. 22, and Pl. 6, fig. 25).

As in C. textile, so in this species, I have only studied the artery passing forward through the nerve collars. This artery (*g.*), after leaving the heart, proceeds forward and obliquely to the right till it reaches the left branch of the visceral commissure (*d.*) anterior to the left visceral ganglion. For some considerable distance, both commissure and artery are surrounded by one sheath of muscular and connective tissue, the artery being on the right of the commissure. Owing to the artery and commissure having been cut through, where surrounded by this sheath, while removing the œsophagus from the nerve collars, I at first mistook the artery for a nerve given off from the commissure, and, indeed, had dissected it out as such, being considerably perplexed by the existence of such a nerve, till on having sections cut I realised my mistake. It is owing to this error that I have been enabled to work on the arterial system at all, for being then engaged on the nervous system, had it from the beginning clearly shown itself to be an artery, most probably it would have been removed without much comment, and some interesting facts remained unknown.

Having branched off from the visceral commissure, the artery passes to the right almost at right angles to the longitudinal body axis, and under the œsophagus. A little distance after its separation from the commissure, a branch artery (*j.*) is given off nearly at right angles and passes upwards to form a loop which partly surrounds the œsophagus, while the end of the loop is bifurcated, one branch running up and the other down.

The main artery proceeds through the three nerve collars, pleuro-subintestinal, pleuro-pedal, and cerebro-pedal, till it arrives at the posterior cleft between the two pedal ganglia. Here a stout but very short branch (*op. l.*) opens into the dorsal surface of the left pedal ganglion at its posterior edge. The artery runs down the dorsal constriction between the two ganglia and over the anterior edge. When about half way along the depression between the ganglia, the artery is slightly expanded, and two branches issue, that to the right (*op. r.*) opening into the dorsal surface of the right pedal ganglion, while the left branch (*w.*), which is much longer, is directed upwards and divides into two, each being again bifurcated and spreading over the radula-sac.

The branches opening into each of the ganglia are of the same size and length, but the left one enters the extreme posterior edge of its ganglion, while the right one opens into the centre of the dorsal surface of the right pedal ganglion.

After leaving the anterior edge of the ganglia, the main artery curves to the left, becomes larger, and runs directly forward, two arteries branching off from it on the right side, then one on the left, and still more anterior to this latter, the main artery is bifurcated.

The first branch (*c. ft.*) to be given off to the right, runs backwards and through some of the pedal nerves, and enters the centre of the foot, where it divides into two branches supplying the central portions of the latter.

The second branch to the right (*v. ft.*) proceeds directly downwards into the foot to its ventral surface, where it forms a T, one arm running forward and the other backward. Slightly anterior to this artery, the main artery sends off the only branch to the left (*te.*), which proceeds to the base of the cephalic integuments. The left bifurcation of the main artery (*y.* 1) runs forward through the dorsal portion of the foot, while the right (*y.*), passing downwards into the foot, attains the extreme right anterior basal surface of the latter.

In section, the main and branch arteries are for the most part oval (Pl. 5, fig. 22), and are composed of two distinct

sheaths or layers, of which the inner (*l. c. m.*) consists of circular muscular tissue lined with endothelium (*end.*), while the outer sheath (*l. m. s.*), which is slightly thicker, is built up of areolar and muscular tissue running longitudinally.

Differences between the Artery in Conus Textile and Conus Tulipa.

Although the position and functions of the artery in both species are roughly the same, there are many points of great difference between them.

In the first place, the artery in C. textile has no connection whatsoever with the visceral commissure, and lies entirely under the œsophagus. In C. tulipa, for some distance, the artery and commissure are enclosed in the same sheath, and the artery traverses the nerve collars in a direct line for the pedal ganglia. In C. textile the main artery passes through the nerve collars some distance to the left of these ganglia, and a branch artery crosses the dorsal surface of the pedal ganglia, but has no connection with them. In C. tulipa this branch is absent, but the main artery opens directly into the dorsal surface of each ganglion, while from the same place as the opening into the right ganglion a stout but short branch proceeds to the radula-sac.

The radula-sac of C. textile is supplied from a branch which is given off from the main artery before the latter passes through the pleuro-pedal nerve collar. This branch is bifurcated, the left arm running backwards and through the pleuro-subintestinal collar and supplies the œsophagus, while in C. tulipa the latter has a direct supply from the main artery posterior to the last-named nerve collar. In this latter species the main artery, after passing over the pedal ganglia, becomes considerably larger, runs directly forward and divides up into five branches, which supply the base of the cephalic integuments, the anterior dorsal surface, the anterior right and basal surface, the central ventral, and the central portions of the foot respectively.

In C. textile, anteriorly and to the right of the left pedal ganglion the main artery forms a cross, the left arm running to the base of the siphon, the central to the anterior and basal surface of the foot, while the right arm, which is twice the length of the other two, proceeds directly backwards to the extreme posterior and dorsal surface of the foot. Lastly, in section, the artery is of a different construction in each species. In C. tulipa it is composed of two layers of tissue, of which the outer, or tunica adventitia, which is slightly thicker than the inner, is built up of areolar and longitudinal muscular tissue. The inner layer, or tunica media, is composed of muscular tissue running circularly round the arterial canal, and having a smooth inner surface lined with endothelium, or pavement epithelium. The reverse in every point, except the endothelial layer, is the case in C. textile. The outer layer, which is half the thickness of the inner, is built up of areolar and circular muscular tissue; the inner layer is a confused mass of circular and longitudinal tissue of the same sort; while the internal surface, which is lined with endothelium, is deeply corrugated, and a section looks more like a vein than an artery. The total thickness of the arterial wall in this species is twice that in C. tulipa.

Though superficially resembling one another, from the foregoing comparison the many and great differences between the arteries in the two species will be observed.

Poli (**21**), pl. xlv, fig. 13, and p. xxxix has illustrated and described in Conus rusticus Linn. an artery which he calls "l'aorte abdominale," which is similar to that in C. textile in some respects. According to Poli's figure, the artery runs forward from the ventricle of the heart beside the left loop of the visceral commissure, across the latter, and forward through the nerve collars, where it is anteriorly split up into several small branches.

In concluding this paper, it is hoped that the contents on the anatomy of two species of the genus Conus may be of use to workers on this group. Since I commenced my work on this genus I have received from various sources a number

of different species, and I hope shortly to be able to start work on them with a view to further determining the anatomical variations between different species.

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EXPLANATION OF PLATES 1–6,

Illustrating Mr. H. O. N. Shaw's paper "On the Anatomy of Conus tulipa, Linn., and Conus textile, Linn."

LETTERING IN ALL THE FIGURES EXCEPT NUMBERS IN FIG. 20.

a. Anastomosis between pleuro-siphonal and anterior branchial nerves. *ac. l.* Left acoustic nerve. *ac. r.* Right acoustic nerve. *ats.* Tooth attachments to sac wall. *B.* Buccal ganglia. *b.* Anterior branchial nerve. *b.* 1. Branch off anterior branchial nerve. *b.* 2. Posterior branchial nerve. *b. a.* Basal attachment of radulæ. *b. c.* Body-cavity. *br. c.* Branchial cavity. *bs.* Barbs of teeth. *C.* cerebral ganglia. *c.*, *c.* 1, *c.* 2, *c.* 3. Parietal nerves to left of body. *c.* 4. Main and posterior parietal nerve. *ca.* Canal. *c. b.* Cerebro-buccal connectives. *ce. pl.* Cerebro-pedal connectives. *c. ft.* Artery to centre of foot. *c. m. s.* Circular muscular sheath. *co. i.* Inner corneal layer. *co. o.* Outer corneal layer. *ct.* Ctenidium. *d.* Visceral commissure. *d.'* Pleuro-sub-intestinal connective. *dt. op.* Duct opening. *dts.* Ducts from unicellular glands. *e.*, *e.* 1. Parietal nerves to right of body. *e.* 2. Columellar nerve. *e.* 3. Right pleural nerve. *end.* Endothelium. *ep.*

Epithelium of tentacles. *ep. cs.* Epithelial cells. *f.* Main pleuro-siphonal nerve. *f.* 1. Lesser pleuro-siphonal nerve. *ft.* Foot. *g.* Artery from heart through nerve collars. *g. c. c.* Granular contents of glands. *h.* Hood-like process of radula-sac. *i.* 1. Left columellar nerve. *i.* 2. Right columellar nerve. *inv.* Invagination into siphon. *inv. ca.* Invagination into canal. *y.* Artery to the œsophagus. *k.* Right pleural nerve. *l.* 1, *l.* 2, *l.* 3, *l.* 4, *l.* 5, *l.* 6. Proboscidean nerves. *l. c. l. m.* Layer of intermingled circular and longitudinal muscle. *l. c. m.* Layer of circular muscle. *le.* Lens. *l. ep.* Lining epithelium. *l. l.* Left lobe of liver. *l. l. m.* Layer of longitudinal muscle. *l. m. s.* Longitudinal muscular sheath. *m.* Hepatic nerve. *m.* 1. Visceral nerve. *m.* 2. Recto-genital nerve. *mh.* Mouth. *ml.* Mantle. *n.* Hepatic nerve from visceral commissure. *n. c.* Nerve collar. *nu.* Nuclei. *o.* Optic nerve. *œ.* Œsophagus. *o. p. d.* Opening of poison duct. *op. l.* Artery opening into left pedal ganglion. *op. r.* Artery opening into right pedal ganglion. *o. r. s.* Opening of radula-sac. *ot. l.* Left otocyst. *ot. r.* Right otocyst. *P.* Left pedal ganglion. *P.* 1. Right pedal ganglion. *pa. pl.* Pleuro-pedal connectives. *p. ft.* Artery to posterior portion of foot. *p. g.* Poison gland. *p. g. d.* Poison-gland duct. *Pl.* Pleural ganglia. *ps.* Proboscis. *r.* Nerve from right pedal ganglion joining tentaculo-pedal connective. *ret.* Retina. *r. g. m.* Recto-genital mass. *rm.* Rostrum. *r. s.* Radula-sac. *r. t.* Row of denticulations. *S.* Supra-intestinal ganglia. *s.* 1. Nerve to anterior portion of poison duct. *s.* 2. Buccal proboscidean nerve. *s.* 3, *s.* 4. Nerves to radula-sac. *s.* 5. Main nerve to poison gland and duct. *s.* 6. Fine nerve to œsophagus. *s. d.* Salivary duct. *s. g.* Salivary gland. *S. i.* Sub-intestinal ganglion. *si.* Siphon. *sp. pa.* Sporozoon parasites. *st.* Stomach. *t.* Main tentacular nerves. *t.* 1, *t.* 2, *t.* 3. Branches of main tentacular nerves. *te.* Artery to cephalic integuments. *tent. mus.* Muscle of tentacle. *t. p.* Tentaculo-pedal connective. *ts.* Tentacles. *u. g.* Unicellular salivary glands. *V.* 1. Right visceral ganglion. *V.* 2. Median visceral ganglion. *V.* 3. Left visceral ganglion. *v. ft.* Artery to ventral surface of foot. *w.* Artery to radula-sac. *x.* Artery to base of siphon. *y.* Artery to anterior base of foot. *y.* 1. Artery to anterior dorsal surface of foot. *z.* Zygoneur between right pleural ganglion and sub-intestinal ganglion.

PLATE 1.

Fig. 1.—Conus tulipa. A general view of the anatomy. The mantle (*ml.*) has been cut open, and reflected to the left showing the ctenidium (*ct.*) lying in a curve along its dorsal surface. The ospradium, which is situated on the inside of the ctenidium, is hidden by the base of the rostrum. This latter (*rm.*) has also been opened along its dorsal

surface, showing its thick muscular walls and tentacles (*ts.*) and the proboscis (*ps.*) situated at its base. The dorsal wall of the body-cavity has been partly removed, and the large poison gland (*p. g.*) will be seen to occupy all the posterior portion. The poison gland duct (*p. g. d.*) is coiled across and anterior to the gland, while the radula-sac (*r. s.*) is anterior and partly dorsal to the poison duct. An incision has been made in the base of the proboscis to show the œsophagus (*œ.*) passing up the centre to the mouth (*mh.*). (As the various organs are situated in the same positions in both Conus tulipa and Conus textile, I have used this figure to illustrate the description of the latter species, as well as the one it depicts.)

Fig. 2.—Conus textile. A view of the poison gland (*p. g.*) and duct (*p. g. d.*), œsophagus (*œ.*), salivary gland (*s. g.*), and radula-sac (*r. s.*) removed and spread out to show the coils and length of poison duct, stomach (*st.*), salivary ducts (*s. d.*)

Fig. 3.—Conus tulipa. A view of the poison gland (*p. g.*) and duct (*p. g. d.*), œsophagus (*œ.*), salivary gland (*s. g.*), and radula-sac (*r. s.*) removed and spread out as in fig. 2, to show the difference in size of poison gland, constricted opening of duct, which latter is shorter and less coiled than in C. textile. Hood-like process (*h.*) of radula-sac, not present in C. textile, stomach (*st.*), salivary ducts (*s. d.*).

Fig. 4.—A tooth of Conus textile. Barbs (*bs.*), basal attachment (*b. a.*).

Fig. 5.—A tooth of Conus tulipa. Barbs (*b. s.*), basal attachment (*b. a.*), row of denticulations (*r. t.*).

PLATE 2.

Fig. 6.—Conus tulipa. A longitudinal section through poison gland, showing layers of muscle-fibres, highly magnified.

Fig. 7.—A transverse section of same.

Fig. 8.—A transverse section of same, highly magnified.

Fig. 9.—Conus textile. A transverse section of poison gland, *ca.* canal, *c. m. s.* circular muscular sheath, *l. ep.* lining epithelium of canal, *l. m. s.* longitudinal muscular sheath.

Fig. 10.—Conus textile. A transverse section of poison duct, showing long club-shaped cells, and irregular surface of canal.

Fig. 11.—Conus tulipa. A transverse section of poison duct showing invagination hanging down into canal, *c. a.* canal, *ep. cs.* epithelial cells, *inv. ca.* invagination into canal, *l. c. m.* layer of circular muscle-fibres, *l. l. m.* layer of longitudinal muscle-fibres.

Fig. 12.—Conus tulipa. A transverse section through the eye. *co. i.* Inner corneal layer, *co. o.* outer corneal layer, *ep.* epithelium of tentacle, *le.* lens, *o.* optic nerve, *ret.* retina, *tent. mus.* muscle of tentacle.

Fig. 13.—Conus tulipa. A transverse section through the œsophagus, showing sporozoon parasites in the lining epithelium, which has shrunk away from the muscle wall. *ca.* Canal, *l. c. m.* layer of circular muscle, *l. ep.* lining epithelium, *l. l. m.* layer of longitudinal muscle-fibres, *sp. pa.* sporozoon parasites.

Fig. 14.—Conus tulipa. A transverse section through the end of poison gland, showing disposition of layers of muscle-fibres and opening of poison duct (*o. p. d.*).

Fig. 15.—Conus textile. A transverse section through œsophagus, thick muscular walls, with a layer of longitudinal and circular muscle-fibres (*l. c. l. m.*) intermixed in the centre.

Fig. 16.—Conus textile. A transverse section through salivary gland showing entry of one of the two main ducts. *dts.* Ducts from unicellular glands, *s. d.* salivary duct, *u. g.* unicellular glands.

Fig. 17.—The above highly magnified, showing a group of five unicellular glands with nuclei and granular contents. *g. c. c.* Granular contents of cells, *dt. op.* duct opening, *nu.* nuclei.

PLATE 3.

Fig. 18.—Conus tulipa. The nervous system. Buccal ganglia (*B.*) and nerves issuing therefrom, with the tentacular and optic nerves given off from the cerebral ganglia (*C.*). The two pleural and the supra-intestinal ganglia are not shown.

PLATE 4.

Fig. 19.—Conus tulipa. The nervous system.—The cerebral ganglia (*C.*), pleural ganglia (*Pl.*), the sub (*Si.*) and supra-intestinal ganglia (*S.*), with the nerves given off from each, also the visceral commissure (*d.*), and the three visceral ganglia (*V.* 1, *V.* 2, *V.* 3,). The pedal ganglia, cerebro-pedal, and pleuro-pedal connectives are not represented.

Fig. 20.—Conus tulipa. The nervous system. The pedal ganglia *P.* and *P.* 1 with the nerves issuing, and the cerebro-pedal (*ce. pl.*) and pleuro-pedal (*pa. pl.*) connectives. Parts of the foot and body to which the nerves (1–41) proceed. 1 to anterior floor of body-cavity in front of the columellar nerves (*i.* 1, *i.* 2), 2 to centre of floor of body-cavity, nerve much crenulated, 3 to left and anterior portion of foot, 4 and 5 to floor of body-cavity, 6 and 7 to left anterior side of foot, 8 and 9

to left anterior extremity of foot (main nerves), 10 to central anterior extremity of foot, 11, 12 and 13 to right anterior extremity of foot, 14 main nerve to right anterior extremity of foot, 15 to anterior and right side of foot, 16 to right side of foot, 17 to extreme light and anterior part of body-wall, 18 to anterior and right side of foot, 19 to extreme right and anterior part of body-wall, 20 to right anterior side of foot, 21 and 22 to centre and right part of foot, 23 to ventral and central surface of foot, 24 to right side of body-wall, 25 and 26 to centre and lower surface of foot (25 main nerve), 27 and 28 to centre of foot, 29 main nerve to extreme posterior end and base of foot, 30 to centre of foot, 31 under operculum, 32 through right corner of body-wall, and to extreme end and base of foot, 33 to right posterior corner of body-wall, 34 to floor and body-wall, 35 to base of posterior wall of body-cavity, 36 to left central side of foot, 37 to posterior wall of body-cavity, 38 to left and central side of foot, 39 to base of posterior body-cavity wall, 40 and 41 to floor of body-cavity, under cerebral ganglia.

PLATE 5.

Fig. 21.—Conus tulipa. The nervous system. Nerve centres. The ganglia with their connectives and nerves. Only nine nerves are shown issuing from the pedal ganglia (*P.* and *P.* 1). The buccal ganglia (*B.*) with the cerebro-buccal connectives (*c. b.*), which lie parallel to the cerebro-pedal connectives (*ce. pl.*), have in this figure, for the sake of clearness, been brought forward. The left and right otocysts (*ot. l.*, *ot. r.*) with their nerves (*ac. l.*, *ac. r.*) can be clearly seen, as also the nerve (*r.*) issuing from the right pedal ganglion (*P.* 1), and united by means of the tentaculo-pedal connective (*t. p.*) to the right tentacular nerve (*t.*).

Fig. 22.—Conus tulipa. A transverse section of the artery to the pedal ganglia.

Fig. 23.—Conus textile. A transverse section of the artery which passes through the nerve collars.

PLATE 6.

Fig. 24.—Conus textile. A general view of the nerve centres (semi-diagrammatic), showing the position and branches of the artery proceeding from the heart through the nerve collars to the foot, etc.

Fig. 25.—Conus tulipa. A general view of the nerve centres (semi-diagrammatic), showing the position and branches of the artery which proceeds from the heart through the nerve collars to the pedal ganglia and foot, etc.

On the Relation between the Structure and the Development of the Centrifuged Egg of the Frog.

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With Plates 7—12 and Text-figs. 1—18.

Contents.

I. INTRODUCTORY.

It is to the structure and constitution of the germ-plasm, of the cytoplasm as well as of the nucleus of the germ-cell, that the path of inquiry into the internal causes of the development

of the organism, the causes which determine in each generation the reproduction of the specific form, inevitably leads back. It is not a little curious that a method of experimental research which has already given something and promises perhaps to contribute still more to the analysis of the factors resident in the egg-cytoplasm should owe its existence to those classical experiments of the physiologist Emil Pflüger, which led their author to formulate the doctrine of the "isotropy" of the ovum, and to deny to the egg-cytoplasm any structure, or at least any significant structure at all.

Normally, the eggs of the frog and toad—Pflüger used the ova of Bombinator—are not free to rotate inside their tightly adherent jelly-membranes when first they are laid. But shortly after and as a consequence of insemination there is developed between the egg and the innermost layer of the jelly a perivitelline space, inside which the egg then rotates until its axis becomes vertical with the heavier yolk-pole below. It will be recalled that Pflüger succeeding in inhibiting the formation of this space, though not the subsequent segmentation and development, by giving each egg only a minimal drop of sperm-containing water. Such eggs could be kept forcibly inverted in any position in which the experimenter chose to place them, with the original axis making any angle with the vertical, and, except when the inversion was absolutely complete, were capable of normal segmentation and development. In cleavage it was found by Pflüger that the first two divisions were vertical and the third horizontal, just as in the undisturbed egg the first two (meridional) and third (latitudinal) are also respectively vertical and horizontal, while later on the dorsal lip of the blastopore appeared just below the actual equator, and travelled down to the actual lower pole in the plane including the actual (vertical) and the original (inclined) egg-axes. The conclusion drawn from these facts—that the embryo may be developed from any part of the egg, that the egg is consequently isotropic, and only undergoes a specific development because it is always brought under the same external conditions—was not allowed to remain unchallenged for long.

First Roux showed that the constant directive influence of gravity might be easily eliminated by keeping the eggs in a perpetual state of slow rotation, under which circumstances their segmentation and development bear the same relation to the original axis and polar structure as is ordinarily the case. And secondly, Born's examination of sections showed at once that in these forcibly inverted eggs there takes place a redistribution of material, the lighter liquid plasma ascending, the heavier yolk-particles descending until there is conferred upon the ovum a new structure with a new axis, which is of course vertical, and has unlike animal or protoplasmic and vegetative or yolk poles. The pigment, however, is not wholly shifted, though some is carried up into the lighter plasma. The upturned white pole remains white, or at most becomes greyish. To this new structure—which may have any relation, make any angle with the original structure—the cleavage and development of the egg has the same relation as normally. With regard to the new axis the first and second furrows are meridional, the third latitudinal, while the head of the embryo appears near the new animal pole. The median plane of the embryo is that plane in which both the original and the new axes lie, for the streaming up and down of the plasma and yolk take place symmetrically about that plane, and the bilaterality so conferred upon the egg-contents persists as that of the embryo.

The very experiments, therefore, which were vainly imagined to prove the isotropy of the cytoplasm have only succeeded in emphasising the significance of the structure of the ovum for the development of the embryo.

This experiment can be performed in what is, I think, a more convenient manner by substituting for gravity a centrifugal force, greater than gravity, but not too large. As soon as the eggs have been inseminated they are placed with sufficient water to allow the jelly to swell and the perivitelline fluid to be exuded, in the tube of the centrifuge, and centrifuged at once for a short time. The eggs, of course, lie haphazard in the tube, with their axes making any angle with the

direction of the force, and since they cannot move until the perivitelline space has been developed their contents are re-distributed as in Pflüger's experiment. They are then removed from the machine. It will be found that they do not turn over, that the first and second cleavages are parallel, the third at right angles to the direction of the force, and that the dorsal lip of the blastopore appears just on the centrifugal side of the (new) equator of the egg.

Wetzel and Hertwig have recently employed this method in the study of the particular case of complete inversion, which does not apparently prevent the formation of the embryo, as stated by Pflüger.

The credit of subjecting the eggs of the frog to a still higher centrifugal force belongs to O. Hertwig. He found that the segmentation of such eggs was meroblastic. A cap of small cells or blastoderm was formed resting upon an undivided though nucleated yolk, and these yolk-nuclei were large and irregular, resembling the giant nuclei found in the large-yolked eggs of fishes and other forms. If removed from the centrifuge in time these eggs developed, though monstrosities (spina bifida) were frequent.

More recently Morgan, Konopacka and McClendon have all made contributions to the problem of the relation between this disturbance of the egg-structure and the development of the embryo.

Morgan has shown that with a still higher speed (1600 revolutions per minute for seven minutes, R = 5 in., f. = 370 g.) the egg of the American species Rana sylvatica develops a grey patch round the animal pole owing to the heavy pigment being driven centrifugally into the interior of the egg like a plate. In these eggs the first and second furrows are approximately meridional, but those of the third phase are abnormal in being again meridional. The dorsal lip of the blastopore is in the normal position, but the yolk plug may be pigmented. The tadpole is antero-ventrally unpigmented, but this defect may be made good in later stages. Morgan refers to but does not follow up nor adequately describe

an interesting abnormality which he says is not uncommon. In this the front end of the nervous system is malformed, the neural folds each terminating in a knobbed extremity. With still longer exposures the yolk fails to segment, but "the more fundamental questions relating to the distribution of the materials of the egg, and the interpretation as to whether these visible substances are organ-forming or organ-determining have not been discussed. It is evident that the black pigment has no such function, but further experiments will be necessary in order to determine what value the other substances in the egg may have"—a conclusion with which we may cordially agree.

The principal object of the work of Konopacka (on Rana fusca) is to discover whether there is any alteration in the sensitiveness of the egg to this disturbing agent during the early stages of fertilisation and segmentation, and it does indeed appear that the number of abnormal embryos is greater when the eggs are centrifuged during the early cleavages than when the operation is performed upon unfertilised intra-uterine eggs, or on eggs in process of fertilisation. Apart from this, however, the paper contains a description and figure, the first published, of the alteration in the structure of the egg-cytoplasm, as well as an account of some of the monstrosities produced. With short exposures to a fairly high acceleration (f = 228 g.) the grey area of Morgan appears, and is seen in sections as a layer of yellow vacuolated hyaloplasm surmounting the inwardly driven pigment and the yolk. With longer exposures a white hyaloplasmatic layer is interpolated between the vacuolated and pigmented layers, while another vacuolated layer appears between the white layer of hyaloplasm and the yolk while the first vacuolated layer becomes folded. No attempt is made, however, to investigate the gradual genesis of these layers, nor to ascertain the chemical nature of the substances composing them. Amongst the abnormalities described are numerous half-embryos, a portion of the egg having remained unsegmented, embryos with persistent blastopores, and tailed but headless monsters.

The last, as I hope to show, are of particular interest, but the author figures only the external appearance of one, whose development has been very much arrested, and gives no detailed description of the anatomy of either this or other stages of the malformation.

The most recent work of all, that of McClendon, marks a very great advance, for here we have for the first time a chemical analysis of the various layers into which the egg cytoplasm is separated by the centrifuge. By the use of a considerable force for a short time (f = 2771 g., for five minutes) the eggs of Rana pipiens and Acris gryllus become divided into zones. In the first there are three zones, A, a yellow centripetal cap (corresponding to the grey area of Morgan and the vacuolated hyaloplasm of Konopacka) which consists of globules—soluble in oils and ether—which are mostly fat but partly lecithin, inasmuch as there is some phosphorus in the alcohol extract of this layer. The water content of this layer is 50 per cent. The second layer, B, is grey, of translucent protoplasm, 82 per cent. of which is water; there is also some fat and lecithin. The third layer, C, is black, it consists of the yolk and pigment, contains 42 per cent. of water, some fat, a good deal of lecithin, and a large quantity of protein which is rich in phosphorus and therefore supposed to be a nucleo-protein. In Acris the first layer consists of a yellow cap and a white ring, while the pigmented portion of the third is divided into three rings.

It goes without saying that the chemical composition of the several layers could not have been ascertained from any investigation of individual eggs. For this purpose a whole mass of eggs was centrifuged, the layers separated and analysed. The method was to take, not, of course, the laid egg with its coating of jelly, but ripe ovarian eggs. In actual practice the whole ovary was removed, including not only the fully grown ova, but all the young ones as well, washed and squeezed through bolting cloth to get rid of the stroma. The pulp so obtained was then centrifuged. This appears to me to introduce a certain error. I must also point

out that McClendon has determined only the phosphorus content of the ethereal and alcoholic extracts and of the solid protein-containing residues, and has given no further proof of the presence of lecithin and nucleo-protein. These points, therefore, and of course many others remain for investigation, but McClendon's work is certainly a beginning, and the priority belongs to him.

I myself had frequently had occasion to examine, somewhat cursorily, the development of the centrifuged eggs of the frog, and it had occurred to me quite independently, as indeed it would naturally occur to anyone with such eggs under his eyes, and before I became acquainted with McClendon's papers, that the layers might be obtained in sufficient quantity for chemical investigation if a mass of egg-pulp were centrifuged. Moreover, if the abnormal development of the embryo really is a consequence of the derangement of the materials of the cytoplasm, it ought to be possible to relate a certain degree of malformation with a certain degree of derangement by comparing on the one hand the composition of the several layers in masses of egg-pulp centrifuged at different speeds, and on the other the development of embryos from eggs centrifuged at corresponding speeds. Such an investigation, though it would not certainly lead to great results, yet seemed well worth undertaking. But before that could even be possible there was a preliminary question to answer. As a result of similar experiments on the ova of various Invertebrates it has been seriously suggested that the polarity of the egg, to which the structure of the embryo bears such a very definite relation, is not determined by the disposition of the various visible and separable constituents of the cytoplasm, which, indeed, so it is maintained, may be driven by the centrifuge to any region of the egg, leaving the original polarity intact, and without prejudice to the normality of development.

It became necessary, therefore, first of all to inquire into the structure of the embryos produced from such ova, and the relation of that to the derangement of the egg materials. For only if it should turn out that the polar structure of the egg

to which the normal development of the embryo is related is determined, in part at least, by a certain arrangement of the visible materials which can be actually separated by the centrifuge, would any attempt to ascertain their chemical nature be of the slightest value, however successful. It was with these objects in view that the experiments which are now to be described were undertaken in March and April last.

I shall deal first with the structure and development of the centrifuged eggs (which in any case have not yet been completely elucidated), and afterwards give such observations as I have made on the chemical composition of the various constituents of the cytoplasm.

A general discussion of these results and of their bearing on the large problem of the nature and origin of the polarity of the ovum and its relation to the development of the embryo will be found in a final chapter.

I will only add here that I am under very great obligations to my friend Dr. Ramsden, not only for permitting me to work in the Laboratory of Physiological Chemistry, but also for giving me the most valuable advice and much personal assistance.

I am also very much indebted to Professor Dreyer for allowing me the use of the centrifuge in his laboratory, and to Dr. Scott for the loan of a freezing microtome.

II. THE EXPERIMENTS.

(A) The Structure and Development of the Centrifuged Egg of the Frog.

(1) Methods.—I have used the eggs of the common English frog. The eggs were taken from the uterus, inseminated, and allowed to remain in water for about an hour until the jelly had swollen and the perivitelline fluid been exuded. The eggs turned into the normal position with the axis vertical and the white pole below. They were then

placed on the centrifuge, which was at first set in gentle motion to turn the axes of the eggs into the direction of the force, and then more rapidly to bring about the desired effect. The eggs were thus centrifuged in the direction of their axes with the animal pole centripetal.

Various speeds were used, but I am unable to state in any case the precise number of revolutions per minute.

In series G the electrically driven machine was used, and at a speed of about 1500 revolutions. In the others a water-driven machine was employed, at speeds varying from about 1100 to 3000 revolutions a minute. These speeds, which I call I, II, III and IV, I being the lowest, were determined by the angle through which the tap of the apparatus was turned, Owing, however, to the inconstancy of the water-pressure they probably varied in the course of the experiments.

The radius in both machines was about three inches.

Different exposures were used, from five to thirty minutes.

After the treatment the ova were removed to vessels of clean water.

(2) Details of the Experiments.

G.

Centrifuged 27 : iii : '13 on the electrically driven machine at bottom speed.

1. One hour after insemination, centrifuged for 5 minutes.

A grey patch appears round the animal pole.

The first two segmentation furrows are normal.

28 : iii : '13.—The eggs have segmented normally.

The grey patch is no longer visible.

8 : iv : '13.—The tadpoles have hatched out, but some are abnormal, the yolk-sac being swollen, and are inert.

All preserved in picric acid.

Of these tadpoles 41 are apparently normal, i. e. like the controls, 12 abnormal. Of the latter 10 are of type (a), 2 of type (b).

G. 1. 8 : iv : '13 (a) (Text-fig. 1, Pl. 11, fig. 19).—The operculum is growing back over the external gills, the tail and fin well-developed. The head is somewhat warty and wrinkled.

Sections show that the anterior head ectoderm is vacuolated, as are also the olfactory pits, the brain, the Gasserian ganglia, the suckers

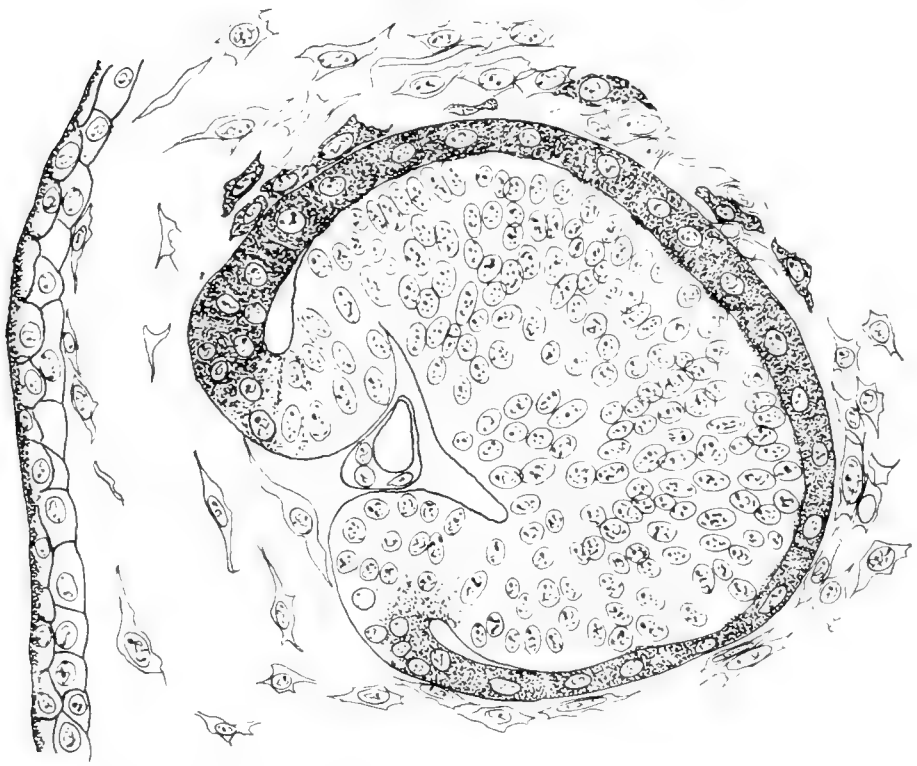
and the wandering mesoderm cells. The anterior end of the brain is single.

The optic cup is some distance from the ectoderm; its cavity is small and encloses a blood-vessel (vitreous body).

There is no lens. The corneal mesoderm is present, but the conjunctiva is not cleared. The infundibulum is present and the pituitary body. The auditory vesicles are well formed.

The notochord begins behind the infundibulum: its structure is normal.

TEXT-FIG. 1.



G. 1. 8 : iv : '13 (a). The lens-less eye. There is a blood-vessel in the small cavity of the optic cup. Corneal mesoderm and conjunctival ectoderm, choroid and sclerotic mesoderm are shown.

Differentiation of the skull and visceral arches has begun, the labial cartilages, Meckel's cartilage, the trabeculæ with the anterior trabecular plate and cornua, the quadrate, the parachordals, the hyoid and branchial arches being all present.

The thyroid is already separated, the trachea and lungs have been formed. There are external gills, and the internal gills are beginning to appear.

The heart is normal. Aortæ and cardinal veins present.

The pronephros has the usual three funnels on each side and a glomus; the ducts open to the cloaca.

The gut is normal.

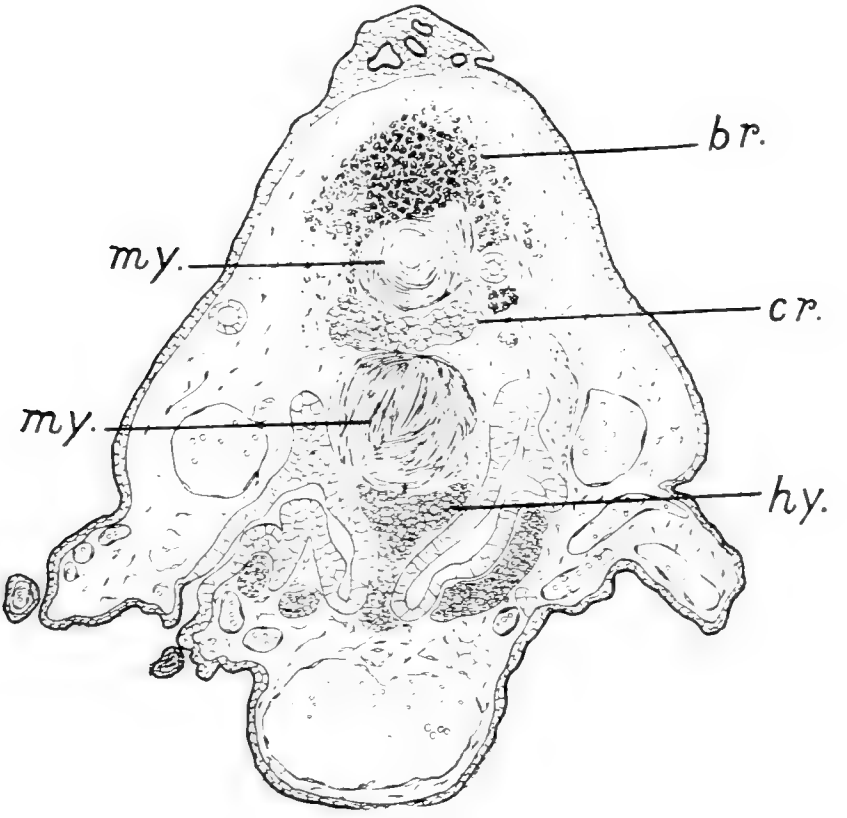
There is a blastema for the pelvis and hind limbs.

Germ-cells are present at the root of the mesentery.

The tadpole is clearly abnormal only in the vacuolation of the anterior ectoderm and its derivatives and in the absence of the lens.

G. 1. 8 : iv : '13 (b) (Text-fig. 2, *a–d*, Text-fig. 4, Pl. 11, figs. 20, 44*a*, 45, 47).—The tail is as well-developed as in the last, but the anterior end

TEXT-FIG 2*a*.



Section of the series through the tadpole, G. 1, 8 : iv : '13 (b). Through the degenerate brain represented by a mass of pigment-cells (*br.*). The rudimentary cranium (*cr.*), the hyoid (*hy.*) and parts of the branchial arches are also shown. Between the parts of the skeleton are bundles of myoblasts (*my.*). The section also shows gill-cleft, external gills, blood-vessels, a large lymphatic ventrally, and dorsally thickened and pitted ectoderm.

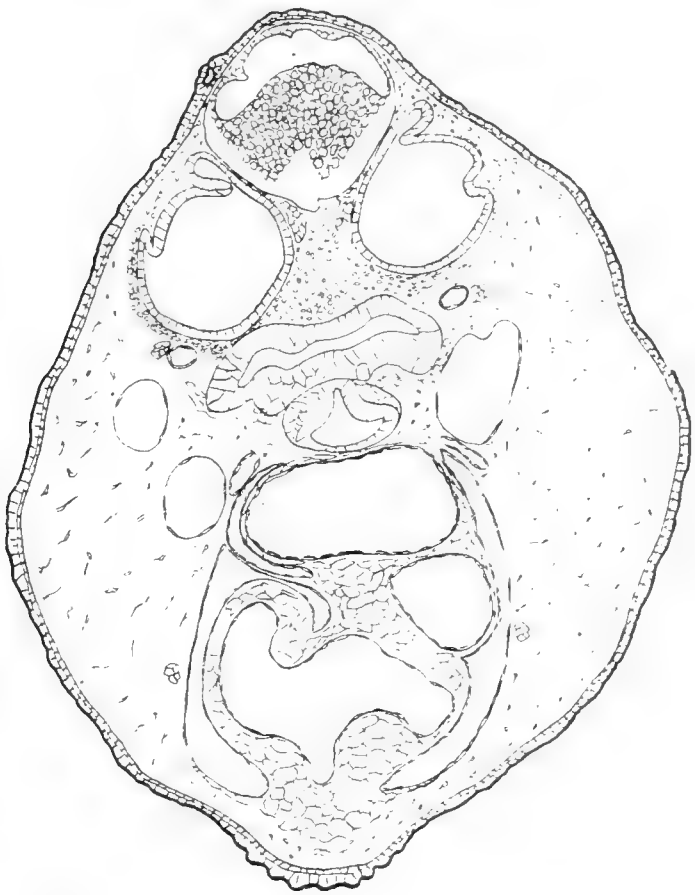
is seriously affected. The head appears to be abruptly truncated, the gills far forwards. The body is swollen. There are no suckers.

Sections show the ectoderm of the head to be folded and much vacuolated.

Olfactory pits, fore-brain, eyes, mid-brain, and part of the hind-brain are all absent, or represented only by masses of degenerating cells.

In the front part of the head is found an aggregation of pigmented and vacuolated cells, some still containing yolk-granules. These cells are the disintegrated residue of the anterior part of the nervous system, for some have the large pale nuclei characteristic of neuroblasts, others the smaller, darker nuclei of spongioblasts. Groups of cells with nerve-fibres proceeding from them are the ganglia of the fifth and seventh

TEXT-FIG. 2*b*.



Section of the series through the tadpole, G. 1, 8 : iv : '13 (b). Hind-brain, with folded roof, auditory vesicles and ganglia, œsophagus, trachea, heart, blood-vessels (aortæ and cardinals). No notochord.

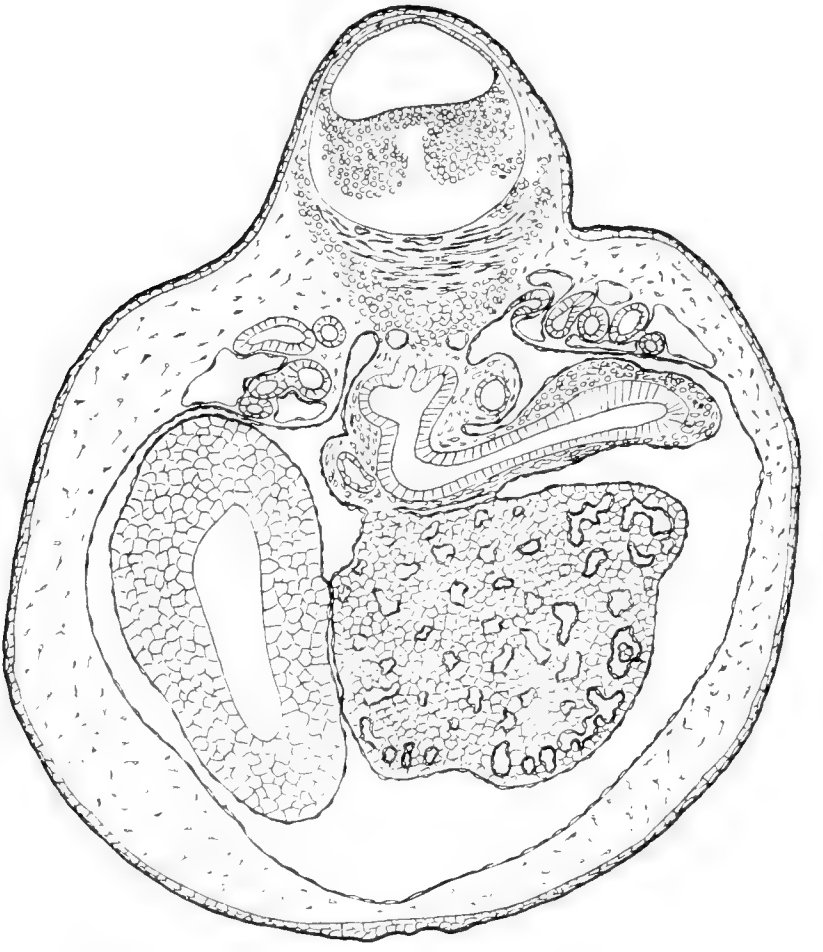
nerves. One or two small vesicles of doubtful significance are also found. In addition to the intact cells are cell-fragments, and chromatic spherules, the remains of nuclear degeneration.

In and around this accumulation are mesoderm cells, some vacuolated, others deeply pigmented (chromatophores).

The auditory vesicles are large and apparently normal; at their level begins that part of the central nervous system which has escaped des-

truction, namely the medulla, continued behind into the spinal cord. Even this, however, is not normal. In outline it is circular, its lumen crescentic. The thin roof is excessively folded, while in the thick floor there is a continuous mass of white matter across the middle line below; next to this comes a layer of neuroblasts and next the lumen a layer of

TEXT-FIG. 2 *c*.



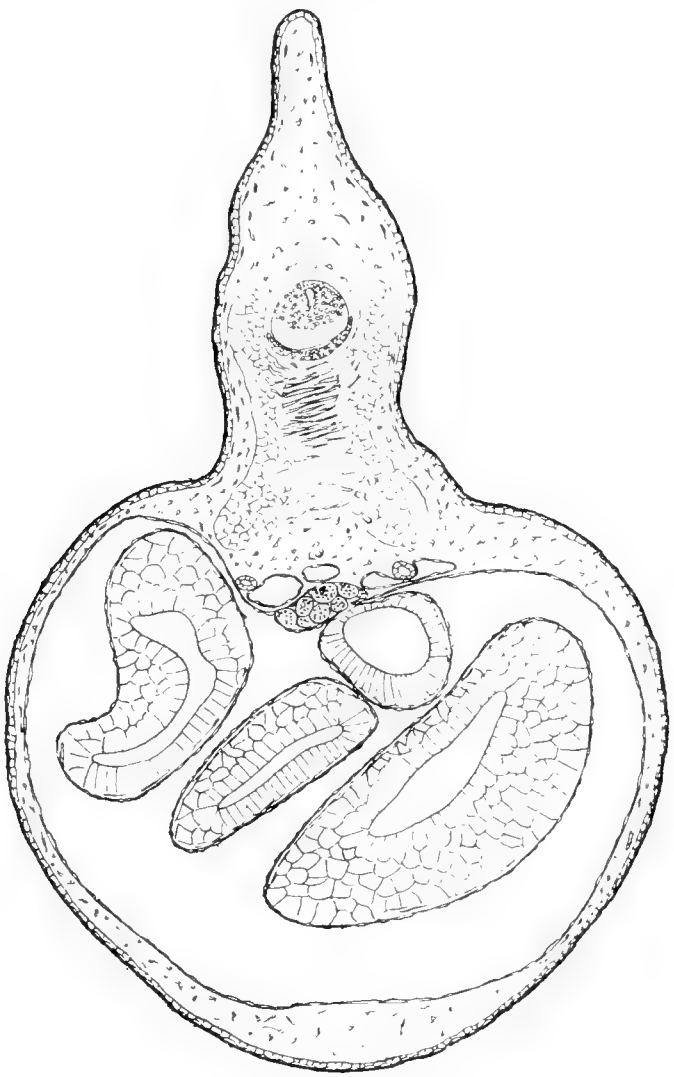
Section of the series through the tadpole, G. 1, 8 : iv : '13 (b). Pronephros (one funnel on right), stomach, liver, intestine, aortæ, cardinals. Fusion of myotomes below medulla. No notochord.

spongioblasts. The spinal cord presents the same histological characters, the lateral tracts of white matter having been apparently forced down into a median ventral position.

The ganglia of the eighth and ninth and tenth nerves are present. The spinal ganglia are abnormal in position, being united ventrally below the cord, an effect, presumably, of the same cause to which the peculiar structure of the cord is due.

The skull has also suffered, being represented only by a small bilateral plate ventral to the degenerate brain, possibly the anterior trabecular plate. Of the visceral skeleton there is a wedge-shaped piece in front of the gut, which may be interpreted as first branchial arch, with

TEXT-FIG. 2 *d*.



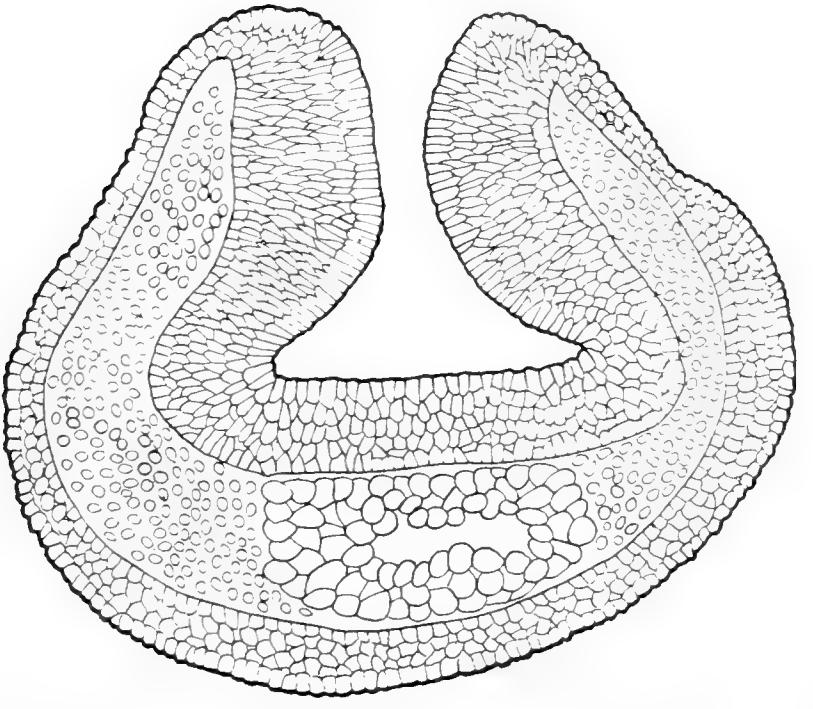
Section of the series through the tadpole, G. 1, 8 : iv : '13 (b). Pronephric ducts, primordial germ-cells, intestine. Spinal cord abnormal, with white matter in a continuous ventral band, and ganglia fused below. Myotomes fused. No notochord.

possibly mandibular and hyoid elements included in it, and behind this, underneath the throat, a basi-branchial bearing two pairs of arches; the relation of these to the gill-slits proves them to be the second and third of the series. Attached to these skeletal elements are masses of myoblasts.

There is no stomodæum, and the fore-gut is in communication with the exterior only by the gill-slits, of which the usual four are present, the last three being open. There is a rudimentary sixth cleft (pharyngeal outgrowth). External gills are borne on the first three branchial arches.

The thyroid, still connected with the throat, lies in a depression of the basi-branchial. The trachea and lungs have been formed. The heart is normal, aorta and cardinal veins present. In front of the pericardium

TEXT-FIG. 3*a*.



Optic vesicles and fore-brain. The pharynx below. I. 1, 3 : iv : '13 (c). Normal.

is a large sinus, lymphatic, partially divided by a septum. It causes the body-wall to protrude. The gut is normal.

The pronephros is well-formed, but on one side there are only two nephrostomes. The ducts open to the cloaca. There is a glomus. The notochord is very imperfectly developed. The myotomes bend round and fuse in a median mass of cells (myoblasts) below the spinal cord; in this mass notochordal tissue may be here and there distinguished, but it is feebly differentiated. Even when present it is not immediately below the spinal cord, but separated from the latter by myoblasts. Germ-cells are found at the root of the mesentery.

2. One hour after insemination, centrifuged for 10 minutes.

The grey patch round the animal pole is more conspicuous.

Segmentation, the first two furrows, may be normal, but is sometimes abnormal.

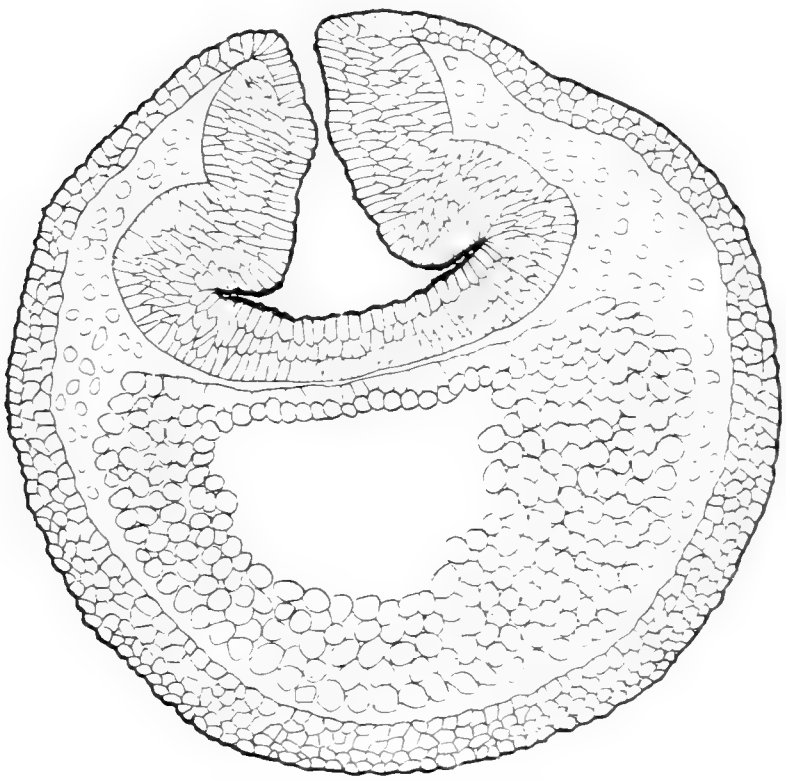
28 : iii : '13.—Segmentation has been normally completed. The grey patch is still visible.

8 : iv : '13.—Many of the tadpoles are retarded in development and have failed to hatch. Those which have hatched are inert.

All were preserved in picric acid.

Of these tadpoles 28 are normal in appearance, 15 abnormal.

TEXT-FIG. 3 *b*.



I. 1, 3 : iv : '13 (a). Optic vesicles and fore-brain. Lumen reduced. The pharynx below.

Of the latter 6 of type (a) resemble externally G. 1. 8 : iv : '13 (a), 4 of type (b) resemble G. 1. 8 : iv : '13 (b), while types (c), (d), (e), (f) and (g) are represented by one each. Suckers and mouth are absent.

G. 2. 8 : iv : '13 (a) (Pl. 12, fig. 46).—The head ectoderm is considerably vacuolated, and so are the mesodermal cells.

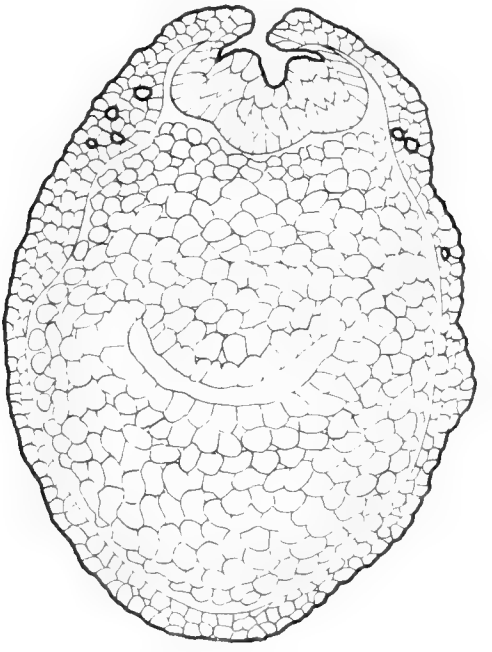
There are no olfactory pits, but two masses of vacuolated cells are found, with nerve-fibres differentiated, each hollow, and connected with the ectoderm by cell-strands.

The fore- and mid-brains and eyes are represented by paired and

median masses of pigmented vacuolated cells. Some of the nuclei have the characters of those of neuroblasts, and nerve-fibres can be seen. The remains of the fifth and seventh ganglia and nerves are also distinguishable.

The brain begins as a solid mass with longitudinal and transverse fibres at this level, and immediately behind, opposite the auditory vesicles, a lumen appears. The hind brain and spinal cord have the structure seen already in G. 1. 8 : iv : '13 (b). The spinal ganglia are

TEXT-FIG. 3 *c*.



L. 1, 6 : iv : '13 (a). Optic vesicles and fore-brain. Very much reduced. The pharynx below. The ectoderm (epidermis) is thickened and pitted.

pressed down and may meet below. The ganglia of viii and ix and x are present. The auditory vesicles are large, the cells vacuolated.

There is no trace of the trabeculæ, but the visceral skeleton is represented by a median plate below the throat bearing three pairs of branchial arches—better developed on one side than on the other—and produced in front into a ring-shaped cartilage which seems to represent the quadrate. To this skeleton are attached bundles of myoblasts, especially anteriorly. Of gill-clefts, the hyo-mandibular and first two branchials (the second open) are present on one side, the first three branchials on the other (the second and third open). There are external gills.

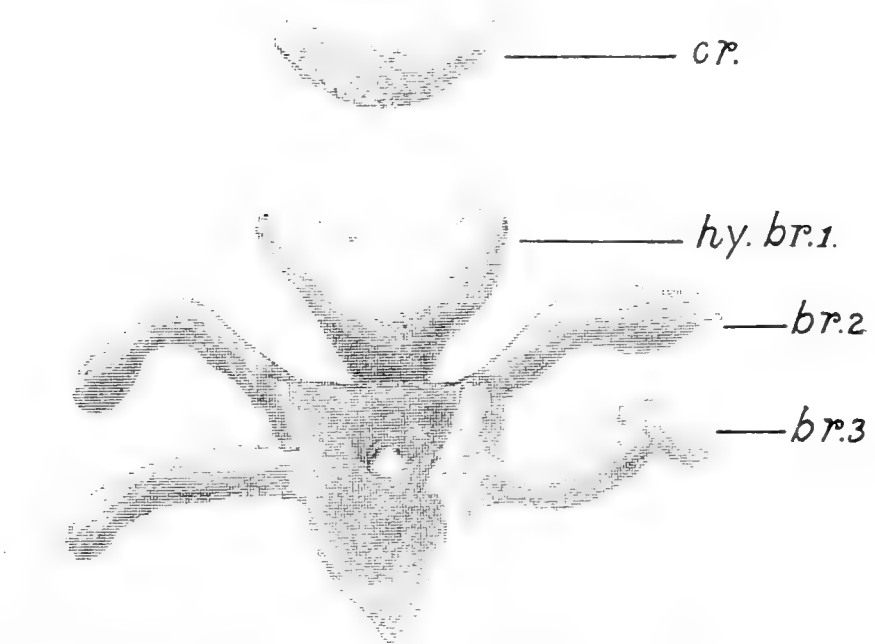
The trachea and lungs have been formed, the heart is small but

twisted. Aortæ and cardinal veins are present. There are three pronephric funnels on one side, but only two on the other. The ducts open into the cloaca. The glomus is absent.

The gut is normal, but the intestine is not yet coiled. There are germ-cells at the root of the mesentery. The myotomes bend down and meet below the spinal cord. In this mass is the notochord, imperfectly differentiated in front, more posteriorly vacuolated. More posteriorly still the notochord is free and the myotomes separate.

The whole body is very œdematous; the connective tissue and the

TEXT-FIG. 4.



Reconstruction of the cranium (*cr.*) and visceral skeleton (*hy. br.* 1, *br.* 2, *br.* 3) of G. 1, 8 : iv : '13 (b).

posterior cardinal vein round the pronephros suffer especially from this accumulation of fluid.

G. 2. 8 : iv : '13 (b) (Text-figs. 8, 11).—Anteriorly the ectoderm is highly vacuolated; so also are the mesodermal cells.

Ventrally the ectoderm is also folded for some little way back.

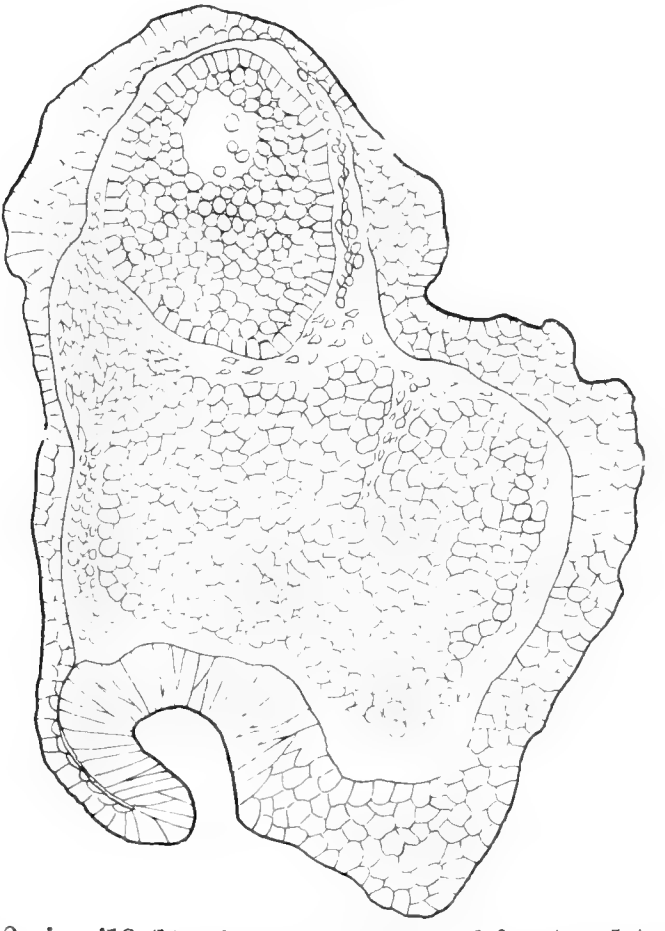
There are no suckers and no mouth. The gills are far forwards. Olfactory pits, eyes, fore- and mid-brains represented only by a heap of pigmented vacuolated cells, with traces of fibres.

The auditory vesicles with ductus endolymphatici. At this level the brain begins. The hind-brain and the spinal cord have the same characters as in (a), but the posterior part of the spinal cord is normal. The auditory, vagus, and spinal ganglia are present, the spinal ganglia being united ventrally.

The visceral skeleton—the only skeleton developed—consists of a median plate bearing three arches, the first, second and third, and an anterior prolongation, situated in the front wall of the pharynx, which represents hyoid and perhaps quadrate elements.

The hyomandibular cleft is absent. The first, second, third, and fourth branchial clefts are present on both sides, the fourth being just open on one side, while on the other the second and fourth are open.

TEXT-FIG. 5.



H. 3. 2 : iv : '13 (b). Not very abnormal front end (section oblique). Brain, neural crest, pharynx, one sucker cut.

There are external gills.

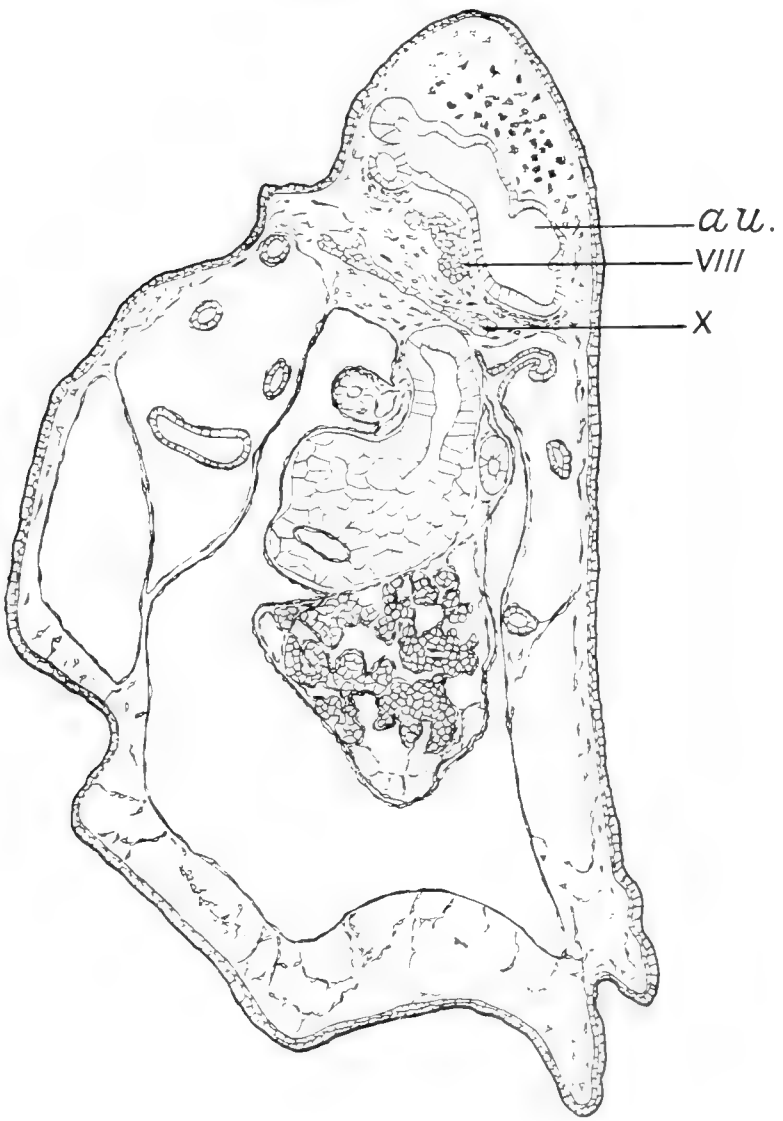
The trachea and lungs are formed, but the trachea is solid.

The heart is also solid, small, and hardly twisted. Aortæ and cardinal veins are formed. The pronephros has three funnels on each side, and a glomus. The ducts open to the cloaca.

The intestine is not differentiated into regions, and in the yolk-cells is a central mass where there are no cell-divisions and the granules are fused into a coagulable liquid.

Germ-cells are found at the root of the mesentery. The myotomes are fused below the spinal cord. The notochord is only distinguishable behind the pronephros. Here it lies immediately ventral to the spinal cord.

TEXT-FIG. 6.



G. 2. 8 : iv : '13 (g). Degenerate brain (pigmented cells). The auditory vesicles united (*au.*); below them the auditory and vagus ganglia (*viii, x*) also united. Œsophagus, lungs, liver, pronephros (one funnel cut on right). Œdematous connective tissue.

In the cœlom and in the posterior cardinal vein round the pronephros is an accumulation of fluid.

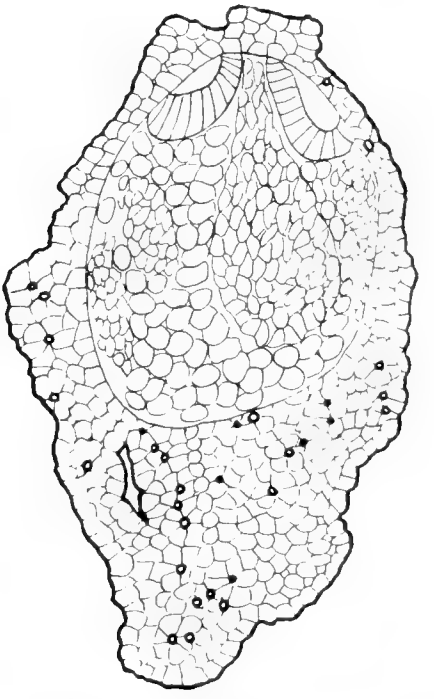
G. 2. 8 : iv : '13 (c) (Text-fig. 10, Pl. 11, fig. 21).—Body much

swollen; tail well developed. Neither mouth nor suckers. The head ectoderm is vacuolated and the mesoderm. The mesoderm s œdematous. Olfactory sacs, eyes and front part of brain represented by an aggregation of pigmented and vacuolated cells, with traces of the ganglia of v and vii, but no nerve-fibres. Hind brain, spinal cord and spinal ganglia as in (a); the spinal cord abnormal to the end.

The auditory vesicles and the auditory and vagus ganglia are present.

A small nodule of perichondrium in the front wall of the pharynx is

TEXT-FIG. 7.



I. 1. 4 : iv : '13 (d). Auditory invaginations. The solid wedge of cells in the middle of the larger endoderm cells is the degenerating fore- and mid-brains. Ectoderm thick and pitted (cut somewhat tangentially on the ventral side).

the only representative of the visceral skeleton. There is no trace of the cranium.

The pharynx has an irregular cavity, but there are no gill-clefts.

The trachea and lungs are present, but the latter are short.

There is no heart.

The pronephros has only two funnels on each side, and there is no glomus. The ducts are open to the cloaca.

The stomach and duodenum are very small.

Germ-cells are present in the mesentery.

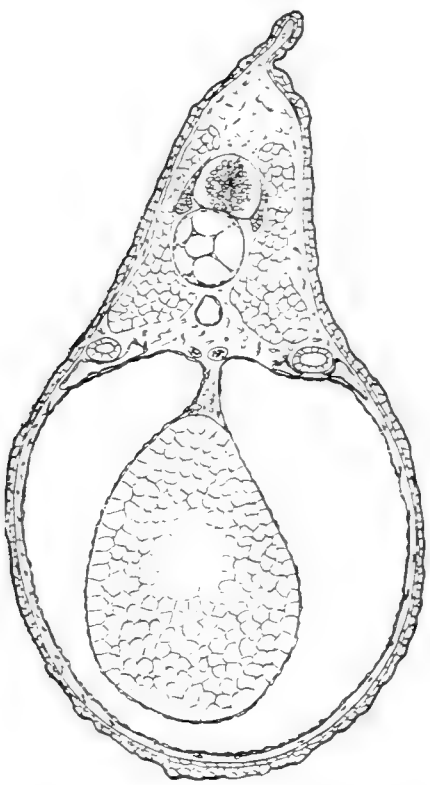
The myotomes, which are fused below the spinal cord, are poorly developed in front.

There is no notochord except in the tail.

There is great œdema of the connective-tissue spaces, of the posterior cardinal veins round the pronephros, of the cœlom, and of spaces (blood-vessels or lymphatics) around the stomach and duodenum.

The ectoderm is distended and flattened.

TEXT-FIG. 8.



G. 2. 8 iv : '13 (b). Hind-end, normal, except for central mass of undivided yolk in intestine and absence of lumen.

G. 2. 8 : iv : '13 (d) (Text-fig. 14 *a*, *b*, Pl. 11, fig. 22).—The body is very stunted; there is a short tail. The blastopore is widely open, and the yolk-plug protrudes.

At the front end the ectoderm is folded, pitted and highly vacuolated.

Internally the front end is occupied by a large cavity with a lining of flattened mesoderm cells. The cavity is probably persistent blastocœl. The hinder wall of this space is occupied by a mass of undifferentiated mesodermal and yolk-cells. Further back are traces of cœlom and blood-vessels.

There is no sign of nervous system, notochord, or myotomes.

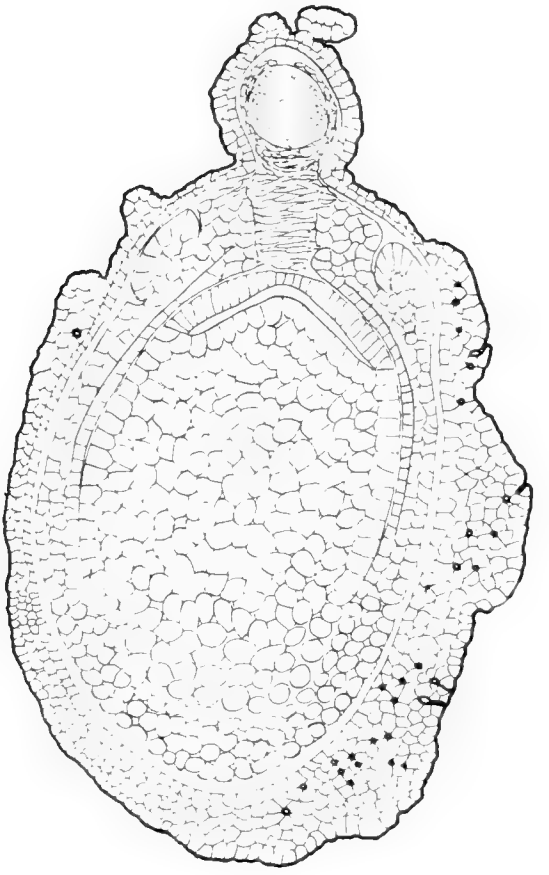
G. 2. 8 : iv : '13 (e) (Text-figs. 12, 13, Pl. 11, fig. 23, Pl. 12, fig. 44 *b*). —Body short; short tail; persistent large yolk-plug. No mouth; no suckers.

Ectoderm of head folded and wrinkled; highly vacuolated.

No olfactory pits nor eyes; no fore- nor mid-brain. These structures are represented by a mass of pigmented cells.

Ganglia of v and vii not to be found. The auditory and vagus ganglion pairs each united across the middle line.

TEXT-FIG. 9.



I. 1. 4 : iv : '13 (d). Thick and pitted ectoderm. Spinal cord solid. Notochord not properly differentiated. Pronephric ridge.

Auditory vesicles well formed, each constricted into two cavities.

The brain begins at the level of the auditory vesicles; it is solid, with fibres ventrally.

The spinal cord has similar characters, but a lumen appears in it here and there. The spinal cord does not reach the hind end of the body. The spinal ganglia are united below.

Fore-gut, but no gill-slits. No lungs.

Gut not much differentiated. In the yolk-cells a central mass without cell-boundaries; here the yolk-granules are fused.

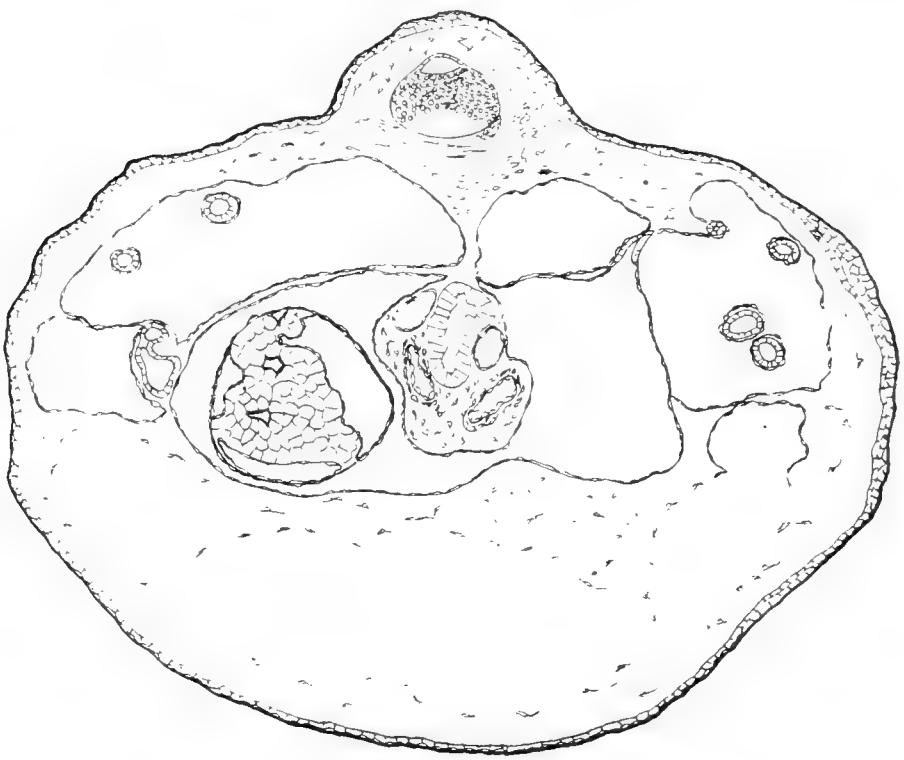
Blood-corpuscles are being formed from the yolk-cells ventro-laterally.

A pericardium is present, but the heart is represented only by a solid cell-mass projecting into this cavity from above. Vitelline veins and cardinal veins are present.

The pronephros has three funnels on each side, and a small glomus. The ducts end blindly.

The pronephric tubules are enormously swollen.

TEXT-FIG. 10.



G. 2. 8 : iv : '13 (c). Pronephros, one funnel cut on right. Abnormal medulla. Fusion of myotomes; no notochord. Œsophagus. Lungs. Liver. Enlargement of posterior cardinal vein and of lymphatics round liver. Œdematous connective tissue.

The myotomes are united across the middle line by a mass of fusiform myoblasts, some of which are vacuolated. No notochord, except for an occasional vacuolation.

The peritoneal cavity is well developed.

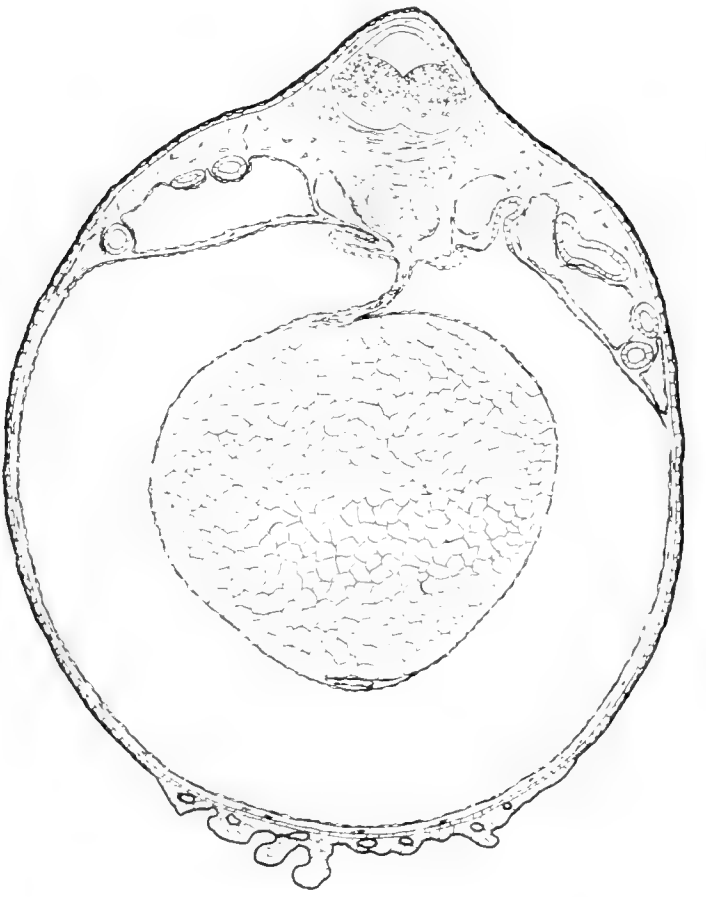
G. 2. 8 : iv : '13 (f).—Body very short and undifferentiated. At one end, presumably anterior, the ectoderm is highly vacuolated and wrinkled. At this end is what appears to be a yolk-plug, but is in reality a yolk-burst.

There is a small archenteron posteriorly opening by the blastopore, a rudimentary pericardium, and the mesoderm is to some extent differentiated—as myoblasts, connective tissue and blood-corpuscles.

No trace of nervous system, nor of sense-organs, nor of notochord, nor of pronephros.

G. 2. 8 : iv : '13 (g) (Text-fig. 6).—Body short, neither mouth nor

TEXT-FIG. 11.



G. 2. 8 : iv : '13 (b). Pronephros, one funnel cut on right, glomus. Cœlom much enlarged. Myotomes fused below medulla. Ventral ectoderm folded.

suckers. Tail with dorsal and ventral fins. There is a side branch to the tail but this does not receive any spinal cord.

The anterior ectoderm is much vacuolated. Neither olfactory pits nor eyes. Fore- and mid-brains represented by a mass of vacuolated pigmented cells. Ganglia of v and vii present, with the nerves developing.

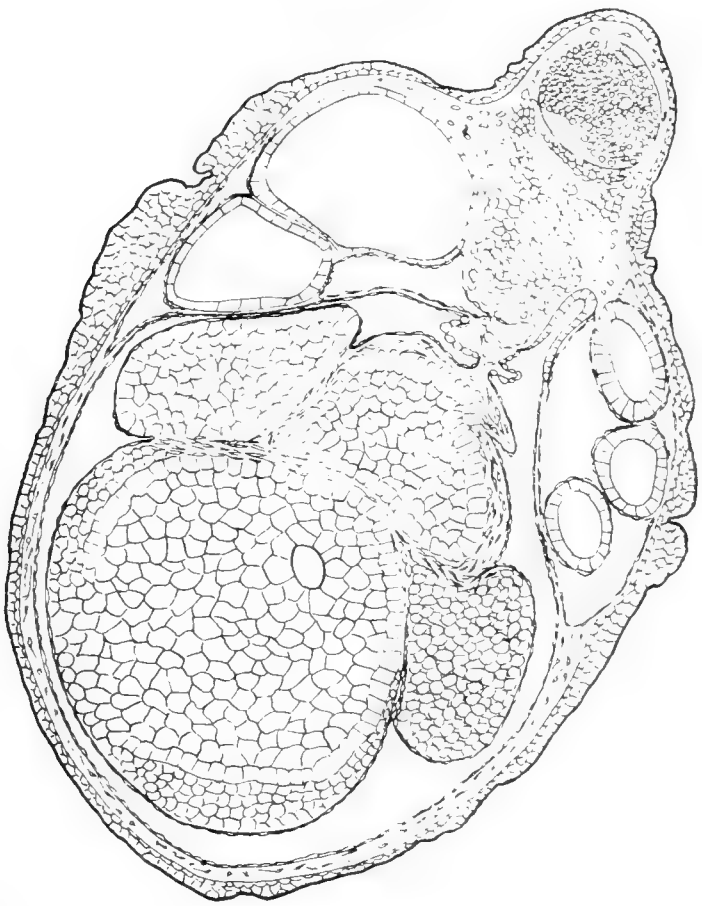
Auditory and vagus ganglion pairs each united below the brain.

The hind-brain, with fibres and neuroblasts stretching across the

thick floor, begins behind the auditory vesicle; the thin roof is much folded. The spinal cord, which has similar characters and a small lumen, is continued into the tail. The ganglia are united below the cord.

The auditory vesicles are large and united, with their cavities also in communication, above the hind-brain.

Text-fig. 12.



G. 2. 8 : iv : '13 (e). Spinal cord nearly solid. Pronephric tubules much swollen, especially on left.

There are external gills, and gill-clefts, two on one side, probably the first and second branchial, the second being open, and three on the other sides, probably the first, second and third branchial, the last two being open.

The visceral skeleton is represented by two pieces: one, anterior to the pharynx and even dorsal to it, probably represents the hyoid and first branchial arches. Muscles (myoblasts) are attached to it. The other is below the pharynx and bears a pair of arches, probably the second branchial.

Trachea and lungs.

Heart and pericardium, the heart straight.

Pronephros with three funnels on each side, the ducts open. The glomus much enlarged.

Stomach and intestine differentiated, liver present, proctodæum open. A central mass of fused granules in the yolk of the intestine.

The myotomes are united below the medullary tube by elongated myoblasts, but there is no trace of a notochord.

Germ-cells at the root of the mesentery.

The connective tissue is œdematous and the posterior cardinal veins much distended.

3. One and a half hours after insemination, centrifuged for 17½ minutes.

A whitish-yellow ring appears round the grey patch.

Segmentation in early stages is normal, except for the inequality of the first or second division, or both.

28 : iii : '13.—A grey and yellow patch is still visible. The animal hemisphere is segmented in some, but not in all. The vegetative hemisphere is segmented in none.

8 : iv : '13.—All the eggs are dead, undeveloped.

4. One and a half hours after insemination, centrifuged for 28 minutes.

As the last; folds appear in the grey patch.

Segmentation is more abnormal than in the last.

28 : iii : '13.—Like the last.

8 : iv : '13.—All the ova dead, undeveloped.

H.

Centrifuged, 28 : iii : '13, on the water-driven machine, about one hour after insemination.

1. For 10 minutes at speed III.

A grey and yellow patch, with folds, appears round the animal pole.

29 : iii : '13.—The yellowish patch is still present. The animal hemisphere is segmented, but the vegetative is not; it is blotched with pigment.

30 : iii : '13.—No blastopore has appeared.

2. For 10 minutes at speed II.

There is a grey patch surrounded by a yellowish ring.

29 : iii : '13.—The animal hemisphere, which still shows the grey patch, is segmented, but the vegetative is not; it is blotched with pigment.

30 : iii : '13.—There is no blastopore.

3. For 10 minutes at speed I.

A grey patch appears round the animal pole, but there is no yellowish margin.

29 : iii : '13.—The grey patch is still present.

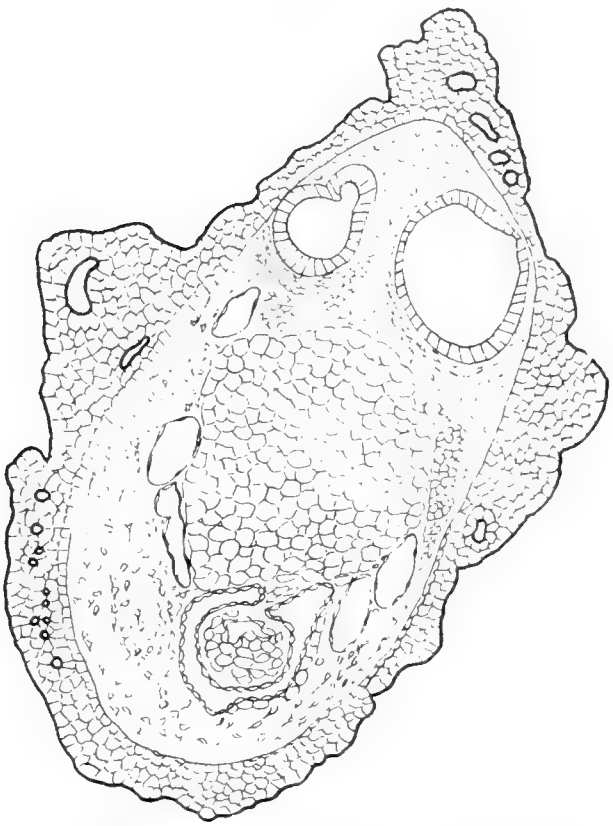
The animal hemisphere is well segmented. The vegetative hemisphere is also certainly segmented in some of the ova. It is blotchy.

30 : iii : '13.—A semicircular blastopore is present.

2 : iv : '13.—Four of the embryos are dead in an early stage. Of the others, some are normal, some abnormal, and of two types, (a) and (b). All these embryos were preserved in picric acid.

H. 3. 2 : iv : '13.—The normal embryos.

TEXT-FIG. 13.



G. 2. 8 : iv : '13 (e). Heart small, fore-gut solid. Auditory vesicles well-formed. Ectoderm very thick and pitted.

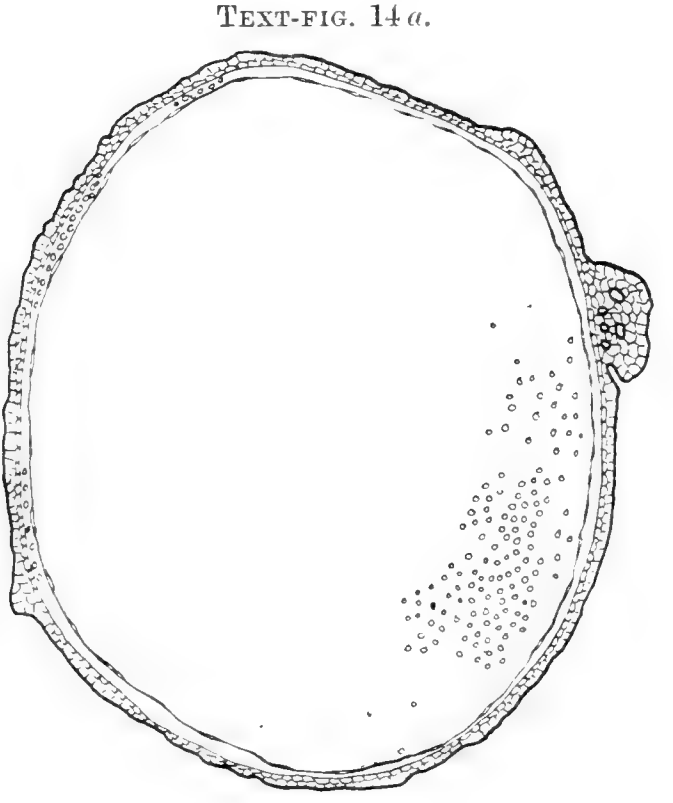
There is a short tail stump. Stomodæum and proctodæum. Suckers. Sections show olfactory pits, optic vesicles, lens thickenings, auditory vesicles in an early stage of invagination, endothelial cells of heart, paired pericardium, pronephric ridge, sclerotome, notochord and sub-notochordal rod. The only abnormality is the mass of fused granules in the centre of the yolk-cells of the gut.

H. 3. 2 : iv : '13 (a) (Pl. 11, fig. 24).—The front end is normal, with olfactory pits, optic vesicle, lens thickening, auditory vesicle, stomodæum, pituitary body, suckers, paired pericardium, endocardial

cells, pronephric ridge, sclerotome, notochord and subnotochordal rod. In the centre of the yolk is a mass of fused granules. Posteriorly there is an exposed yolk-plug bounded above by the tail—containing spinal cord, notochord and mesoderm—and in front and on the left by a small knob which may be a second tail, inasmuch as it contains two rounded cell masses and some mesoderm.

H. 3. 2 : iv : '13 (b) (Text-fig. 5, Pl. 11, fig. 25).—The anterior end is abnormal. The ectoderm here is highly vacuolated, the olfactory pits

TEXT-FIG. 14 *a*.



G. 2. 8 : iv '13 (d). Section of front end through the enlarged head-vesicle lined by a thin layer of mesoderm and containing some scattered mesoderm cells. This is the persistent segmentation cavity.

very shallow, the fore- and mid-brain represented by a solid mass of vacuolated cells, and there is no sign of the infundibulum nor of the optic vesicles. The neural crest goes forwards into this region. The lumen of the medullary tube appears first in the hind-brain just in front of the auditory vesicles, and is continued into the spinal cord, which is normal.

The auditory vesicles are just invaginated, ductus endolymphatici being present.

There is no stomodæum; the suckers are very far forwards.

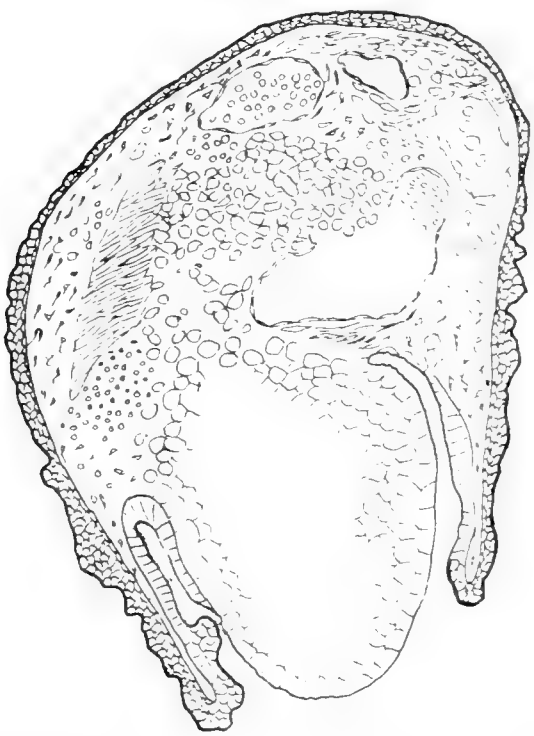
Fore-gut with incipient gill outgrowths, but no clefts.

Heart-tube between the two sides of the paired pericardium. Slight peritoneal cavity, pronephric ridge, sclerotome, notochord and subnotochordal rod.

What appears to be an enlarged liver outgrowth extends forwards below the heart.

A mass of fused yolk-granules in gut. Hind-end normal with tail gut and neurenteric passage (a streak of pigmented cells).

TEXT-FIG. 14 *b*.



Posterior end of the same as Text-fig. 14 *a*, through the yolk-plug, with deep blastoporic involution. Some myoblasts and blood-vessels have been differentiated.

I.

Centrifuged 31 : iii : '13 on the water-driven machine at speed IV.

1. Fifty minutes after insemination, centrifuged for 10 minutes.

A grey patch appears round the animal pole; it is surrounded by a yellowish-white ring, and in its centre is a yellowish spot. Segmentation is normal.

2 : iv : '13.—The grey patch is still visible. There is a crescentic blastopore.

3 : iv : 13.—The grey patch is still present.

Medullary folds formed, blastopore widely open and yolk-plug protruding. Three embryos preserved, (a), (b) and (c).

I. 1. 3 : iv : '13 (a) (Text-fig. 3*b*, Pl. 11, fig. 26).—The anterior ectoderm thickened and vacuolated. The brain-cells are also vacuolated. The optic vesicles are abnormally thick-walled, and have an abnormally narrow lumen.

Nerve crest, sense-plate and gill-plate present. The notochord is distinct.

In the yolk is a central mass of fused granules. The yolk-plug is lateral (the blastopore has closed on the right, and the medullary tube has grown back).

I. 1. 3 : iv : '13 (b) (Pl. 11, fig. 27).—Anterior ectoderm thickened and vacuolated. The medullary groove shallow, deeper in front, but the optic vesicles have not yet been evaginated. There is a neural crest. The notochord is hardly distinct from the mesoderm.

There is a central mass of fused yolk-granules in the gut.

I. 1. 3 : iv : '13 (c) (Text-fig. 3*a*).—The anterior ectoderm and brain vacuolated as in (a). The optic vesicles have a lumen of nearly normal size. There is a neural crest, and the notochord is beginning to be vacuolated. The vertebral plate is beginning to be separated from the lateral plate.

There is a central mass of fused yolk-granules in the gut.

4 : iv : '13.—Some are normal or nearly so, but many have large, persistent yolk-plugs.

All these embryos were preserved.

Three are apparently normal, (a), ten abnormal. Of the latter there are seven of type (b), and one each of types (c), (d) and (e).

I. 1. 4 : iv : '13 (a).—Normal except for the vacuolation of the head ectoderm and the fusion of yolk-granules in the centre of the yolk-cell mass.

Olfactory pits, optic vesicles, slight lens thickening, auditory invaginations, stomodæum, pituitary body, notochord, pharynx, gill-outgrowths, pericardium and heart-tube, suckers, liver, pronephric ridge, slight peritoneal cavity, subnotochordal rod, proctodæum and neurenteric streak of pigmented cells.

I. 1. 4 : iv : '13 (b) (Pl. 11, fig. 28).—Like (a), except for the yolk-plug.

I. 1. 4 : iv : '13 (c) (Pl. 11, fig. 29).—Head ectoderm vacuolated. The front part of the brain degenerate, but the optic vesicles can be distinguished as small, almost solid outgrowths.

Shallow olfactory pit, auditory invaginations. Suckers absent.

There are indications of a pericardium, but there is no heart.

The notochord is distinct. There is a pronephric ridge. The hind-

end is fairly normal except for the large yolk-plug. The spinal cord ends in the middle line, it does not pass into the caudal swellings.

There is fusion of yolk-granules in the centre of the yolk-cells.

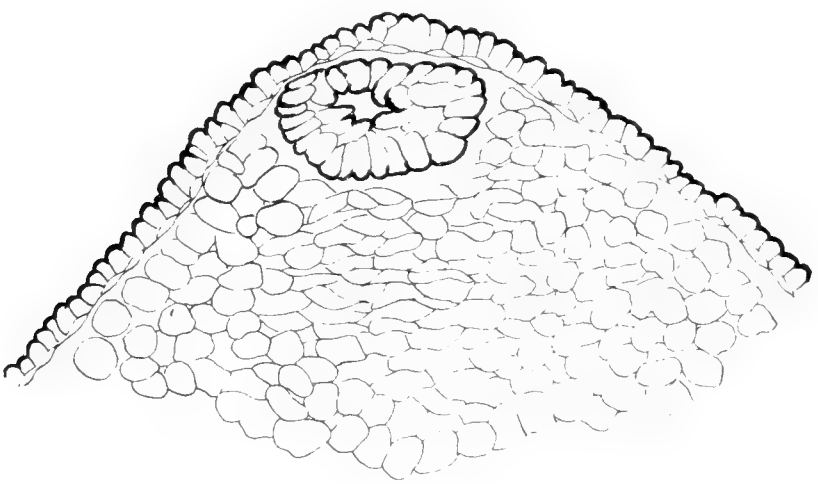
I. 1. 4 : iv : '13 (d) (Text-figs. 7, 9).—The head ectoderm is highly vacuolated, thickened and pitted.

The fore- and mid-brains are represented only by a mass of pigmented cells which are mingled with an aggregation of yolk-containing cells, the front wall of the fore-gut.

In this region are found the two auditory ingrowths.

The medullary tube begins behind the latter as a solid cord, but further back a lumen appears, nearer the dorsal than the ventral side.

TEXT-FIG. 15.



L. 1. 6 : iv : '13 (c). Spinal cord very small. Notochord not differentiated from the mesoderm. The mesoderm of the vertebral plates united across the middle line by cells which are beginning to elongate.

The spinal cord is similar, but anteriorly the lumen is only present here and there; at the hind end it is better developed.

Suckers, heart, and pericardium are absent.

The neural crest is lateral, not ventral.

The pronephric ridge is well developed. There is no notochord; the myotomes are united across the middle line by horizontally elongated myoblasts. This median cell-mass is, like the myotomes, segmented.

The gut has a lumen. There is a mass of fused yolk-granules. The yolk-plug protrudes.

I. 1. 4 : iv : '13 (e) (Text-fig. 16, Pl. 11, fig. 30).—The anterior ectoderm is very thick, vacuolated and pitted.

No fore-brain nor mid-brain, nor even auditory vesicles. There are no suckers. The medullary tube is narrow, almost solid, and passes into one lip of the widely open blastopore, round one side and there ends. There is no notochord. Dorsal and ventral mesoderm are present, but the former is not differentiated into vertebral and lateral plates.

Neither heart nor pericardium are found, nor a pronephric ridge.

At the ventral lip of the blastopore is a short ectodermal involution, presumably the proctodæum.

The gut has a narrow crescentic lumen.

2. Seventy-five minutes after insemination, centrifuged for 30 minutes.

There appears a yellow-grey patch round the animal pole; it is much folded. Round it is a double whitish ring, and round this again a grey ring.

Segmentation is abnormal. The first and the second divisions are not meridional, and the grey patch may be cut off as a separate "cell."

Some fail to segment.

2 : iv : '13.—Only one or two have segmented and developed further; the blastopore, if present, is a wide semicircle.

3 : iv : '13.—No further development. Cellular disintegration setting in.

The zones are still distinguishable.

I. Controls, 2 : iv : '13.—Semicircular blastopore.

J.

Centrifuged, 1 : iv : '13, on the water-driven machine, at speed I, 50 minutes after insemination.

1. For 10 minutes (Pl. 7, fig. 7.)

2. For 20 minutes.

The results are similar in the two cases. A faint grey patch appears round the animal pole with a margin of a rather lighter colour.

Segmentation is perfectly normal.

8 : iv : '13.—The tadpoles are ready to hatch, and as normal as the controls.

K.

Centrifuged 2 : iv : '13 on the water-driven machine at speed II, 70 minutes after insemination.

1. For 10 minutes (Pl. 9, fig. 13).

A grey patch appears round the animal pole with central, or sometimes excentric, yellowish-white spots, and surrounded by a lighter marginal ring.

Segmentation is normal.

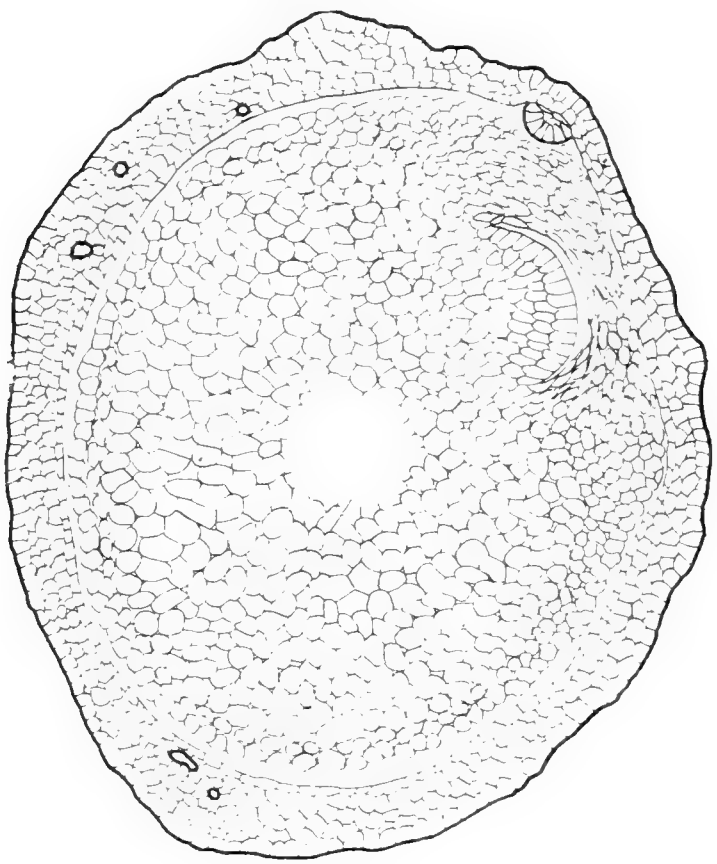
2 : iv : '13.—The grey patch is still visible. The ova appear to be as well segmented as the controls.

3 : iv : '13.—The grey patch is faintly visible. The blastopore is three quarters of a circle.

4 : iv : '13.—The medullary folds are closing. Fairly normal.

6 : iv : '13.—Fairly normal.

TEXT-FIG. 16.



I. 1. 4 : iv '13 (e). Spinal cord reduced to a minimum. Gut lumen very narrow. Dorsal and ventral mesoderm differentiated, but no notochord. Ectoderm thickened and pitted. Central undivided yolk-mass.

8 : iv : '13.—Fairly normal, with stomodæum, suckers, nostril, gills, tail and fin.

These tadpoles were not preserved.

2. For 20 minutes.

The grey patch becomes folded. The marginal ring is more marked, and may be confluent with the spots inside the grey patch.

Segmentation is not normal, the first and second furrows not being meridional, and the rate of division is retarded.

2 : iv : '13.—The animal hemisphere alone is segmented. It is streaked with white or grey. The vegetative hemisphere is blotched with pigment.

3 : iv : '13.—The blastopore has not yet appeared.

4 : iv : '13.—The embryos resemble those of I. 1. 3 : iv : '13. The medullary folds are developed, the yolk-plug is exposed. The grey patch is still to be seen at the anterior end.

6 : iv : '13.—Dead in an early stage.

These embryos were not preserved.

L.

Centrifuged 1 : iv : '13 on the water-driven machine at speed III.

1. Forty minutes after insemination, centrifuged for 5 minutes.

The grey patch round the animal pole has a whitish spot in the centre and is surrounded by a faint marginal ring.

Segmentation is normal in form but slightly retarded on the controls.

2 : iv : '13.—The grey patch and whitish spot are still visible. Segmentation has proceeded not quite as far as in the controls.

3 : iv : '13.—The grey patch is still present. In some eggs the dorsal lip of the blastopore has appeared.

4 : iv : '13.—The blastopore is circular, the yolk-plug large and protruding.

6 : iv : '13.—There is a tail stump. The blastopore is open in many.

Twelve of these embryos were preserved.

Of these, 2 are normal, with nostrils, suckers, stomodæum and incipient gill-clefts, 9 are monstrous, and 1 undeveloped. Of the 9 monstrous embryos, 1 is of type (a), 1 of type (b), 1 of type (c), 2 of type (d), 1 of type (e), 1 of type (f), and 2 of type (g).

L. 1. 6 : iv : '13 (a) (Pl. 11, fig. 31, Text-fig. 3 *c*).—Anteriorly the ectoderm is very thick and vacuolated. A solid in-growth of this represents the fore-brain, produced into minute optic vesicles.

There are traces of olfactory pits.

The in-growth of ectoderm becomes grooved behind this point, and the groove deepens : this is the mid-brain. Then the groove closes, in the region of the hind-brain.

The auditory vesicles are in an early stage of invagination.

There are no suckers.

The heart and pericardium are not formed yet.

The notochord is normal, slightly vacuolated.

The mesoderm is also normal, with somites, lateral plate and pronephric ridge.

There is a small lumen to the gut, open at the blastopore. There is no proctodæum.

The medullary tube and notochord pass to one side of the protruding yolk-plug. Neural crests small. In the yolk-cells there is a central mass of fused granules.

L. 1. 6 : iv : '13 (b).—The vacuolated anterior ectoderm is very thick and crinkled.

Neither nostrils nor suckers are present.

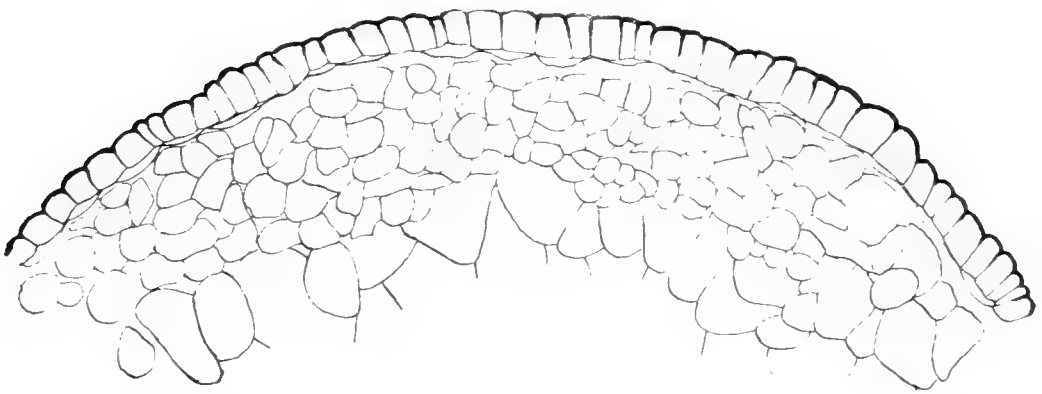
There is hardly any medullary tube in front of the auditory invaginations, and that is solid.

The cells of the hind-brain are vacuolated, the spinal cord is normal. The neural crests are small.

There are no gill-clefts.

There is a pericardium but no heart.

TEXT-FIG. 17.



L. 1. 6 : iv : '13 (g) (1). No nervous system. No differentiation of the notochord from the dorsal mesoderm.

Somites, lateral plate and pronephric ridge are present.

The notochord goes as far forwards as the auditory vesicle.

The proctodæum is open.

There is a mass of fused yolk-granules in the yolk-cells; the large yolk-plug is one-sided.

L. 1. 6 : iv : '13 (c) (Pl. 11, fig. 32, Text-fig. 15).—Anterior ectoderm thick and vacuolated.

No olfactory pits, no auditory vesicles, no suckers.

The medullary tube very asymmetrical in front; this is presumably the hind-brain, but in the absence of the auditory vesicles it is impossible to say with certainty.

The lumen of the medullary tube is very small and absent in places. Behind is an open medullary groove.

The notochord is not distinguishable in front, barely so behind.

There is a pericardium, but no heart.

The pronephric ridge is indicated.

The fore-gut is much folded and crumpled.

The gut lumen disappears behind.

There is the usual mass of fused granules in the middle of the yolk-cells, and a large yolk-plug.

L. 1. 6 : iv : '13 (d) (1) (Pl. 11, fig. 33).—Medullary tube and notochord both absent. On one side of the large yolk-plug is a slight protrusion: this is probably dorsal and represents the tail. The mesoderm in this tail is continued forwards into the body; ventral mesoderm is being differentiated.

There is no cœlom.

There are no suckers.

The anterior ectoderm is thickened, pitted and vacuolated, and there is a central fusion of yolk-granules. The gut is limited to the small archenteric cavity round the yolk-plug.

L. 1. 6 : iv : '13 (d) (2).—This is similar to the last. In the dorsal mesoderm there are indications of the differentiation of a median tract, the notochord.

L. 1. 6 : iv : '13 (e) (Pl. 11, fig. 34).—Like (d), except that the blastopore is closed, and the tail protuberance bilobed.

Of the two pits seen at the base of this tail one is quite superficial, a mere fold of ectoderm, while the other is a deep involution which passes in some way to end blindly. It is the proctodæum.

The vacuolated ectoderm is at the other (anterior) end.

L. 1. 6 : iv : '13 (f) (Pl. 11, fig. 35).—This is similar to (e) except that the rudiment of the tail is not bilobed. Of the two depressions at its base, one is a mere superficial folding, the other a deep passage leading into a cavity lying behind the yolk-mass, a rudimentary archenteron.

L. 1. 6 : iv : '13 (g) (1) Text-fig. 17).—This resembles (d) (2). There is an indication of the differentiation of the notochord—as a median band of smaller cells—from the dorsal mesoderm. There is a rudimentary archenteron opening under the dorsal lip of the blastopore.

L. 1. 6 : iv : '13 (g) (2).—Similar to the last and to (d) (1). There is no trace of a separation of the notochord.

8 : iv : '13.—The remainder were preserved.

One is undeveloped, 2 are normal in form with the tail longer than before, and 9 are monstrous. Among these tadpoles types (a), (b) and (e), (f) and (g) are represented each by one, types (c), (d), each by two specimens.

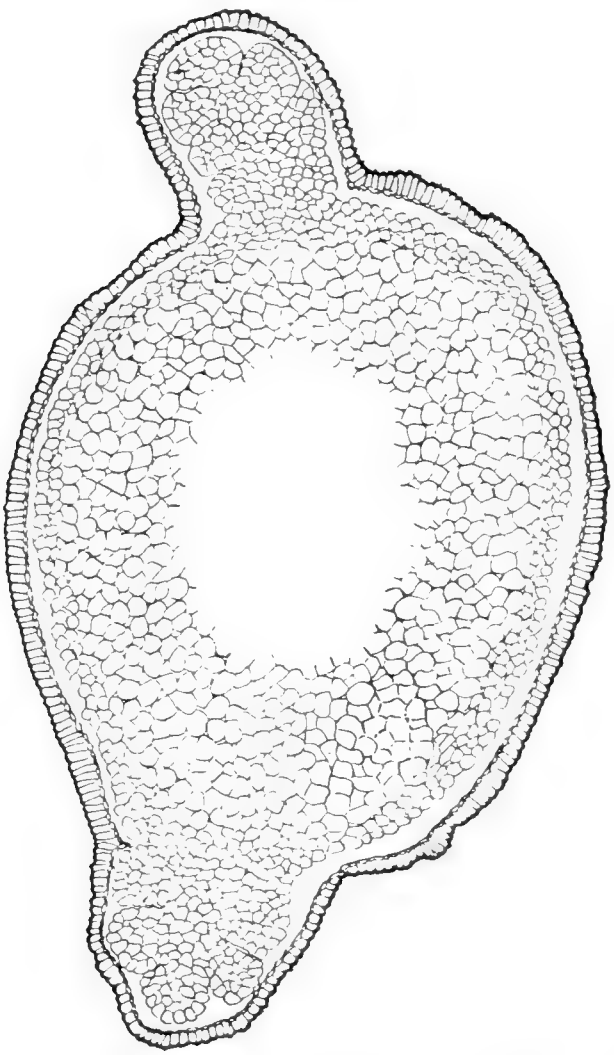
L. 1. 8 : iv : '13 (a) (Pl. 11, fig. 36).—The anterior ectoderm is very thick and pitted, and highly vacuolated.

There is no nervous system nor sense-organs. From the dorsal lip of

the blastopore a thick band of mesoderm stretches forwards; in front this becomes thinner. There is no trace of a notochord in it.

There is ventral mesoderm and the beginning of a pericardium.

TEXT-FIG. 18.



L. 1. 8 : iv : '13 (g). Both tails are cut. Signs of segmentation of the mesoderm in them. In the body mesoderm has been differentiated from the yolk-cells, in which is a central undivided mass of fused yolk-granules.

There is no sucker.

The blastopore is widely open; on one side is a deep involution, but except for this no gut. The yolk-plug is large. In the centre of the yolk there is a mass of fused granules.

L. 1. 8 : iv : '13 (b) (Pl. 11, fig. 37).—The head ectoderm is

vacuolated and thick. The fore-brain, with the optic vesicles and infundibulum, is present. The mid-brain is also present, and the hind-brain and spinal cord. All parts of the brain show cell-vacuolation.

Auditory vesicles, and ganglia, vagus ganglia, and ganglia of v and vii all formed, and neural crest. The suckers are present.

The notochord, normally vacuolated, extends to in front of the auditory vesicles. Both it and the spinal cord pass into the tail.

There is no stomodæum. The gill-slit outgrowths are solid.

The pericardium is large, but the heart rudiment is solid. The peritoneal cavity is still small. The pronephric tubules are developing, but are still without lumina and funnels. There is a liver diverticulum, but the lumen of the gut is obliterated.

There is fusion of yolk-granules in the yolk-mass, and a large yolk-plug.

L 1. 8 : iv : '13 (c) (1).—This resembles (a), but there is a deep involution underneath each lateral blastoporic lip. Otherwise there is no gut.

There are signs of the differentiation of a notochord.

There is no pericardium in the ventral mesoderm. The nervous system is absent, the longitudinal pleats looking like medullary folds being merely ectodermal ridges.

L. 1. 8 : iv : '13 (c) (2) (Pl. 11, fig. 38).—Like the last, but a rudimentary nervous system is present. A solid median mass of ectodermal cells with paired appendages represents the brain and ganglia. Imbedded in this are two small auditory vesicles. Behind this point a solid spinal cord runs back and enters one of the caudal swellings.

The dorsal mesoderm is a thick plate; it shows no signs of a notochord. The ventral mesoderm is present, but neither pericardium nor heart. The pronephric ridge is indicated on one side. The gut has no lumen. In the yolk-cells a central fusion of yolk-granules.

L. 1. 8 : iv : '13 (d) (1).—The anterior end can only be determined by the vacuolation of the ectoderm.

The segmentation cavity persists in front and dorsally. Below it is the mass of yolk-cells; its roof is formed of a band of mesoderm cells which passes back into the dorsal lip. This band stops before the front end is reached so that there the segmentation cavity is roofed only by ectoderm.

Ventral mesoderm is being differentiated. There is an open blastopore and persistent yolk-plug; the archenteric cavity is very small. There is neither notochord nor nervous system.

L. 1. 8 : iv : '13 (d) (2).—The segmentation cavity is obliterated and there is an archenteron, narrow, but longer than in the last. Otherwise the same.

L. 1. 8 : iv : '13 (e) (Pl. 11, fig. 39).—Body rounded, bearing a

short tail. No nervous system nor sense-organs. Dorsal mesoderm, but no notochord. Dorso-laterally the mesoderm is thickened and these two thickenings pass back into the tail. In each is a cavity (cœlom).

The ventral mesoderm is developed, but slight. There is an archenteron on the dorsal side with a postero-ventral extension. The latter is in communication with the exterior by an ectodermal involution (proctodæum).

The anterior ectoderm is vacuolated.

L. 1. 8 : iv : '13 (f) (Pl. 11, fig. 40).—The body consists of a solid mass of yolk-cells (in the centre of which is a region in which the granules are fused together), enclosed by a vacuolated ectoderm. On one side of the large yolk-plug is a tail. In this tail are paired segmented somites, with indications of myocœl. There is, however, no notochord. On the opposite side of the blastopore is a much shorter tail-like structure. The nervous system is completely absent. Dorsally and ventrally there are thin layers of mesoderm.

L. 1. 8 : iv : '13 (g) (Text-fig. 18, Pl. 11, fig. 41).—Body stumpy, with two tail-like structures, one on each side of the large blastopore.

Each of these tails contains mesoderm, which shows signs of being divided into pairs of somites. Otherwise this embryo resembles the last.

The pit at the anterior end is a mere infolding of the ectoderm.

2. Forty minutes after insemination, centrifuged for 10 minutes.

The central yellowish spot is surrounded by folds of the grey patch, the marginal white ring broader.

The first two furrows are normal in form, but retarded, not only on the controls, but slightly on L. 1.

2 : iv : '13.—The grey patch and yellowish spot are still visible. The animal hemisphere is as well segmented as in L. 1; the vegetative hemisphere is, however, divided into only a few large cells.

3 : iv : '13.—The grey patch is still present. The dorsal lip of the blastopore has not yet appeared.

4 : iv : '13.—There are indications of a blastoporic groove at the edge of the pigmented area.

6 : iv : '13.—The yolk is still exposed.

3. Seventy-five minutes after insemination, centrifuged for 16 minutes.

The folding of the grey patch round the yellowish spot is more marked; the spot appears to sink in. The marginal ring is more conspicuous and flecked with dark spots.

The first and second segmentation furrows may be normal in form, but there are many irregularities; the furrows of the third phase are also irregular, for instance, parallel to the first. These meridional furrows fail to reach the vegetative pole.

2 : iv : '13.—The grey patch and yellowish-white ring are still visible, but the foldings seem to have been smoothed out.

The animal hemisphere imperfectly segmented, the vegetative not at all.

3 : iv : '13.—No change.

4 : iv : '13.—There is still no sign of a blastopore.

4. Seventy-five minutes after insemination, centrifuged for 25 minutes.

The marginal zone becomes broader still.

Segmentation as in 3, or even more irregular. The yellowish spot may become cut off as a separate "cell," the folds being incorporated in the divisions or else entirely smoothed out.

2 : iv : '13.—The white ring is still visible.

Segmented in the animal hemisphere.

3 : iv : '13.—As 3.

4 : iv : '13.—There is no sign of a blastopore.

6 : iv : '13.—The white ring is now very faint. No other change (Pl. 9, fig. 14).

M.

Centrifuged 2 : iv : '13 one hour after insemination at speed II on the water-driven machine.

1. For 10 minutes.

A circular grey patch appears round the animal pole, containing sometimes a yellowish spot; it is surrounded by a paler border (Pl. 7, fig. 8*a*).

The first and second furrows are meridional as normally, but sometimes either of these divisions is unequal. They appear later than in the controls.

The furrows of the third phase are abnormal in being meridional. All these furrows reach the vegetative pole.

3 : iv : '13.—Segmentation is completed; the cells of the vegetative hemisphere are rather large.

The grey patch has disappeared.

4 : iv : '13.—There is a blastopore, three quarters of a circle or circular; it is rather large.

6 : iv : '13.—The medullary folds are closing (when they have been formed). The blastopore is still open in some cases.

Six embryos were preserved at this stage, one each of types (a) and (b), two each of types (c) and (d).

M. 1. 6 : iv : '13 (a).—Rounded body, yolk-plug still exposed. A slight back-growth of the body on the dorsal side of the blastopore is the beginning of a tail. The archenteron is short and does not extend in front of the middle of the body.

Fusion of yolk-granules occurs.

There is no medullary plate. The dorsal mesoderm is hardly differentiated from the roof of the archenteron. There is no distinct notochord; ventral mesoderm is present.

Anteriorly the ectoderm is thickened but not so remarkably as in later stages. It is slightly pitted.

M. 1. 6 : iv : '13 (b).—The anterior ectoderm is vacuolated. There are no suckers.

Neither olfactory pits nor eyes are present. The auditory vesicles are curved plates, slightly detached from the ectoderm.

A mass of pigmented cells in front probably represents the fore- and mid-brains. Similar lateral masses would be the ganglia of this region, possibly also the optic vesicles. The median mass is continued into a fold, and this passes behind into a solid cord which presently enlarges. The lumen of the hind-brain appears in this. The lumen is dorsally situated. The hind-brain is continued in turn into the spinal cord, in which the lumen is also excentrically dorsal; posteriorly, however, it is in the normal position.

The neural crests come fairly far down but do not meet below the cord.

The vertebral plates are united across the middle line by horizontally elongated cells. There is no notochord.

A pronephric ridge is present and there are indications of a splanchnocœl in the lateral plate.

Ventral mesoderm is present but there is as yet no pericardium.

The enteron is narrow but extends far forwards.

There is a central fusion of yolk-granules.

There is a proctodæum, and a streak of pigment-cells indicates the neurenteric passage.

The yolk-plug has been withdrawn.

M. 1. 6 : iv : '13 (c).—As (a), but there is no back-growth above the yolk-plug, and the archenteron is shorter.

M. 1. 6 : iv : '13 (d).—As (b), but the medullary tube is smaller and the lumen minute.

There is a yolk-plug.

8 : iv : '13.—A tail is developed in most. The head is deficient.

Seven embryos were preserved, two each of types (a) and (c) and one each of types (b), (d) and (e).

M. 1. 8 : iv : '13 (a).—Anteriorly the ectoderm is vacuolated. Here there is a large cavity bounded by a layer of flattened cells. The cavity is the persistent segmentation cavity, the lining cells mesodermal elements which have been differentiated from the yolk-cells and passed into the cavity, while the yolk-cells have remained in their original position.

Posteriorly the mesoderm cells pass into the main mass of yolk-cells in which is the enteron.

There is a central mass of fused yolk-granules.

Dorsal mesoderm is present and differentiated into vertebral and lateral plates. A median notochordal tract is distinguishable from the vertebral plate in places.

Pronephric tubules are in course of formation.

Ventral mesoderm not well developed.

There is no nervous system. What appears from the outside to be such is a median fold of ectoderm enclosing some mesodermal elements. Proctodæum large but not open into enteron.

M. 1. 8 : iv : '13 (b) (Pl. 11, fig. 42).—Anterior ectoderm vacuolated, enclosing a persistent segmentation cavity. In front this is bounded by the ectoderm alone, but further back a thin layer of cells appears lying inside the ectoderm. This layer is continued into the mass of yolk-cells behind. In the latter a central mass of fused granules.

The dorsal mesoderm passes back into the tail, and is there segmented. There is no notochord. Ventrally the mesoderm is poorly developed.

The cavity of the enteron is small and short. The proctodæum opens into it.

There is no nervous system.

M. 1. 8 : iv : '13 (c) (Pl. 11, fig. 43).—As in (a) and (c) there is anteriorly a persistent segmentation cavity. It is not, however, lined in whole or in part by a sheet of mesoderm, but merely includes a certain number of mesodermal cells.

The yolk plug is exposed, and there is a short archenteron opening by the blastopore.

The tail springs from a thick mass of mesoderm lying beneath the lip of the blastopore on one side; there is a similar mass of mesoderm on the opposite side, and the two become confluent in front.

Correct orientation of this embryo is very difficult as there is neither nervous system nor notochord, but assuming that the mesodermal thickenings are lateral the tail is either right or left. The side on which the two masses meet may then be dorsal.

M. 1. 8 : iv : '13 (d).—The anterior vacuolated ectoderm encloses a solid mass of yolk-cells, with peripheral mesoderm. The segmentation cavity does not persist.

The mesoderm becomes concentrated posteriorly into three masses which project wedge-like into the yolk-cells. At the hinder end these three pass into the blastoporic lip, where they become continuous with small cells on the surface of the yolk-plug. Enteron, nervous system and notochord are all absent.

M. 1. 8 : iv : '13 (e).—The anterior vacuolated ectoderm encloses a

solid mass of yolk-cells. Peripherally mesoderm is differentiated. Posteriorly there is a short tail springing from what appears to be the dorsal side, since the archenteron is on this and the proctodæum on the opposite side.

The proctodæum ends blindly.

The tail contains mesoderm which springs from a dorsal median, partly also from a lateral concentration of mesoderm.

Nervous system and notochord both absent.

2. For 20 minutes.

The grey patch is larger. A grey ring appears outside the white ring.

Segmentation as in 1 (Pl. 7, fig. 8, *b*).

3 : iv : '13.—The grey patch is still to be seen.

The vegetative hemisphere is imperfectly segmented.

4 : iv : '13.—No blastopore has appeared.

3. For 30 minutes.

There is a yellowish central spot inside the grey patch.

Segmentation more irregular. The second furrow, for instance, may be parallel to the first, and the divisions do not reach the vegetative pole.

3 : iv : '13.—The grey patch is still visible. The vegetative hemisphere is not segmented.

4 : iv : '13.—No blastopore has yet been developed.

N.

Centrifuged 2 : iv : '13 on the water-driven machine one and a half hours after insemination at speed IV.

1. For 10 minutes.

The circular grey patch contains a yellowish spot surrounded by folds. Round it is a yellowish-white margin.

By the time segmentation has begun the folds have disappeared and the central spot is not very distinct.

The first furrow may be normal but is not always so. The second also may be meridional, but is not so always. Later furrows are irregular, sometimes meridional, sometimes circular—that is, circumscribing a small area in the animal hemisphere. The meridional furrows do not reach the vegetative pole.

3 : iv : '13.—The animal hemisphere alone is segmented, and that incompletely.

4 : iv : '13.—No development has occurred.

2. For 20 minutes.

There is a grey ring outside the white margin.

The first two furrows may be meridional but are not necessarily so. They do not reach the vegetative pole. Later furrows are very irregular.

3 : iv : '13.—As 1.

4 : iv : '13.—No development.

3. For 30 minutes.

The grey patch is surrounded by a groove. Outside this is first a white, then a grey ring. Segmentation is very seldom regular, even in the earliest stages. Divisions are often parallel to a meridian, and circular furrows are common. The vegetative hemisphere is not segmented.

3 : iv : '13. } As 2.
4 : iv : '13. }

O.

Centrifuged 3 : iv : '13 on the water-driven machine at speed IV.

All these eggs were preserved in formol immediately or in early cleavage stages.

1. One hour after insemination, centrifuged for 5 minutes (Pls. 7, 8, 10, figs. 1, 9, 16).

A circular grey patch appears round the animal pole; in it are radial dark striæ converging towards a central yellowish spot. The patch is separated by a groove from the pigmented area.

The patch is sometimes grooved or folded. The first and second furrows may be meridional, but are often parallel to a meridian. Later furrows are very irregular. Some, but not all of the meridional furrows reach the vegetative pole.

2. One hour after insemination, centrifuged for 10 minutes (Pl. 7, fig. 2).

The central spot is slightly depressed and surrounded by folds of the grey patch. The patch is marked by radial striæ, and surrounded by a groove. It has a narrow, faint whitish border.

Segmentation as in 1.

3. One hour after insemination, centrifuged for 15 minutes (Pls. 7, 8, 10, figs. 3, 10, 17).

The central spot is sinking in and being covered over by the folds. The radial striæ are still present. The white border is broader. Outside the groove is a grey ring.

Segmentation is more irregular than in 2. The furrows hardly reach the vegetative pole.

4. One and a quarter hours after insemination, centrifuged for 20 minutes (Pls. 7 and 8, figs. 4, 11).

The central spot is no longer visible, the folds having grown over it. The grey patch is still radially striated. External to the marginal groove is a second white ring, just above the grey ring and derived from it.

The first furrow may reach the vegetative pole, the others do not. Circular furrows are seen.

5. One and a quarter hours after insemination, centrifuged for 25 minutes.

The folds have nearly met and fused. Internal to the white border of the grey patch is a yellowish ring.

The furrows, when meridional, do not pass beyond the pigmented area (Pl. 7, fig. 5).

6. One and a half hours after insemination, centrifuged for 30 minutes (Pls. 7, 8, and 10, figs. 6, 12, 18).

The grey patch is now homogeneous, the radial striæ having disappeared. It is smaller, and immediately surrounded by a groove.

Outside this groove is a wide white ring which appears to be due to the confluence of the two white rings of the earlier stage.

Then comes the grey ring subdivided into three zones, of which the middle is darker.

Segmentation very irregular. The first furrow may begin at the side instead of at the animal pole. Circular furrows are found.

(3) The Effect produced by the Centrifuge on the Structure, Segmentation, and Development of the Egg.

From the details of the experiments that have been given in the preceding section it will be clear that the effect produced by the centrifuge upon the structure of the ovum, and upon its segmentation and development, varies with the degree of acceleration and the length of exposure.

A strict comparison is, of course, only possible between eggs of the same batch, centrifuged on the same machine, at the same time, at different speeds and exposures. Obviously, however, these conditions cannot always be observed, and then, owing, as already explained, to diurnal, if not hourly inconstancies in the water pressure, as well as to variations in the eggs themselves, it may well happen that on some occasion an exposure to a higher will not effect a greater change, will effect, perhaps, even a less change than an exposure of the same length to a lower acceleration.

Fortunately, in my series of experiments, this has not occurred, except, perhaps, once.

The accompanying table gives a résumé of the experiments performed with the water-driven centrifuge :

Speed.	Exposure in minutes. 5.	10.	15.	20.	25.	30.
I	—	H. 3	—	—	—	—
	—	J. 1	—	J. 2	—	—
II	—	H. 2	—	—	—	—
	—	K. 1	—	K. 2	—	—
	—	M. 1	—	M. 2	—	M. 3
III	—	H. 1	—	—	—	—
	L. 1	L. 2	L. 3	L. 4	—	—
IV	—	I. 1	—	—	—	I. 2
	—	N. 1	—	N. 2	—	N. 3
	O. 1	O. 2	O. 3	O. 4	O. 5	O. 6

On referring back to the preceding section it will be found that in nearly all cases the same acceleration and exposure produce the same, or nearly the same, effect upon the egg.

Thus in H. 3 and J. 1 there is a grey patch only, without a markedly white border. In H. 2, K. 1 and M. 1 the patch is surrounded by a lighter ring. In K. 2 and M. 2 the patch is folded round a central lighter spot, but in M. 2 there is an additional grey ring, which in K. 2 is absent. In H. 1 and L. 2 the patch is deeply infolded. In I. 1, N. 1, and O. 2 there is a central yellowish spot in the grey patch, and the latter has a lighter border. In N. 2 and O. 4 the grey patch is deeply infolded, the white border marked, while in I. 4, N. 3, and O. 6 the grey patch is surrounded by a broad white border, and this by a composite grey ring. When the same speed is employed the effect is always increased by prolonging the exposure. Conversely, when the exposure is constant the effect ought to vary with the acceleration, but this has not proved to be the case invariably. Thus the change in J. 2 is less than in K. 2, and less in K. 2 than in L. 4, and similarly in the series J. 1, K. 1, L. 2, and, again, less in M. 3 than in O. 6, but H. 1 and L. 2 are very like O. 4, L. 4 like O. 6, so that here the case is realised of a lower acceleration having caused a greater alteration than a higher in two different lots of eggs, the exposure being the same.

The same discrepancy appears in the subsequent develop-

ment, the embryos of I. 1 being as well if not better developed than those of L. 1 and M. 1 (which are much alike). Development only occurs in the series J. 1 and J. 2, H. 3, I. 1, K. 1, L. 1, M. 1, and G. 1 and G. 2. The ova of the series H. 2 did not develop though it would have been expected that they should. O. 1, N. 1 and O. 2 would very possibly have developed if they had been kept.

Of these J. 1 and J. 2 develop best, then H. 3, then G. 1, then G. 2, then I. 1, L. 1 and M. 1. The position of the embryos of K. 1 in the list is uncertain since they were unfortunately not preserved. There is, however, sufficient evidence that in the main the capacity to develop is progressively decreased as the acceleration is raised and the exposure prolonged. The experiments of the G series can only be assigned a position amongst the others by the extent to which the egg-structure and development are altered. In G. 1 and 2 there is a simple grey patch, and development is much better than in any except J. 1 and J. 2. They probably lie very near H. 3. G. 3 and G. 4 have the ring round the grey patch and fail to develop.

We may now proceed to discuss in order the effects which the operation produces upon the structure of the egg, its segmentation and its development.

a. The Effect Produced upon the Egg-structure.

The Structure of the Normal Egg.—As is well known, the spherical egg of the frog has a radially symmetrical structure about an axis, which axis has unlike poles. The polarity is determined first by the distribution of the superficial pigment, which only occupies about two thirds of the egg-surface, and second, by the disposition of the plasma (protoplasm) and yolk, the plasma being mainly, though not exclusively, situated in the pigmented region, the yolk-granules larger and more abundant in the unpigmented region, though found, of course, in the plasmatic portion as well. The line drawn through the centre (at the surface) of the pigmented

and plasmatic region, the centre of the egg itself, and the centre (at the surface) of the unpigmented yolky region is the axis, and its unlike plasmatic and yolky poles respectively the animal and vegetative. Internally there is also diffuse pigment, aggregated a little more intensely in the axis and in the animal hemisphere. The nucleus of the full-grown but immature oocyte lies, of course, axially, but excentrically, in the animal hemisphere, and it is in the same place that the pronuclei meet in fertilisation. All this is, of course, a common place of embryology, but the distribution of certain other substances in the ovum has not, as far as I am aware, ever been described. I allude to the fat (including lecithin, which, as I shall have occasion to show later, is present) and the glycogen. The fat appears to be uniformly distributed in the shape of small globules, variable in size, lying in the plasma between the yolk. These globules are easily demonstrated in sections of eggs, preserved in formalin and frozen, by means of the fat stain Sudan III. The yolk-granules remain uncoloured by the dye.

In respect of the glycogen the egg is, however, polarised, for this substance, present in the form of small spherules, is more abundant in the plasma of the animal than in that of the vegetative region, the spherules being larger and more numerous in the former, while in the latter they are excessively minute and very scanty (Pl. 10, fig. 15).

The glycogen, therefore, like the plasma in which it is embedded, gradually decreases in concentration from the animal to the vegetative pole, while the concentration of the yolk increases in the opposite direction.[1]

This polar structure is seriously affected when the egg is centrifuged, in my experiments in its axis. Roughly speaking what happens is that the lighter fat comes to the centripetal, that is, the animal pole; the next lightest, the plasma with the

[1] The distribution of glycogen in the course of development has not yet been worked out. I have only observed that in tadpoles in which the operculum is closing this substance is found only in the myotomes, in the tubules and duct of the pronephros and in the roof of the medulla.

glycogen, forms a layer next to the fat; while the heavy yolk and pigment are driven to the centrifugal, the vegetative pole. Hence the various zones or strata into which these eggs become divided. The somewhat complex details of the changes are most readily made out in a series of eggs centrifuged at the same acceleration with successively longer exposures, as, for example, the series O. The acceleration was here considerable: the exposures were 5, 10, 15, 20, 25 and 30 minutes. The ova were preserved in formol; from this material good sections are obtainable, either by the paraffin method, or after freezing. The latter are necessary for the study of the fat.

In O. 1 (Pl. 7, fig. 1, *a*, *b*), there is developed round the animal pole a circular grey patch radially striated with dark lines converging towards a central yellowish spot. The patch is separated by a groove from the surrounding pigment. The radial striæ are the lines along which the pigment is streaming away from the centripetal pole, while the lighter fat and plasma is moving in the opposite direction.

The yellowish spot may be excentric, and the grey patch may be folded.

A meridional section shows, beginning at the animal pole (Pls. 8 and 10, figs. 9, 16), (1) a superficial layer of rather finely vacuolated plasma, staining violet with hæmatoxylin. In it there is an occasional yolk-granule. Some of the original pigment remains in this layer, being disposed in (α) a denser sheet at the surface, (β) more diffusely below.

This layer is the grey patch seen from the surface.

(2) A more coarsely vacuolated layer of the same violet staining plasmatic substance. In it is some pigment and a few yolk-granules. At the level of this layer is one enormous vacuole (*v.*) which presses out layer 1 on the centripetal side. The floor of this vacuole is formed of the next layer. Both the large vacuole and the smaller vacuoles of layers 1 and 2 contain fat, staining vividly with Sudan III, and it is the large vacuole which is seen from the outside, shining through the first layer, as the yellowish spot.

(3) A layer of a violet-staining plasmatic substance which appears to have an alveolar structure (though this may be the effect of one of the reagents used). This layer is composite, there being denser sheets with a good deal of pigment running through lighter patches which contain little pigment. Fat vacuoles, singly and in groups, occur in this layer, and a good deal of yolk remains in it.

Neither the second nor the third layer comes to the surface, the first layer being here co-terminous with the fourth.

(4) The yolk and pigment.

Immediately below layer 3 is a sheet of pigment from which dense streamers depend into the yolk below. The original sheet of superficial pigment remains at the surface.

(5) The unpigmented region round the vegetative pole forms a fifth layer, but this, of course, was not produced by the centrifuge.

A good deal of fat and plasma remain still in the yolk.

In O. 2 the central spot is slightly depressed, and a white border is beginning to appear around the edge of the grey patch (Pl. 7, fig. 2, *a*, *b*).

Sections show the following layers:

(1) With the same characters as in O. 1. Below it a large fat-vacuole.

(2) With the same characters as O. 1, but with still less pigment, hence the appearance of a white border round the grey patch. This layer has probably been reinforced by some plasma from the third layer seen in O. 1 from which the yolk-granules have been drawn away.

(3) A broad band with numerous small yolk-granules—those found in the third layer of O. 1—and some vacuolated plasma. The latter has presumably just come up from the yolk below. This layer lies immediately centrifugal to the layer of pigment.

(4) The rest of the yolk with large granules. The superficial pigment is unaltered. As layer—

(5) may be distinguished the original unpigmented area.

In O. 3 the central spot begins to sink in below the sur-

rounding folds of the grey patch, while the white border to the latter is more distinct. Immediately outside this there is in the pigmented area a grey ring (Pl. 7, fig. 3, *a*).

The section shows the following layers (Pls. 8 and 10, figs. 10, 17) :

(1) The coarsely vacuolated pigmented layer in which the large vacuoles are embedded. This is the grey patch which is folded. The folds appear to arise by the accumulation of large globules or vacuoles of fat. As these are forced more and more centripetally the folds which are caused by them pass over the central depressed portion of the grey patch, which itself appears yellow owing to the accumulation of fat there.

In some of the vacuoles of this layer there is a coagulated fluid, which is stained pink with eosin. This appears to be a protein distinct from the material of which the walls of the vacuoles are composed. Whether the same vacuoles also contain fat is not certain ; in that case this might be a protein material accompanying the fat—the thin envelope, perhaps, of the fatty globules. On the other hand, the eosinophilous material may be normally associated with the other proteins of the plasma, and only dissociated from it by a certain degree of centrifuging. We shall see later that there is evidence for the existence of more than one kind of protein in the plasma.

(2) A layer which appears homogeneous under a low, finely alveolar under a high power. It stains violet with hæmatoxylin. In it are a few fat-vacuoles, but it contains no yolk-granules. Pigment is scattered through it, and the remains of the superficial pigment is seen at its external surface. It is the white border of the grey patch. This is identical with layer 2 in O. 2, but is thicker, and has lost nearly all its fat.

(3) Below this stretches a thin sheet of pigment, and immediately below this a broad vacuolated layer. The walls of the vacuoles are formed of plasma—staining with hæmatoxylin—with yolk-granules embedded in it; the cavities of the vacuoles are occupied by fat. Pigment is scattered through this layer, but sparsely, and at the external surface the

denseness of the original pigment sheet is much diminished. Externally this appears as the grey ring.

This layer is evidently derived from layer 3 of O. 2. What has happened is that more fat-globules have been driven centripetally and accumulated here in vacuoles underneath the dense plasma of layer 2, through which at present they are unable to pass.

In this layer are found local accumulations of deeply staining (violet) plasma. The plasma is, of course, being driven centripetally. There are also vacuoles containing eosinophilous coagulum.

(4) The pigmented region of the yolk. The lower edge of the pigment sheet is driven inwards.

(5) The pigment-free yolk.

O. 4. (Pl. 7, fig. 4, *a*, *b*). The folds of the grey patch have nearly met. A second white ring, derived from the grey ring, lies just external to the white ring of the grey patch.

The section (Pl. 8, fig. 11) reveals the same layers as in O. 3. The third layer—of fatty vacuoles with yolk-granules in the walls—is emancipating itself from the sheet of pigment, and sending streamers into the plasmatic layer (layer 2) above. The yolk-granules are evidently adherent—for the moment—to the fat-globules, and caught up in the centripetal movement. This gives the white division of the grey ring. Layer 2 is now practically devoid of pigment. The other layers are as in O. 3.

O. 5 (Pl. 7, fig. 5, *a*) presents very little change beyond the close approximation of the lips of the folds of the grey patch, but in—

O. 6 great alterations have occurred.

The grey patch—no longer radially striated—is immediately surrounded by a deep groove. Outside this lies a very broad white ring, and then a grey ring, compounded of three zones, the middle of which is darker than the others (Pl. 7, fig. 6, *a*).

Sections (Pls. 8 and 10, figs. 12, 18) show that (1) the grey

patch is composed of the same coarsely vacuolated pigmented material as before, and encloses a large fat-vacuole; (2) outside the groove which bounds this patch is another layer of vacuolated material, but this includes but little pigment. The vacuoles contain fat.

(3) There follows the finely alveolar plasmatic layer, almost devoid of pigment, with a few yolk-granules, and here and there a fat-vacuole. With the transitional condition O. 4 before us it seems easy to understand what has happened. The fat of layer 3, which was there beginning to penetrate the plasmatic layer 2, has completely passed through the latter and given rise to the present layer 2—a layer which, as we should expect, contains but little pigment. In so doing it has shaken off the yolk-granules, which have passed back in the opposite direction, although a few remain entangled in the third layer. The second and third layers then form together the broad white wing. In this process the original groove external to the white ring (layer 2 of O. 3 and O. 4) disappears, while a fresh groove is formed round that portion of the fatty layer in which a considerable quantity of pigment is still entangled, namely, in the grey patch.

(4) Underneath the homogeneous plasma layer is a sheet —the uppermost sheet of the yolk—from which a good deal of the pigment has disappeared. This is the grey ring.

In it a finely vacuolated layer can be distinguished above from a layer which is not so vacuolated. The former is the paler upper zone of the grey ring, and is due to a fresh agglomeration of fat-globules in vacuoles below the plasma layer. In other words, the process seen in O. 4 is about to be repeated. It must be remembered that there is still a good deal of fat left in the yolk. With Sudan III the yolk stains a faint orange, and proper examination reveals the fat-globules between the yolk-granules (Pl. 10, fig. 18 *c*).

I have not succeeded in finding in the sections the lower pale zone of the grey ring.

The other layers are as before.

We can now form some idea of the changes that take

place when the egg is centrifuged at this high acceleration for progressively longer periods.

First the pigment and yolk are driven centrifugally while the fat and plasma are urged in the reverse direction. The fat is, however, lighter than the plasma, so that the former occupies the most centripetal position. At the same time the globules become agglomerated into larger masses, some of which are enormous; a certain amount of pigment remains obstinately adherent to the fat. Hence the grey patch and yellow spot. The plasma forms a layer next to this, and gradually rids itself of fat and yolk, in opposite directions. When it is free from pigment it appears as the white border. At the same time the pigment is spread out in a third layer, which sends streamers into the yolk below. This fact, coupled with the frequent mottling of the original unpigmented area, suggests that the pigment is perhaps heavier than the yolk.

The supply of fat and plasma in the vegetative region is, however, by no means yet exhausted, and a fresh accumulation is soon spread out beneath the barrier, at present impenetrable, of the plasma layer. But eventually this gives way, the fat passes through, dragging at first some yolk-granules with it, but these are quickly discarded and driven back. The condition seen in O. 6 is thus reached, though this is by no means the final stage, since there are evident preparations for a fresh conglomeration of fat. What the end would be is clear enough. The fat—with adherent pigment and plasma—would be centripetally disposed, the yolk and pigment centrifugally, while the plasma, including, I may perhaps now say, the glycogen, would lie between. A discussion of the chemistry of the components of these layers must, however, be reserved for a later chapter.

The same kind of effect is produced with a smaller acceleration, but, as a rule, the white ring appears before the central spot—as, for example, in series M (Pl. 7, fig. 8). Probably considerable force is required to make the fat-globules cohere in one or more large masses.

A section of K. 1 (Pl. 9, fig. 13) shows the grey patch as a coarsely vacuolated layer, with pigment, the pale border as a plasma layer, with some pigment, and below this a sheet of pigment and the yolk.

When the acceleration is smaller still, as in series J (Pl. 7, fig. 7), the only alteration revealed by the sections is an immigration of pigment round the animal pole. This causes the faint grey patch seen in these eggs.

As a rule the various zones and rings become somewhat confused, and the folds of the grey patch disappear after a short interval; but a grey or blotched patch usually persists for a considerable time, and may often be seen at the anterior end of the embryo.

There is, in fact, a slight re-diffusion of the disarranged materials through one another, but the normal arrangement is never regained—not at least in eggs centrifuged so soon before segmentation.

b. The Effect produced upon the Segmentation of the Egg.

With the lower acceleration the segmentation of the egg is normal except for a slight retardation, as for example in L. 1, L. 2, M. 1 and M. 2 (Pl. 7, fig. 8), but when greater force is applied irregularities appear (Pl. 7, figs. 1 *c*, 2 *c*, *d*, 3 *b*, 4 *c*, *d*, 5 *b*, 6 *b*, *c*). Even though the first two furrows are meridional, those of the third phase may be meridional instead of latitudinal (as in L. 4) or parallel to the first. The first and second are often parallel to a meridian, and with greater accelerations the normal sequence is almost entirely abandoned. The first furrow may begin at the side instead of at the pole (O. 6), and circular furrows, cutting off a small region of the animal hemisphere completely or incompletely, are frequently observed (O. 4, O. 6, L. 4). Many or all of the meridional furrows fail to reach the vegetative pole, and as a result the segmentation becomes more or less meroblastic; in extreme cases, indeed,

it ends in the formation of a blastoderm resting upon an unsegmented yolk, as Oscar Hertwig pointed out; as mentioned by the same author the nuclei in the yolk become enlarged, irregular in shape and highly chromatic, so resembling the yolk nuclei of Elasmobranchs and Teleostei, bodies which are concerned in the elaboration of the yolk alone, and do not play any part in the formation of the embryo.

A section (Pl. 9, fig. 14) through one of these eggs (L. 4, 6 : iv : '13) shows that though five days have elapsed since the operation no differentiation has occurred. The egg has merely continued to segment slowly.

Some of the strata can still be recognised.

Round the animal pole is the grey patch, lightly pigmented and vacuolated. This region alone is properly segmented, and even here it is not possible to distinguish cell-boundaries between the nuclei of the deeper layers. There are three or four layers of nuclei in all.

The grey patch passes into the remains of the plasmatic layer, but this contains now masses of yolk-granules and a good deal of pigment, both of which have evidently returned from the inferior position to which they were driven. In this layer are numerous large vacuoles, some clear (these evidently contained fat), and some filled with a coagulated liquid which stains with the plasma dye (picro-indigo-carmine). The fat-globules are presumably due to the breaking up of the larger vacuoles of the centrifuged egg—again a return of a substance towards its original position. This layer contains nuclei, many of which are homogeneous and highly chromatic, while others are very large and irregularly amœboid.

Below is the yolk, in the upper (centripetal) region of which are a few nuclei, of a large but not excessive size.

c. The Effect produced upon the Development of the Embryo.

We have at our disposal embryos and larvæ obtained from the series J, H. 3, G. 1, G. 2, I. 1, L. 1 and M. 1.

In the series J the acceleration was small and the tadpoles were normal.

In the series G. 1 a larger acceleration was (probably) employed, and out of 53 tadpoles 12 were abnormal. These tadpoles were twelve days old when preserved. The H. 3 ova were subjected to about the same force as those of G. 1, but the embryos were killed at an earlier stage when five days old. In G. 2 the same force was used as in G. 1 but the exposure was longer: out of 43 larvæ (killed at 12 days) 15 were abnormal. The force employed and the effect produced in the remaining series were greater, the degree of abnormality being progressively larger in I. 1 (10 abnormal out of 13), L. 1 (17 abnormal out of 21), and M. 1 (all abnormal). The numbers are, however, too small to be genuinely significant, and all the embryos of these three series may be placed in one class. Those of I. 1 were preserved on the third and fourth days, those of L. 1 on the fifth and seventh days, and those of M. 1 on the fourth and sixth days after the operation.

The available material should, therefore, provide an opportunity for the study of the genesis of these aberrations of development.

The distortion of development produced by the centrifuge is of a very striking kind, and is, moreover, one which cannot be induced as far as I am aware by any other method. It consists essentially of, first, a disturbance at the anterior end, which may manifest itself merely by a vacuolation of the ectoderm and of the nervous system and other structures in that region, but more usually takes the form of a total disintegration of the front part of the head: the olfactory pits, the fore-brain and eyes, and the mid-brain, the skull and the mouth, all disappear as such, and the nervous system begins in the region of the medulla. Secondly, the yolk is affected. The only sign of any derangement may be a tract of undivided yolk in which the granules have become fused into one mass, but the closure of the blastopore is often prevented, or at least delayed, and there is a more or less persistent yolk-plug. When it is remembered that the yolk-plug is derived from

material situated in the vegetative region of the egg while the head end of the embryo is developed near the animal pole, the significance of the relation between these malformations and the structural derangement produced by the centrifuge along the axis of the ovum will be sufficiently obvious. While these abnormalities are proceeding in the head and round the blastopore the middle region may be developing normally, such structures as the auditory vesicles, medulla and spinal cord, pharynx and gill-clefts, branchial skeleton (in part), lungs, heart and blood-vessels, alimentary canal, pronephros, germ-cells and tail may all be of ordinary appearance. (The tail, I may remind the reader, though, of course, posterior, is developed from the lateral lips of the blastopore—that is, from material placed originally in the equatorial region of the egg.) There is, however, one curious malformation in this region, which may best be described as a fusion of paired structures in the middle line. It is seen in the abnormal arrangement of neuroblasts in the medulla and spinal cord, in the fusion below the spinal cord of the paired spinal ganglia, sometimes of the posterior cranial ganglia too, and in the fusion below this, again, of the mesodermal somites, or rather of the myotomes. The last leads to the obliteration of the notochord. Exceptionally the auditory vesicles may unite above the medulla. All these changes occur when the ova have been subjected to a moderate degree of force (as in the G. series) : when a greater force is employed, the derangement is more serious ; the whole of the nervous system and the organs of the middle region of the body may disappear, leaving an embryo with perhaps a very short archenteron and an undifferentiated mesoderm. A longer or shorter tail may, however, grow out ; sometimes it is double, and the mesoderm contained in it may show traces of segmentation.

These monsters occur mainly in the L. 1 and M. 1 series; that they are not merely retarded embryos of an early stage, which might possibly have developed further, is indicated by their age—four to seven days—and by the extreme irregularity of their appearance.

The Vacuolation of the Anterior Ectoderm (Pl. 12, fig. 44).

This alteration occurs at the front end, extending back some little way on both dorsal and ventral sides, but never reaches quite to the posterior end though the ectoderm is here often folded. Where the centrifuging has been more severe it is more extensive.

In slight cases the ectoderm remains two-layered and only the epidermal layer is affected (as in G. 1, 8 : iv : '13 [a and b]), but after a more violent operation the ectoderm becomes thickened by an increase in the number of layers and at the same same time folded and pitted and the cells in all layers are affected. At the same time the vacuoles are larger (as in G. 2, and the embryos of the I., L. and M. series). There can be no doubt that in the fresh condition these vacuoles were full of the fat forced by the centrifuge to the animal pole. Other structures at the anterior end may be similarly vacuolated ; the olfactory pits (G. 1, 8 : iv : '13 [a]), the brain (G. 1, 8 : iv : '13 [a], I. 1, 3 : iv : '13 [a, b and c]), the optic vesicles (I. 1, 3 : iv : '13 [a and c]), the auditory vesicles (G. 2, 8 : iv : '13 [a]), the ganglia of cranial nerves, and mesoderm cells (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a]).

The Degeneration of the Front Part of the Head.

In G. 1, 8 : iv : '13 (a) (Pl. 11, fig. 19) the brain and skull are normal and there is a mouth. The only abnormality, indeed, to be noticed is the absence of a lens. The optic cup has a narrow mouth and is some little distance from the ectoderm (Text-fig. 1).

In such early stages as I. 1, 3 : iv : '13 (a, b, and c), H. 3, 2 : iv : '13 (a) (Pl. 11, figs. 24, 26, 27), both fore-brain and mid-brain are present and apparently normal; while normal olfactory pits are found in H. 3, 2 : iv : '13 (a), I. 1, 4 : iv : '13 (a and b), and optic vesicles in H. 3, 2 : iv : '13 (a), I. 1, 3 : iv : '13 (b).

As a rule, however, these structures, together with the front part of the hind-brain, suffer disintegration by the time such stages as G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (a, b, c, e, and g) (Pl. 11, figs. 20, 21, 23) are reached. All that remains of them is then a mass of pigmented vacuolated cells lying in the front of the head (Text-fig. 2, *a*), some of which have the large pale nuclei characteristic of neuroblasts (Pl. 12, fig. 45), and from some of which nerve-fibres proceed. Mingled with the cells is a débris of cell and nuclear fragments (the latter having undergone chromatolytic degeneration into deeply staining spherules), yolk- and pigment-granules.

The ganglia of this region (v and vii) often preserve their individuality more or less completely (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, c, g]); they appear as groups of vacuolated pigmented neuroblasts, and the appropriate nerves can often be traced (Pl. 12, fig. 46). In one case (G. 2, 8 : iv : '13 [a]) the olfactory sacs remain as two vacuolated fibrous masses, still retaining their connection with the ectoderm. No trace of the eyes is found in these embryos.

At an earlier stage the rudiments of these organs were laid down; their degeneration seems to set in almost at once. Thus in H. 3, 2 : iv : '13 (b), L. 1, 6 : iv : '13 (a), the front part of the brain is a solid wedge of cells (the mid-brain region in the last-mentioned is an open groove), in L. 1, 6 : iv : '13 (b) the brain is very small, the olfactory pits are very shallow in H. 3, 2 : iv : '13 (b), I. 1, 4 : iv : '13 (c), and the optic vesicles thick-walled (I. 1, 3 : iv : '13 [a]) or much reduced (L. 1, 6 : iv : '13 [a], I. 1, 4 : iv : 13 [c]) (Text-fig. 3). Associated with débris of the nervous system and sense organs are of course mesodermal elements, such as wandering connective tissue cells and chromatophores. Mesodermal cells may suffer vacuolation (G. 1, 8 : iv : '13 [b] (Pl. 12, fig. 45 *b*, *c*).

The mouth is almost always absent. It is found in the nearly normal G. 1, 8 : iv : '13 (a), and in early stages a stomodæum is present (H. 3, 2 : iv : '13 [a] I. 1, 4 : iv : '13 [a and b]). The pharynx then communicates with the exterior

only by the gill-clefts, which are often well developed (Text-fig. 2, *a*).

The head skeleton is also profoundly modified (except in G. 1, 8 : iv : '13 [a]). Properly speaking, the cranium is entirely absent, with the exception of a small plate of cartilage situated below the débris of the brain, which appears to represent some part of the cranial floor—perhaps the anterior trabecular plate—in G. 1, 8 : iv : '13 (b). The branchial skeleton is, however, better developed in a few cases. In G. 1, 8 : iv : '13 (b) it consists of a wedge-shaped piece embedded in the anterior wall of the pharynx, and a triangular plate placed below the pharynx, with a median depression, in which the thyroid is lodged, a backwardly directed apex, vertical lateral edges, and two pairs of branchial arches. From their relation to the gills and gill-clefts these appear to be the second and third branchial arches. The anterior piece then represents the first branchial and the hyoid, with, perhaps, an admixture of mandibular (quadrate) elements. Bundles of myoblasts connect these two pieces to one another and the rudiment of the skull (Text-fig. 4).

In G. 2, 8 : iv : '13 (a) there is a plate under the throat bearing branchial arches, not symmetrically nor fully developed: on one side is a large third branchial and small fourth, second and first branchials, and a hyoid, the last two being united a little way from the median plate; on the other side are long second and third, short fourth and first branchials, and a short hyoid. In front the plate is produced into two curved pieces, which may be extensions of the hyoid. Bundles of myoblasts pass from this anterior to the more posterior parts of the apparatus.

In G. 2, 8 : iv : '13 (b) there is a plate bearing three pairs of short branchial arches, the first and second being united on each side. In front, embedded in the anterior wall of the pharynx, is a wedge-shaped piece, resembling that of G. 1, 8 : iv : '13 (b), but here united to the main plate. It appears to be hyoidean.

In G. 2, 8 : iv : '13 (g) there is a similar anterior piece,

with, however, irregular anterior prolongation. Behind, and below the pharynx, is a plate with one pair of irregular arches. Lastly, in G. 2, 8 : iv : '13 (c) the branchial skeleton is reduced to a small nodule in the front wall of the pharynx. Gill-slits are absent in this larva.

Suckers are almost invariably absent. They are found, indeed, only in G. 1, 8 : iv : '13 (a), L. 1, 8 : iv : '13 (b) (Pl. 11, fig. 37), and in comparatively early stages, such as H. 3, 2 : iv : '13 (a and b), I. 1, 4 : iv : '13 (a and b) (Text-fig. 5).

The Changes at the Yolk and Blastopore End.

In G. 2, 8 : iv : '13 (b) (Text-fig. 8), and in all embryos which are as much or more malformed, a central region is observable in the mass of yolk-cells in which cell divisions are absent and the yolk-granules fused together. This would seem to be due to withdrawal of plasma. Another consequence of the operation is the delay in the closure of the blastopore, seen, for instance, in G. 2, 8 : iv : '13 (d, e), H. 3, 2 : iv : '13 (b), I. 1, 3 : iv : '13 (a, b, c), I. 1, 4 : iv : '13 (b, c, d, e), and in most of the very severely affected embryos of the L and M series (Pl. 11, figs. 22, 23, 25, 26, 27, 28, 29, 30). In L. 1, 6 : iv : '13 (e and f), in L. 1, 8 : iv : '13 (e), in M. 1, 6 : iv : '13 (b) and in M. 1, 8 : iv : '13 (b and e) the yolk-plug is, however, withdrawn in spite of the very serious arrest of development (Pl. 11, figs. 34, 35, 39, 42).

Fusion of Paired Structures in the Middle Line; the Hind-brain and Spinal Cord, the Myotomes and Notochord.

In those embryos in which the front part of the nervous system has disintegrated, the brain begins at the level of the auditory vesicles or sometimes a little in front of or behind this point (Text-fig. 2 *b*). Inasmuch as the ganglia of v and vii, or the remains of them, are found with the rest of the débris in the head, we must suppose that part only of the hind-brain has escaped destruction.

This part has the structure of the medulla with a thin roof and a thick floor, but is still not quite normal since the floor is excessively thick, often projecting into the lumen, while a mass of white matter occupies the whole of its ventral side, uninterrupted by any cells. The cells lie above this, the spongioblasts next the lumen, the neuroblasts above the fibres. The lumen is itself further dorsal than it should be. The roof is excessively folded.

In this region are found the auditory and vagus ganglia; they often approach one another in the middle line and may meet (G. 2, 8 : iv : '13 [e and g]) (Text-fig. 6).

The auditory vesicles (Text-fig. 2 *b*) are well developed. In G. 2, 8 : iv : '13 (e) each is constricted into two distinct parts, and in G. 2, 8 : iv : '13 (g) the vesicles of opposite sides are united in front of the hind-brain (Text-fig. 6). They are found, of course, in earlier stages (H. 3, 2 : iv : '13; I. 1, 4 : iv : '13 [d]) (Text-fig. 7). The spinal cord (Pl. 12, fig. 47) (Text-fig. 2 *d*) has the same defects as the medulla—that is to say, the lumen is driven dorsally by an excessive thickening of the floor across which runs a continuous band of fibres; next to this comes a layer of neuroblasts, and then the spongioblasts next the lumen (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, b, c, e, g], M. 1, 6 : iv : '13 [b]). Posteriorly, however, it may be normal (G. 2, 8 : iv : '13 [b], M. 1, 6 : iv : '13 [b]) (Text-fig. 8), and may be normal throughout (L. 1, 6 : iv : '13 [a] ; 8 : iv : '13 [b]). The abnormality is interesting; it is as though the lateral tracts of fibres had come down, forced the neuroblasts of the ventral cornua upwards towards the canal, and then met in a continuous ventral band. In other words, there has been a median fusion of paired structures, and the same phenomenon is seen in the fusion below the cord of the spinal ganglia (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, partial], [b, in front], [c, e, g]). The ganglia, however, still retain their proper relations with the cord in that the fibres of the dorsal root pass upwards from their cells to enter the cord at the side.

Like the ganglia the myotomes unite below the cord

(Text-figs. 2, *c*, *d*, 10, 11) in a mass of fusiform, horizontally placed myoblasts (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, not posteriorly], [b, c, e, g], I. 1, 4 : iv : '13 [d], M. 1, 6 : iv : '13 [b, d]). There is little doubt that these latter cells by which the junction is effected are cells which should have, but have not, given rise to the notochord; for first the notochord is absent in these cases or only represented by an occasional vacuolation (as in G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [e and g]), except where some part of the original material has been saved (as in G. 2, 8 : iv : '13 [b]), and a notochord is seen lying above the median conjunctive mass, or where there has been no fusion (as in the hind-end of G. 2, 8 : iv : '13 [a]). In the second place the beginning of the process is seen in such early arrested stages as L. 1, 6 : iv : '13 (c) (Text-fig. 15), M. 1, 6 : iv : '13 (b, d), where the vertebral plate mesoderm of the two sides is continuous across the middle line in a median mass of cells which are already beginning to elongate.

In early stages (of slightly centrifugalised eggs) there is a normal notochord (H. 3, 2 : iv : '13 [b]), and sometimes in others (L. 1, 6 : iv : '13 [a, b] ; 8 : iv : '13 [b] ; I. 1, 4 : iv : '13 [a, b, c]).

Lastly, in one case, already referred to, the two auditory vesicles are united, while a fusion of the auditory and vagus ganglia may also occur.

I am unable to suggest any explanation of this curious change.

The Organs of the Middle Region of the Body.

With the exceptions already noted, the posterior part of the nervous system, the auditory vesicles, and the muscles of the back are well developed. The same may be generally stated of the pharynx, lungs, alimentary canal, heart and blood-vessels, pronephros, germ-cells and tail.

The pharynx is provided with gill-evaginations, some of which may be open (Text-fig. 2, *a*). In G. 1, 8 : iv : '13 (b) all five pairs are present, and the last three (second, third and fourth branchials) are open. In G. 2. 8 : iv : '13 (a) the first

three branchials are present on one side (the second and third open) and on the other the second, third and fourth branchials (the last two open). In G. 2, 8 : iv : '13 (b) the first, second, third and fourth branchials are present on both sides (the last two open on one side, the last hardly open on the other), and in G. 2, 8 : iv : '13 (g) the first, second and third branchials are present on one side (the second and third open), while on the other are the first and second branchials (the second open). Thus the hyomandibular evagination is found only in the first tadpole; in the remainder branchial clefts in numbers which differ in individuals, and on the two sides of the same individual.

The embryos of Series H and I are too young to show gill-clefts. In L. 1, 8 : iv : '13 (b) there are solid outgrowths, but no perforations.

As already pointed out, there is a correlation between the presence of clefts and the development of a branchial skeleton, as would, of course, be expected.

External gills are found in G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (a, b and g). They are placed very far forwards (Pl. 11, fig. 20). In G. 1, 8 : iv : '13 (b) there is a small opercular fold.

The trachea and lungs (Text-figs. 2 *b*, 6, 10) are found in the older embryos (G. 1, 8 : iv : '13 [b]. G. 2, 8 : iv : '13 [a, b, c, g, but not e]), whose development is not too much arrested. The pericardium and heart (Text-figs. 2 *b*, 13), with the principal blood-vessels, aortæ and cardinal and vitelline veins, are found in the better developed embryos and are usually well formed (G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, b, e, g], L. 1, 8 : iv : '13 [b]), but in G. 2, 8 : iv : '13 (b) the heart is small, in (g) not twisted, and in (e) and L. 1, 8 : iv : '13 (b) very small and solid. Aortæ and cardinal veins may be present when the heart is absent (G. 2, 8 : iv : '13 [c]).

In G. 2, 8 : iv : '13 (d and e) there are irregular blood-vessels (Text-fig. 14 *b*) and a structure, which is possibly the heart, lying on one side of the pericardial cavity. In the younger but fairly normal embryos (H. 3, 2 : iv : '13 [a, b], I. 1, 4 : iv : '13 [a, b, c], L. 1, 6 : iv : '13 [b, c]), the peri-

cardium is still small, and the heart in either a very early stage or quite undeveloped.

The peritoneal cavity is frequently well formed (Text-figs. 2 *c*, *d*, 6, 8) (as in G. 1, 8 : iv : '13 [b], G. 2, 8 : iv : '13 [a, b, c, e, g]), and in communication with the exterior by the pronephros (Text-figs. 2 *c*, 6, 10, 11). The full number of pronephric tubules and funnels is found in G. 2, 8 : iv : '13 (a, b, e, g), but in G. 1, 8 : iv : '13 (b) there are only two funnels on one side, in G. 2, 8 : iv : '13 (c) two only on both sides. The tubules are bathed, as usually, in the capillaries of the posterior cardinal vein. The glomus is found (except in G. 2, 8 : iv : '13 [a and c]). The ducts open into the cloaca (except in G. 2, 8 : iv : '13 [e]).

In the younger embryos (H, I, L. 1, 6 : iv : '13 [a, b, c], M. 1, 6 : iv : '13 [b, d]), only the pronephric ridge is found (Text-fig. 9). In L. 1, 8 : iv : '13 (b) differentiation of the tubules has not gone very far.

The gut is well differentiated (with stomach, liver and intestine) (Text-fig. 2 *c*) ; in G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (c, g), less differentiated in G. 2, 8 : iv : '13 (a, b, e), L. 1, 6 : iv : '13 (c). In the H and I series little differentiation, has occurred beyond the formation of the liver diverticulum, and the embryos L. 1, 6 : iv : '13 (a), 8 : iv : '13 (b), M. 1, 6 : iv : '13 (b, d) are in the same early, probably arrested, condition.

A proctodæum, not open to the gut in the early or arrested stages, is found always except where the blastopore persists.

Primordial germ-cells are found at the root of the mesentery in G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (a, b, c, g) (Text-fig. 2 *d*).

A tail is found, provided with a fin, in G. 1, 8 : iv : '13 (b), G. 2, 8 : iv : '13 (a, b, c) (Pl. 11, figs. 20, 21). In the young embryos it is of course only a short stump.

Œdema.

Many of these tadpoles suffer from œdema or an accumulation of fluid in the connective-tissue inter-cellular spaces,

or in cavities (Text-figs. 6, 10, 11, 12). Such an accumulation is seen in the connective-tissue in G. 2, 8 : iv : '13 (a and c), in the cœlom (G. 2, 8 : iv : '13 [b, c, g], in the posterior cardinal vein round the pronephros (G. 2, 8 : iv : '13 [a, g]), in the lymphatics or blood-vessels round the gut and liver (G. 2, 8 : iv : '13 [c]), and in the pronephric tubules (G. 2, 8 : iv : '13 [e]). In G. 1, 8 : iv : '13 (b) there is a large ventral cavity, in front of, but quite independent of, the pericardium, partially divided by a median septum, which is probably due to the same causes. In several cases where the development has been more seriously interfered with, the segmentation cavity persists. This, perhaps, belongs to the same category. The persistent blastocœl may contain a few scattered mesodermal cells, but otherwise retain its original character, its front wall being formed of the small ectodermal cells of the animal hemisphere, its hind wall of the large yolk-cells (M. 1, 8 : iv : '13 [c]), or the mesodermal cells which have advanced into it may give it a lining of its own, incomplete (M. 1, 8 : iv : '13 [b], L. 1, 8 : iv : '13 [d] [1]), or complete (M. 1, 8 : iv : '13 [a], G. 2, 8 : iv : '13 [d]) (Text-fig. 14 *a*). The cavity in question has no communication with the alimentary canal, which is, indeed, in most of these cases very small, or restricted to the blastoporic groove.

The Changes after Severer Treatment.

While it is thus possible, if the centrifugal force applied be not too great, to obtain tadpoles which, though deformed anteriorly and in the region of the blastopore, are yet more or less normal in the middle portion of the body and in the tail, in individuals which have suffered more seriously there is witnessed a gradual loss of structure, until eventually no more differentiation occurs than is involved in the production of some dorsal and ventral mesoderm and a slight blastoporic overgrowth.

The heart usually goes before the pericardium, or, to express it in a better way, the latter may be developed while the former

is not. Thus in L. 1, 8 : iv : '13 (b) the heart is solid, the pericardium large, and in I. 1, 4 : iv : '13 (c), L. 1, 6 : iv : '13 (b, c), the former is absent while the latter is present. In the more seriously injured embryos neither is found, though blood-vessels may be (G. 2, 8 : iv : '13 [d]). The pronephros holds out perhaps a little longer, as it is formed not only in the embryos just mentioned, but also in M. 1, 6 : iv : '13 (b, d), which possess no pericardial cavity. The peritoneal cavity appears to persist in these same embryos, but is not found in the more degenerate individuals (except possibly in L. 1, 8 : iv : '13 [e]). The absence of the heart and pericardium in L. 1, 6 : iv : '13 (a), while the nervous system and auditory vesicles are present, may indicate that in general the former organs are affected before the latter. The auditory vesicles are found almost as long as the central nervous system persists. Indeed, in G. 2, 8 : iv : '13 (e) they are well developed, while the hind-brain and spinal cord are reduced. On the other hand, in I. 1, 4 : iv : '13 (e) and in L. 1, 6 : iv : '13 (c) they have disappeared, while the hind-brain and spinal cord have remained.

The successive steps in the degeneration of what is left of the nervous system are easy to follow. The hind-brain has a very small lumen in I. 1, 4 : iv : '13 (d), is nearly solid in I. 1, 4 : iv : '13 (a, b, c, e), L. 1, 6 : iv : '13 (a), M. 1, 6 : iv : '13 (b, d), and quite solid in G. 2, 8 : iv : '13 (e), L. 1, 8 : iv : '13 (c) (2). The spinal cord is solid in the last-mentioned and nearly so in the others ; in L. 1, 6 : iv : '13 (c) it is still open behind (Text-figs. 15, 16). In all other cases there is no sign of the nervous system at all (Text-fig. 17). The gut may remain—even if only as an archenteron—when the other organs have disappeared. Thus it is found as a narrow but fairly long cavity in L. 1, 6 : iv : '13 (c), M. 1, 6 : iv : '13 (b, d), which still possess a nervous system (Text-fig. 16). In G. 2, 8 : iv : '13 (f), L. 1, 6 : iv : '13 (f) (Pl. 11, fig. 35), L. 1, 8 : iv : '13 (d, e) (Pl. 11, fig. 39), M. 1, 6 : iv : '13 (a, c), and M. 1, 8 : iv : '13 (a, b, c, d, e) (Pl. 11, figs. 42, 43)—none of which have any nervous system—it is a very short cavity, opening by the proctodæum or by the blastopore. In G. 2, 8 : iv : '13 (d) (Pl. 11, fig. 22), L. 1, 6 : iv :

'13 (d), L. 1, 8 : iv : '13 (a, b) (Pl. 11, figs. 36, 37), (c, f, g) (Pl. 11, figs. 40, 41), it is reduced to the blastoporic involution (Text-fig. 14 *b*), while in L. 1, 6 : iv : '13 (e) it is altogether absent, though a proctodæum is present.

The tail remains in many of these extremely stunted forms as a longer or shorter stump, as in G. 2, 8 : iv : '13 (d), L. 1, 6 : iv : '13 (f), L. 1, 8 : iv : '13 (a, b, e), M. 1, 6 : iv : '13 (a, b, d), M. 1, 8 : iv : '13 (a, b, c, e), and it is sometimes double (L. 1, 6 : iv : '13 [e], L. 1, 8 : iv : '13 [f, g]). The double rudiment of the tail is seen of course in those cases where it is represented only by two caudal swellings (I. 1, 4 : iv : '13 [c], L. 1, 6 : iv : '13 [a], L. 1, 8 : iv : '13 [c]) (figs. 29, 31, 38). These tails, though devoid of nervous system and notochord, may yet display traces of a metameric segmentation of the mesoderm (as in L. 1, 8 : iv : '13 [f, g], M. 1, 8 : iv : '13 [b]) (Text-fig. 18).

In the rest there is not even a tail. Dorsal and ventral mesoderm are, however, always developed. The dorsal mesoderm may give indications of the differentiation of a median notochordal tract (L. 1, 6 : iv : '13 [g], 8 : iv : '13 [c], [1]).

(B) On the Chemical Nature of the Substances in the Frog's Egg which may be Separated from one another by the Centrifuge.

The inquiry into the chemical nature of the substances in the several layers which appear in the centrifuged egg can only be prosecuted with any hope of success by centrifuging a large quantity of egg-material in vitro. For this purpose the eggs must be obtained free from their coating of mucin-jelly, that is, before they have entered the oviduct.

Such eggs are not, strictly speaking, in quite the same physiological condition as the fertilised eggs, inasmuch as in them the maturation divisions have not yet occurred. If, however, the eggs be taken after their release from the ovary—while they are still in the peritoneal cavity—or immediately before that release, it will be found that the germinal vesicle has already broken down and dispersed its

contents into the cytoplasm, while the first polar spindle has come to the surface of the egg. In their cytoplasm such eggs are probably not so very different from those that have become completely mature.

My first idea was to employ only cœlomic ova, but I soon found that I could not obtain anything like a sufficient quantity of material in this way, since at any one moment but few eggs are found in the body-cavity, some being still retained in the ovary, while others are already in the oviduct. I, therefore, adopted the plan of removing the ovaries, washing them well in Ringer's solution to remove blood and peritoneal fluid, and then leaving them in a quantity of the same solution at a low temperature until the eggs dropped out. I was able to get considerable numbers of eggs by this method, which seems to me to be preferable to that employed by McClendon; this, as already pointed out, involves the inclusion of all the young ova with the old ones.

From the eggs so obtained the Ringer's solution was poured away as completely as possible. The ova were then ground to a fine pulp in a mortar, and this egg-pulp was centrifuged. In the first experiments a fairly high velocity was used (about 3200 revolutions a minute) for about twenty minutes; in later experiments, to which I shall refer presently, different and lower accelerations and exposures were employed and their effects compared.

The centrifuged mass becomes separated into three distinct layers:

1. A centripetal dark yellowish-grey layer.
2. A middle light grey or opalescent layer.
3. A very thick black centrifugal layer.

The first of these consists of fatty substances and some protein, the second of proteins and glycogen, and the third of pigment and yolk with an admixture of fatty substances.

Layer 1.

(i) This layer is slimy and viscid, hardened at its surface into a thin crust.

(ii) Microscopical examination: Pigment-granules, refringent

globules entangled into angular masses in some other material, and some fluid.

The refringent globules are soluble in chloroform and acetone, and blacken with osmic acid. They appear, therefore, to consist of a fatty material. The material in which the globules are entangled and by which they are partly obscured is soluble in 1 per cent. NaOH. The globules then become more evident.

Some of the globules are nearly or quite colourless, others of a yellow colour. There are no yolk-granules in this layer.

(iii) When this stuff is boiled for some time in alcoholic potash a solution of soap is formed which may be salted out with NaCl. By $CaCl_2$ the soap is precipitated, and on the addition of acetic a layer of fatty acid rises to the surface.

(iv) A portion of the material was mixed with ·75 per cent. NaCl and filtered.

A. The filtrate—

(1) Filters quickly.
(2) Is opalescent.
(3) Gives Millon's reaction slightly.
(4) Gives Heller's reaction slightly.
(5) Gives the xanthoproteic reaction slightly.
(6) Gives a slight heat coagulum.
(7) Gives a slight glycogen reaction with iodine.

B. The residue—

(1) Gives Millon's reaction.
(2) Gives the xanthoproteic reaction.
(3) Dried to a black colour and washed well with ether. The ether becomes yellow. The residue is now grey.
(α) This grey residue gives the xanthoproteic reaction.
(β) This residue was now washed with hot alcohol, dried and incinerated. The ashes were dissolved in dilute HNO_3. On the addition of ammonium molybdate yellow crystals of ammonium phosphomolybdate appear.

(v) Another portion of this material was placed in alcohol.

a. A flocculent coagulum appears at once. The coagulum gives Millon's reaction.

b. The material was then boiled repeatedly in alcohol. The alcohol becomes yellow, and several large yellow globules fall to the bottom without dissolving.

Filtered hot—

A. (i) The filtrate, which becomes cloudy on cooling, was now boiled

for half an hour with BaOH, the baryta soap filtered off, the barium removed by passing CO_2, the $BaCO_3$ filtered off and the filtrate evaporated to dryness.

A minute fragment of the evaporate placed on a slide under a cover-glass in IKI showed clouds of black globules and then rectangular black crystals. This is choline ennea-iodide, and proves that part at least of the fatty material is lecithin.

(ii) On evaporating a portion of the alcoholic filtrate a yellow-brown residue is left. This residue is soluble in ether, but not in acetone. A small piece placed in water slowly swells and puts out finger-shaped processes.

When incinerated and dissolved in dilute HNO_3 it gives crystals of ammonio-phosphomolybdate with ammonium-molybdate and coffin-lid crystals of ammonio-magnesio-phosphate with magnesia mixture.

B. The residue washed with ether. The ether becomes yellow.

(i) While acetone gives no precipitate with this yellow filtrate, the choline reaction can nevertheless be obtained from the dried residue.

(ii) The second residue, after washing with ether, was dried and placed in 1 per cent. NaOH, in which it dissolves. The solution—

(α) Gives the xanthoproteic reaction.

(β) Gives Millon's reaction.

(γ) Gives a pink biuret reaction.

(δ) Does not give the iodine reaction for glycogen.

The centripetal layer therefore contains fatty substance, protein and a little glycogen. Part of the fatty substance is lecithin, which can be precipitated by acetone from alcoholic but not from ethereal solution, which will give choline, and from which phosphorus may be obtained. Part seems to be fat, as some of the globules are soluble in acetone.

The proteins seem to include a globulin, but others are possibly present; for instance, the solid protein in which the fat-globules are embedded, which may be the same as the eosinophilous coagulum seen in the vacuoles of the egg-cytoplasm. The phosphorus obtained from the protein-containing residues may be due to inorganic phosphates or perhaps to a phospho-protein or a nucleo-proteid. No purine

bases have, however, so far been satisfactorily demonstrated in any constituent of this layer.

This layer obviously corresponds to the grey patch in the highly centrifugalised eggs.

The admixture of a good deal of melanin pigment is an additional difficulty in the investigation of the proteins of this layer.

Layer 2.

A. (i) This layer is opalescent and liquid.

(ii) The greyish colour is not due to pigment, but to angular masses enclosing refringent globules of fatty material. These masses are not very numerous. They are soluble in 1 per cent. NaOH.

(iii) The liquid plasma is coagulated by alcohol, the coagulum being finely granular.

(iv) Distilled water produces a finely granular precipitate, the granules being arranged in a reticulum in which the angular masses are emmeshed. The greater part of the plasma remains liquid.

(v) The plasma may be coagulated by heat.

(vi) It may be precipitated by sublimate, and by 2 per cent. acetic.

(vii) HNO_3 gives a white precipitate. This becomes yellow on heating, and with NH_3 an intense yellow.

B. A portion of this layer is dissolved in distilled water and filtered.

(i) The filtrate—

(*a*) Is perfectly clear under the microscope.

(*b*) Is slightly alkaline.

(*c*) Is heat coagulable.

(*d*) HNO_3 produces a slight opalescence.

(*e*) Gives the xanthoproteic reaction.

(*f*) Gives Millon's reaction.

(*g*) Gives a purple biuret reaction.

(*h*) Gives a slight glyoxylic reaction (Adamkiewicz).

(*i*) Boiled with 40 per cent. NaOH and treated with lead acetate gives no sulphur reaction.

(ii) The residue, incinerated, gives the ammonium molybdate phosphorus reaction.

C. A portion of this layer is dissolved in ·75 per cent. NaCl and filtered; it filters slowly.

(i) The filtrate—
  (*a*) Is opalescent.
  (*b*) Is alkaline.
  (*c*) Gives a heat coagulum.
  (*d*) Gives no precipitate with 2 per cent. acetic.
  (*e*) Gives Heller's HNO_3 reaction.
  (*f*) Gives the xanthoproteic reaction.
  (*g*) Gives Millon's reaction.
  (*h*) When boiled with H_2SO_4 the vapours (of furfural) turn anilin acetate red.
  (*i*) Acidified with acetic, iodine gives an abundant red colour, which disappears on heating but reappears on cooling.
  (*j*) This glycogen reaction is not given after the liquid has been digested with saliva.
  (*k*) After incineration gives the ammonio-phosphomolybdate and ammonio-magnesio-phosphate reactions.

(ii) The residue, dried and washed with ether—
  (*a*) Gives the xanthoproteic and—
  (*b*) Millon's reactions.
  (*c*) Washed again, in hot alcohol, the residue, incinerated gives the two phosphorus reactions.

It appears, therefore, that the second layer contains proteins, a good deal of glycogen, presumably in solution, and a small quantity of fatty substance, of which a part may be lecithin.

The evidence, so far as it goes, seems to point to the existence of at least two heat coagulable proteins, of which one is soluble in water, the other not. There is also the solid protein, in which the fat-globules are entangled. The phosphorus may be due to inorganic phosphates, or to phosphoproteins or to nucleo-proteins, but no satisfactory proof of the existence of the last has been obtained.

This layer is represented in the eggs by the white circle outside the grey patch.

Layer 3

A. (i) Is thick and pasty.
  (ii) Microscopically examined, yolk-granules, pigment and fat-globules are seen.

The yolk-granules are soluble in—

(*a*) NaCl 10 per cent., 5 per cent., $2\frac{1}{2}$ per cent., but in $1\frac{1}{4}$ per cent. only swell a little and lose their refringency without disappearing. In saturated solution the yolk-granules swell and become less refringent, but remain distinct.

(*b*) $(NH_4)_2SO_4$ $\frac{2}{5}$ saturated solution, but not in $\frac{1}{2}$ saturated or saturated solution.

(*c*) NaOH 1 per cent.

(*d*) Na_2CO_3 1 per cent., 2 per cent.

B. A watery extract is made and filtered.

The filtrate—

(*a*) Is opalescent.

(*b*) Is alkaline.

(*c*) Is coagulable by alcohol.

(*d*) When acidified and boiled gives a heat coagulum.

(*e*) Gives Heller's reaction.

(*f*) Gives the xanthoproteic reaction.

(*g*) Gives Millon's reaction.

(*h*) Gives a purple biuret reaction.

(*i*) Re-filtered, the filtrate still gives a heat coagulum, and Heller's, Millon's, and the xanthoproteic reactions.

(*k*) Gives no glycogen reaction with iodine.

C. An extract is made in ·75 per cent. NaCl and filtered.

The filtrate—

(*a*) Is opalescent.

(*b*) Is alkaline.

(*c*) Gives a heat coagulum when acidified and boiled.

(*d*) With HNO_3 gives a cloudy precipitate, which partially clears on boiling and reappears on cooling.

(*e*) This turns yellow on addition of NH_3.

(*f*) Gives Millon's reaction.

(*g*) Gives a purple biuret reaction.

(*h*) Is precipitated by distilled water.

(*i*) Is precipitated by alcohol.

(*j*) Does not give the anilin acetate reaction.

D. An extract is made with 1 per cent. NaOH and filtered.

(i) The filtrate gives a precipitate with 2 per cent. acetic, completely when the liquid has been neutralised.

(ii) Re-filtered, the filtrate—

(*a*) Is coagulated by alcohol.

(*b*) Gives the xanthoproteic reaction.

(*c*) Gives Millon's reaction.

(*d*) Gives a purple biuret reaction.

E. A quantity of the material is ground up in a mortar and washed with ether. The ether becomes yellow.

It is then boiled in alcohol, which extracts still more fatty substance.

The residue—

(*a*) Dissolved in 10 per cent. trichloracetic acid, and filtered. The filtrate gives no phosphorus reaction.

(*b*) Incinerated and the ashes dissolved in dilute HNO_3;

(1) Ammonium-molybdate gives crystals of ammonio-phosphomolybdate.

(2) The crystals washed in water and dissolved in NH_3. Magnesia mixture gives crystals of triple phosphate.

(*c*) Boiled with H_2SO_4. The fumes do not give the anilin acetate reaction.

F. A quantity of the stuff is treated with ether and hot alcohol until the fat and lecithin has been extracted.

It is then subjected to the following treatment, borrowed with slight modification from Fridericia, in order to see whether purine bases are present:

[(1) Hydrolysed by boiling for fifteen hours with 1 per cent. H_2SO_4.

(2) NH_3 added till alkaline.

(3) Boiled till no alkaline vapours are given off.

(4) Two per cent. acetic is added till the reaction is acid, and the liquid is boiled.

(5) The coagulated proteins are filtered off.

(6) Equal parts of 40 per cent. sodium bisulphite and 10 per cent. copper sulphate are added. The whole is boiled for three minutes.

(7) Filtered. The residue washed repeatedly with boiling water until the water is no longer blue.

(8) Filter-paper and residue are now put back into the same flask that was used for the copper precipitation, water is added, the whole brought to the boiling-point, and excess of sodium sulphide added.

(9) Acidified with acetic and the H_2S boiled off; filtered. The residue washed with boiling water which is added to the filtrate.

In this filtrate are the purine bases if any.]

The copper precipitate obtained from the material of layer 3 was a bright Indian red colour. The final filtrate (9) gave the following reactions:

(*a*) NH_3AgNO_3 added. A gelatinous precipitate came down at once. This is the silver compound of the purine base.

(*b*) The precipitate of (*a*) was washed and dissolved in hot 33 per cent. HNO_3. A crystalline precipitate slowly settled.

(*c*) Evaporated with HNO_3 to dryness it became yellow. The addition of NaOH turned this a bright red, which colour heat converted to a brownish-red. This points to xanthine.

(*d*) Chlorine water and a drop of HNO_3 was added. The whole is evaporated. The residue was white. The addition of NH_3 turned this first yellow then dark red on heating. This again points to xanthine.

(*e*) It was precipitated by ammoniacal basic lead acetate but not by basic lead acetate alone.

(*f*) It was precipitated by NaOH; the precipitate was soluble in excess.

(*g*) With HCl a white crystalline precipitate was given.

(*h*) With Zn and HCl no red colour was given.

While, therefore, there is no doubt that a purine base may be extracted from the yolk-granules, it is not certain which one is present. The reactions appear to point to xanthine.

The third layer consists, therefore, of pigment and yolk, and some fat. There is no glycogen. There may be a little plasma left between the yolk-granules, which would give the proteins found in the watery extract.

The solubilities of the yolk-granules point to their protein being a globulin or a nucleo-protein. They certainly consist of or contain a nucleo-protein, from which a purine base, probably xanthine, can be obtained.

The third layer obviously corresponds to the pigmented yolk region of the centrifuged egg.

These data do not, of course, pretend to be in any sense a complete account of the chemistry of the frog's ovum, but they are, I hope, a beginning which may serve as the basis of future work. Even as they stand they may throw some light on the relation between the stratification of the egg-cytoplasm and the abnormal development of the embryo.

Before concluding this section I will refer briefly to the results of one or two other experiments in which the thickness of the layers was determined in egg-pulp centrifuged at different

speeds, and at the same time, and for the same length of time, as the eggs of the series H. 1, 2 and 3 and I. 1 and 2.

These results are embodied in the accompanying table :

	Thickness in millimetres : Layer 1.	Layer 2.	Layer 3.
H. 1 (10 minutes at speed III)	8		24
H. 2 (10 minutes at speed II)	4		26
H. 3 (ten minutes at speed I)	2		22
I. 1 (10 minutes at speed IV)	1	$2\frac{1}{2}$	$27\frac{1}{2}$
I. 2 (30 minutes at speed IV)	1	5	31

It was not possible in the H series to distinguish accurately the boundary between the first and second layers.

In the next table the relative volumes of layers 1 and 2 taken together and of layer 3 are given, and the ratios of the volume of the third to that of the others.[1]

	Relative volumes. Layers 1 and 2.	Layer 3.	Ratio $\frac{\text{layer 3.}}{\text{layers 1 and 2.}}$
H. 1	8	$21\frac{1}{2}$	2·7
H. 2	4	$23\frac{1}{2}$	5·9
H. 3	2	$19\frac{1}{2}$	9.75
I. 1	$3\frac{1}{2}$	25	7·14
I. 2	6	$28\frac{1}{2}$	4·75

The relative volume is a measure of the disturbance. Since the embryos of H 3 develop nearly normally, while those of I. 1 develop, but not so normally, but the remainder not at all, it may be said that centrifuged eggs are only able to develop when in a mass of egg-pulp centrifuged at the same acceleration and for the same time the combined volumes of the

[1] In calculating the volume allowance must, of course, be made for the hemispherical bottom of the tube.

layers of fat and plasma are not more than one seventh of the volume of the layer of yolk.

I have only to add that the few quantitative determinations I was able to make show, as we should expect, that the water content and the fat content of the third layer diminish with increased centrifuging. The fat was extracted in a Soxhlet apparatus. I give the figures in tabular form :

	Percentage of dry substance in layer 3.	Fat in layer 3 given as a percentage of the dry substance.
H. 1	32·6	22·4
H. 2	31	24·4
H. 3	29·9	—
I. 1	32·4	—
I. 2	34·9	—
Egg-pulp not centrifuged	30·4	25

That there is a smaller percentage of dry substance in the third layer of H. 3 than in the uncentrifuged egg-pulp must be attributed simply to the fact that different lots of eggs were used in these two determinations :

(c) Conclusions to be Drawn from the Foregoing Experiments.

The egg of the frog has a structure which depends on a certain arrangement of visible materials—the plasma, the glycogen, the yolk and the pigment—this arrangement being such that the plasma and the glycogen gradually decrease in concentration from one point on the surface towards the diametrically opposite point, while the yolk gradually increases in concentration in the same direction. The pigment lies in a superficial sheet in the plasmatic two thirds of the egg. In respect of the distribution of these substances, therefore, the egg has a polar structure, or polarity, or is radially symmetrical about an axis with unlike poles. As a result of fertilisation this polar is replaced by a bilateral structure

when the grey crescent appears on one side of the egg at the margin of the pigmented area.

To this original polar and superimposed bilateral structure the structure and symmetry of the embryo are definitely related in ordinary development in such a way that the head of the embryo is formed near that pole towards which the concentration of the plasma is increasing—the animal pole—the blastopore closes near the vegetative pole, that towards which the concentration of the yolk is increasing, while the dorsal side is that on which the grey crescent appeared. Ventral, right and left sides are therefore also simultaneously determined.

From Pflüger's experiment, and the variant of it in which a centrifugal force is substituted for gravity, it appears that a new polarity, and, indeed, a bilateral symmetry, may be conferred upon the egg merely by redistributing the heavier and lighter constituents of the cytoplasm. The new polar structure resembles the old in being determined by a similar graduated arrangement of materials, and to it the segmentation of the egg and the symmetry of the embryo bear precisely the same relation as do the cleavage furrows and the parts of the embryo to the structure of the egg in normal development.

We might reasonably suppose, therefore, that any disarrangement of these materials, severe enough to alter their normal graduation, would bring about a distortion of development or might even prevent it altogether. And this, as we have seen, actually occurs.

By the centrifuge the materials of the egg are driven past one another in opposite directions, the fat towards the centripetal (animal) pole, with some entangled pigment and some plasma, the yolk and pigment towards the centrifugal (vegetative) pole, while the movement of the plasma is opposite at one end to that of the fat, at the other to that of the yolk.

Where the separation of materials is nearly or quite complete no development is possible nor even a normal cleavage. In

less severely centrifuged eggs, while there must in any case be an excess of fatty substances round the animal pole, and a deficiency of plasma, glycogen and yolk, an excess of yolk and a deficiency of plasma and fat round the vegetative pole, it is possible that in the equatorial region the relative concentration of the different stuffs, their graduation, may remain normal. Such eggs may develop, but monstrously.

The irregularities of cleavage and development may be directly traced to this disturbance.

As far as cleavage goes it appears that yolk deprived of its plasma cannot divide, as, of course, is seen in normally meroblastic eggs, though nuclei may be present. In the enlargement of these yolk-nuclei, their excess of chromatin and their apparently amœboid movements, there is an interesting analogy with the yolk-nuclei of Elasmobranch and Teleostean ova. The distortions of development are seen primarily at the head and blastopore ends—that is, in the parts developed from materials situated at the animal (centripetal) and vegetative (centrifugal) poles. In front there is a fatty vacuolation of the ectoderm and of other organs, which increases in the more severely handled eggs. This is obviously due to the accumulation of fat around the animal pole. In the yolk towards the other end there is seen a mass where there are no cell-divisions and the yolk-granules have fused. This is to be attributed to the deficiency of plasma. Where the derangement of materials has been greater the front part of the head is degenerate, and the blastopore fails to close, the yolk-plug remaining exposed. The first of these malformations must be assigned to the excess of fat and lecithin, the deficiency of plasma and possibly of glycogen, and probably also of yolk, since the yolk contains a store of nucleo-protein which is presumably normally used in the production of fresh nuclear material. The persistence of the yolk-plug, on the other hand, is caused ultimately by a deficiency of plasma. In the normal process of the closure of the blastopore the yolk-cells are not merely passively pushed beneath the overgrowing blastoporic lip, but creep actively inwards, as Kopsch

showed long since. Anything which diminishes their vitality, as, for example, in experiments of another kind, heat or solutions of salt and other substances, will inhibit the process and the yolk-plug will persist. In the present case it is the deficiency of the plasma; the cells are too large and overloaded with yolk. But while at the animal and vegetative poles, or head and blastopore ends, the development is being distorted in this way, the middle region of the body may remain fairly normal. As already pointed out, the distribution of materials may remain approximately normal in the equatorial tract while altered at the poles, and the development of the middle part be regular for this reason. With more centrifuging, however, the usual distribution of stuffs in this region also is lost and then the power of development disappears; the heart, pronephros, auditory vesicles, nervous system and alimentary canal all go, and we are left with an embryo in which no development has occurred beyond the differentiation of some mesoderm. The significance of the experimental results obtained by Konopacka is now apparent. When the egg is centrifuged in an early stage (prior to fertilisation), sufficient time (several hours) elapses before cleavage begins, a return of the materials to their original position is possible, and development is normal. But when the disturbance takes place immediately before cleavage, or during its early phases, there is no time for recuperation and there are many abnormalities. That such a return does take place, or at least begins to do so, is shown by the blurring of the zones and rings, even in highly centrifuged eggs, by the time cleavage begins.

A certain arrangement of most of the visible materials of the cytoplasm appears therefore to be a condition of normal development: the concentration of the plasma, glycogen and yolk must be graduated in a certain way, that of the fat must be uniform. The position of the pigment is alone inessential, as Morgan has pointed out. But with this exception the factors to which the egg owes its visible polar structure are also the causes of the production from that egg of a normal organism.

[Though it does not fall within the scope of these experiments, it seems probable that the relation between the bilateral structure of the ovum and the bilateral symmetry of the embryo might be expressed in similar terms. It seems likely that at the time of the formation of the grey crescent there is some redistribution of the materials in the cytoplasm : it is, indeed, known that the grey crescent is due to an immigration of pigment.]

The visible materials of the egg are partly living, the plasma, partly not, the yolk, glycogen and fat. Though the latter are not properly to be termed organo-genetic, yet they condition development in the manner described. In the living plasma, on the other hand, there may be distinct organo-genetic bodies, arranged in a certain way, and each causally related to the formation of some particular organ, and the disarrangement of these would necessarily involve malformation. No evidence for their existence is, however, brought forward by these experiments, and we must postpone the discussion of this problem until we have inquired how far in other cases also a certain arrangement of materials not only bestows a polarity upon the egg, but is also a condition of the normal development of the embryo.

(III) ON THE RELATION BETWEEN THE CYTOPLASMIC STRUCTURE OF THE EGG AND THE DEVELOPMENT OF THE EMBRYO IN GENERAL.

The effect upon development of deranging the cytoplasmic materials of the egg has now been investigated in the ova of several Invertebrates.

We turn first to the experiments carried out by Lyon, and by Morgon and Spooner, upon the eggs of the sea-urchin, Arbacia, in which there is a diffuse red pigment.

If the ripe but unfertilised ovum be strongly centrifuged (f = 6400 g) four strata appear. The red pigment passes to the centrifugal pole ; next to this is a grey granular layer, blackened by osmic acid, then a fluid hyaline layer in which

lies the nucleus, while the centripetal pole is occupied by a cap of opaque white material. The new axis of stratification which is thus produced by the operation may make any angle with the original axis as determined by the micropyle. When removed from the centrifuge the strata begin to re-mingle, but the first and fourth return to their original positions very slowly, if at all. The second and third layers, on the other hand, intermingle with one another rapidly, and it is apparently necessary that they should do so before segmentation and development can occur, for if the egg be broken into two portions between them, then neither portion can be fertilised (Lyon).

In segmentation it is the axis of stratification which determines the direction of the furrows, since the first three, which are at right angles to one another as in the normal egg, either include or are at right angles to this axis, or to put it in another way, the axis of stratification coincides with one line of intersection between some two of these three divisions. At the next division the micromeres are formed at that intersection of two furrows which is at the anti-micropylar pole or nearest to it (when the axis of stratification is oblique to the original egg-axis). It appears therefore that some invisible polarity of the egg has remained unaffected by the centrifugal force, and that this determines, or at least helps to determine, the symmetry of the embryo, since the micromere pole becomes the blastopore pole and the original egg-axis the gastrula axis, or as nearly as possible (Morgan). The pigment is found in any part of the larva, right or left, dorsal or ventral, anterior or posterior. It is not, therefore, essential to the development of any particular part.

It may be added here that the yellow pigment band of Strongylocentrotus is equally unnecessary. Normally it is subequatorial and passes into the archenteron, but it may be meridional or oblique to the egg-axis and so become incorporated wholly or partly in the ectoderm (Garbowski).

Experiments of a like kind on other eggs have yielded very similar results, for while the existence of an invisible structure

has been revealed, a structure which is not disturbed by the centrifuge and is definitely related to the subsequent differentiation, that differentiation has been shown to be independent of some at least of the visible constituents of the cytoplasm.

Thus Lillie, by centrifuging the egg of Chætopterus during the first maturation division, produced in it three layers—a small grey cap at the centripetal pole, a clear layer, and a yellow granular hemisphere (on the centrifugal side). These strata, it was found, might occupy any position with regard to the egg-axis (as defined by the polar bodies), yet in fertilisation the sperm always entered at the vegetative pole and cleavage was always normally related to that axis. The grey cap is derived from the contents of the germinal vesicle, the clear band from the microsomes of the endoplasm, and the yellow granules from the coarser endoplasmic constituents. It would be interesting to know something of the further fate of these centrifuged eggs.

This we do know in some other cases.

The ovum of the Lamellibranch Cumingia contains a red pigment and an oily green material, both scattered through the cytoplasm. When the egg is centrifuged during the first polar division (Morgan) these go to opposite poles, the red pigment to the centrifugal, the green oil to the centripetal. Between the two is a broad hyaline layer. Maturation proceeds and the polar bodies are extruded. With the egg-axis, as so determined, the axis of stratification may make any angle. Fertilisation occurs, and in the subsequent cleavage the planes of division bear the normal relation to the axis of the egg. The strata persist so that the red pigment may be in the AB or the CD cell and so on, the green oil on the opposite side. Development follows, and these two coloured materials are found in the trochophore larva in any position opposite to one another. The structure of the trochophore has the normal relation to the cleavage system and therefore to the original axis. There does, however, appear to be some tendency for the green stuff to redistribute itself.

So, again, in Pulmonate eggs (Physa, Planorbis, Limnæa) Conklin has by the same means produced three strata—a grey finely granular zone at the centripetal pole, a narrow clear band, and a yellow granular centrifugal hemisphere. When segmentation and development take place the strata make any angle with the first and subsequent furrows, and any angle with the principal planes of the embryo. Conklin has, however, added the important observation that the possibility of obtaining a normal development is largely dependent on the redistribution to or towards their original positions of some of the disturbed cytoplasmic materials, for it is only when the operation is performed prior to maturation or during its earliest stages—only, that is, when some time elapses between the operation and cleavage—that development is afterwards normal. Eggs centrifuged during the extrusion of the first polar body or later either die or give rise to monstrous embryos. It appears, further, that during the interval the clear substance disappears into the grey or the yellow layer or both—a readjustment which cannot occur unless sufficient time be allowed.

In another mollusc, Crepidula, on the other hand, as well as in the Ascidian Cynthia, Conklin has found it possible, by prolonged centrifuging, to shift the original polar axis (the position of which in the experiments just quoted is left unaltered) without prejudice to normal development. The symmetry of cleavage and of differentiation are, it seems, determined by the new polarity, as in the frog's egg.

In Cyclops (Spooner) the centrifuge separates the cytoplasm into three similar zones—a greenish-white layer at the centripetal pole, a middle clear stratum, and the blue yolk-granules. These eggs are stated to develop normally, even when continuously centrifuged.

From Hegner's observations it appears that in centrifuged beetle eggs the embryo is developed at the centripetal end, whether this be the morphologically anterior or posterior end; also that when the eggs are centrifuged violently enough to produce well-marked layers the development is abnormal.

In the Rotifer Hydatina (Whitney) the polar zones are pink and grey, the middle clear as in the foregoing instances. The stratification may have any relation to the original axis, and the first cleavage is, as in the normal egg, transverse to this axis. Normal young are produced, become mature and reproduce in their turn.

Lastly, in the centrifuged egg of Ascaris similar strata appear. The normal egg is telolecithal. There are in the cytoplasm also some clear spherules and pigment-granules. The layers that appear after centrifuging are a sheet of yolk at the centripetal end (the yolk is here lighter than the cytoplasm), a layer of clear spherules, clear plasma, and finally at the centrifugal pole a cap of brown pigment. When strongly centrifuged the egg becomes flattened against the slide on which it is placed (the centrifugal force is perpendicular to the slide), and, if still subjected to the action of the force, the fertilisation spindle places itself at right angles to the direction of the latter—that is, parallel to the stratification (and in the clear zone), and the division is meridional. If, on the other hand, the egg is removed from the machine (or is less strongly centrifuged), it resumes or keeps its spherical shape, and the spindle returns more or less completely to its proper axial position, and the first division is equatorial (or oblique).

It is suggested by Boveri and Miss Hogue (to whom the experiment is due) that there is an invisible polarised structure in the cytoplasm which is not affected by the operation and with the axis of which the stratification of the movable substances can make any angle. Into the axis of this invisible polarity the spindle is supposed to return if and when the egg is allowed to resume its spherical shape. The facts do not appear to necessitate this view, for when placed on the machine the whole egg rotates inside its shell until the heavier animal pole is centrifugal, and then the stratification of the cytoplasmic materials begins. As long as the force is operating the spindle is compelled to place itself parallel to the stratification, but when released from the force,

returns or attempts to return to its usual position, namely, in the egg-axis, that is, in the stratification axis. The obliquity of the spindle in those cases where the return to the normal position is incomplete would then be the result of two tendencies, at right angles to one another, urging the spindle to place itself the one perpendicular, the other parallel to the stratification.

When the spindle returns (more or less completely) to its normal situation the division is equatorial or oblique, and a normal embryo is developed, in spite of the stratification. When, however, the spindle remains parallel to the stratification the first division is meridional, and each cell behaves as the P_1 (vegetative) cell of an entire ovum. (The greater part of the pigment cap is usually extruded from these eggs at the centrifugal pole as a "ball.") Each half blastomere divides into two, which can be recognised as *E. M. St.* (endo-meso-stomoblast) and P_2 (meso-gonoblast), by the chromosomes being diminished in the one, intact in the other, and by their subsequent behaviour, and so gives rise to what is essentially a blastula without ectoderm. (The ectoderm is normally derived from the absent S_1, the sister cell of P_1.) It might be imagined that the ectodermal material had been extruded with the "ball," but apparently this is not so, since development is the same when (as may happen) no ball is extruded.

Now, when we survey the results of these experiments, it certainly does seem at first sight as though the most incontrovertible proof had been brought forward for the existence of a polar structure in the cytoplasm, invisible, not to be shifted by the centrifuge, and not therefore dependent on any graduation or stratification of heavier and lighter materials in opposite directions, a structure, moreover, to which the symmetry of the embryo is definitely related, while it bears no such relation to the visible fat and yolk and pigment, which, indeed, may be driven to any part of the egg without prejudice to the eventual normality of development.

The evidence is, however, not altogether flawless. For, in the first place, we notice that in certain cases (Arbacia, the

Pulmonates) a return of the materials towards their original positions is described—a return which, moreover, appears certainly in the Pulmonates and possibly in the sea-urchin to be a condition of normal development as in the frog. In the second place, it does not follow that because the position of the egg-axis has not been hitherto affected by the centrifugal force employed it never can be so moved. Conklin, indeed, has succeeded in shifting it in Crepidula and Cynthia. The polar structure to which it belongs may therefore eventually prove to be dependent, to quote Conklin's own words, on "the heteropolar arrangement of certain oöplasmic substances," though these are indistinguishable to the eye, and need not, of course, be of sufficiently different specific gravities to allow the force applied to them to overcome their viscosities. When it is remembered that a solute can be centrifugally separated from its solvent, there is no need to despair of the possibility of still further separating from one another the materials of the egg cytoplasm.

Thirdly, the development of these embryos and larvæ has not always been followed either sufficiently closely or sufficiently long to vindicate the claim that is made for their normality.

In the present state of our knowledge, therefore, it is still possible that that polarity of egg-structure to which the development of the embryo is so intimately related is in these cases also itself dependent on a graduated concentration of different materials, and renewed investigation may justify the generalisation of this conception. That would probably enable us at once to express the polarity of the developing and the polarity of the regenerating organism in common terms. For the "gradation of materials," a formula originally proposed by Morgan himself, is certainly the best hypothesis yet put forward to explain one of the most constant features of regeneration—the formation of a regenerated structure with the same characters in the same direction as the original. Polarity may, of course, be reversed, but the reversal can usually be accounted for by supposing that the concentration

of a particular substance at a certain point has overcome the direction of gradation of the other substance (as, for example, when two Hydras are grafted together by their head ends, one cut off after the union is complete close to the graft plane, and a head instead of a foot regenerated from the cut surface).

Now though the polarity of an organism is often compared to the polarity of a crystal, experiment has made it abundantly clear that there is no real parallel between the two phenomena. Any fragment of a crystal has, of course, the same optical properties with the same orientation as the whole from which it was taken, no matter from what part of the larger piece it came; and any such fragment will regenerate the whole when suspended in the mother liquor. But with organisms it is not so. In certain cases (Protozoa, Hydroids, Planarians, some Oligochæts) any piece that is not too small, or at least any piece transversely cut out, will give rise to a complete organism, and the original polarity is observed in the process, but the proportions are not necessarily the same as in the original, and differ with the level or region of the whole from which the part was removed; and, indeed, the influence of region is frequently so great that in certain parts of the body the original structure cannot be replaced at all, as in the earthworm, where a head is never regenerated at an anterior cut surface behind a certain level, but a tail instead—another instance of region, or substance, overcoming polarity or the graduation of another substance. The hypothesis, of course, though the best available, remains a pure speculation, for the graduation of materials cannot be seen here as it can in the ovum.

It is not, however, only by means of the centrifuge that the causal relation between the polarity of the egg-cytoplasm, a certain arrangement whether of its visibly different or of its indistinguishable materials, and the formation of the organs of the embryo can be demonstrated. There is a whole series of experiments of another kind in which some

definite part of the ovum being removed, some definite organ or system of organs of the embryo is defective or lacking. Not only, therefore, does the polarity of the egg-cytoplasm determine the orientation of the parts of the embryo, but certain materials in it are causally associated with the eventual appearance of certain organs; the materials in question may, but need not be, situated in that region in which the organ will be developed, as in Dentalium, where factors on which the formation of both the anterior sense-organ and the trunk of the larva depend are both resident in the vegetative polar lobe of the egg.

In the cytoplasm, therefore, are placed, on both lines of evidence, the causes on which the primary differentiation of the embryo depends. Not that it is proved or even necessary to suppose that every separately inheritable organ or character of an organ is represented by some distinct material, for there is evidence that fresh structures may be developed by the interactions of already existing parts on one another. Given, indeed, some heterogeneity to start with, and the rest, however complex it may be, will follow in a regular and orderly sequence.

And this primary heterogeneity resides in the cytoplasm, which, therefore, is a vehicle of inheritable characters. The characters which the cytoplasm of the egg-cell thus transmits appear to be those which give the organism its rough outline, the large features which place it in its proper phylum, class, order, family, perhaps, which make it an Echinoderm and not a Vertebrate or a Mollusc, an Echinoid and not an Asteroid, and such general characters are probably transmitted only by the cytoplasm, and, therefore, only by the female cell, if we may trust the evidence of heteregeneous hybridisations. The smaller characters, of course—generic, specific, varietal—which might be more correctly described as so many modifications of the larger ones, are obviously transmissible equally by the germ-cells of the two sexes; that is, are carried by the chromosomes or smaller chromatic elements of their nuclei. But even the different chromatic

elements require a difference in the cytoplasm before they can exert each their appropriate activity, and call forth each the character to which each is beforehand appropriate. For every cell in the body contains in its nucleus a complete set—indeed, in sexually produced organisms two complete sets—of the chromatin elements or determinants characteristic of the species, since nuclear division is quantitative always, with the exception of the maturation division of the germ-cells. It is hard to conceive of the several chromatic determinants coming into operation, each at the right place and time, save in a heterogeneous medium. Given such a medium, then, this nuclear element will become active here, and that there, this will produce a specific modification of one character, that of another. And it is in the cytoplasm that this primitive and necessary heterogeneity is found, and from the cytoplasm that the prime factors of differentiation may, we hope, be isolated, and perhaps by means of the centrifuge.

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EXPLANATION OF PLATES 7–12,

Illustrating Dr. J. W. Jenkinson's paper "On the Relation between the Structure and the Development of the Centrifuged Egg of the Frog."

PLATE 7.

Fig. 1.—O. 1. *a*, from the equator; *b*, from the animal pole; *c*, segmentation.

Fig. 2.—O. 2. *a*, from the equator; *b*, from the animal pole; *c*, *d*, segmentation.

Fig. 3.—O. 3. *a*, from the animal pole; *b*, segmentation.

Fig. 4.—O. 4. *a*, from the equator; *b*, from the animal pole; *c*, *d*, cleavage, from the equator and from the animal pole.

Fig. 5.—O. 5. *a*, from the animal pole; *b*, cleavage.

Fig. 6.—O. 6. *a*, from the equator; *b*, cleavage from the equator; *c*, cleavage from the animal pole.

Fig. 7.—J. 1. From the animal pole.

Fig. 8 *a*.—M. 1. 4 cells. 8 *b*.—M. 2. 4 cells. Both from the equator.

PLATE 8.

Figs. 9–13.—Meridional sections of centrifuged eggs.

Fig. 9.—O. 1. 1–5, the strata or layers (see text); v., enormous fat-vacuole.

Fig. 10.—O. 3. 1–5, the layers described in the text; v., vacuole.

Fig. 11.—O. 4. Figures and letters as before.

Fig. 12.—O. 6. 1–4, the layers described in the text; 5, yolk with pigment; 6, the original unpigmented portion of the yolk. (5 and 6 correspond to 4 and 5 of the previous figures); in 3 there is no fat; 5 and 6 have not yet been exhausted of their fat.)

PLATE 9.

Fig. 13.—K. 1. 1, vacuolated fatty layer with some pigment; 2, plasma; 3, yolk and pigment; 4, unpigmented yolk.

Fig. 14.—Meridional section through the meroblastically segmented egg, L. 4, 6 : iv : '13. There is a cap of cells (*bl.*) lying on a vacuolated unsegmented plasmatic mass in which are patches of yolk-granules and some pigment. The nuclei here are large, chromatic and irregular. Below is the yolk, with nuclei only in its more animal region. Of the vacuoles some are filled with a (protein) coagulum, while others (clear) presumably contained fat.

PLATE 10.

Fig. 15.—Glycogen spherules amongst the yolk-granules: *a*, from the animal; *b*, from the vegetative region of the normal egg.

Figs. 16–18.—Minute structure of the layers in the centrifuged eggs.

Fig. 16.—*a*, layer 1; *b*, layer 2 and *c*, layer 3 (with some yolk-granules) of O. 1.

Fig. 17.—O. 3. *a*, Eosinophilous coagulum in vacuoles of layer 1; *b*, plasma of layer 2, finely alveolar, with some pigment and a few vacuoles; *c*, the vacuoles of layer 3, with yolk-granules in the walls; *d*, lump of plasma from layer 3.

Fig. 18.—O. 6. *a*, layers 2 (above) and 3 (below); a few yolk-granules in 3; *b*, the upper vacuolated sheet of the grey ring (layer 4); *c*, fat-globules (stained with Sudan III) amongst the yolk-granules of the vegetative moiety of the egg.

PLATE 11.

Figs. 19–43.—Development of the centrifuged eggs.

Fig. 19.—G. 1. 8 : iv : '13 (a).
Fig. 20.—G. 1. 8 : iv : '13 (b).
Fig. 21.—G. 2. 8 : iv : '13 (c).
Fig. 22.—G. 2. 8 : iv : '13 (d).
Fig. 23.—G. 2. 8 : iv : '13 (e).
Fig. 24.—H. 3. 2 : iv : '13 (a).
Fig. 25.—H. 3. 2 : iv : '13 (b).
Fig. 26.—I. 1. 3 : iv : '13 (a).
Fig. 27.—I. 1. 3 : iv : '13 (b).
Fig. 28.—I. 1. 4 : iv : '13 (b).
Fig. 29.—I. 1. 4 : iv : '13 (c).
Fig. 30.—I. 1. 4 : iv : '13 (e).
Fig. 31.—L. 1. 6 : iv : '13 (a).
Fig. 32.—L. 1. 6 : iv : '13 (c).
Fig. 33.—L. 1. 6 : iv : '13 (d).
Fig. 34.—L. 1. 6 : iv : '13 (e).
Fig. 35.—L. 1. 6 : iv : '13 (f).
Fig. 36.—L. 1. 8 : iv : '13 (a).
Fig. 37.—L. 1. 8 : iv : '13 (b).
Fig. 38.—L. 1. 8 : iv : '13 (c) (2).
Fig. 39.—L. 1. 8 : iv : '13 (e).
Fig. 40.—L. 1. 8 : iv : '13 (f).
Fig. 41.—L. 1. 8 : iv : '13 (g).
Fig. 42.—M. 1. 8 : iv : '13 (b).
Fig. 43.—M. 1. 8 : iv : '13 (c).

PLATE 12.

Fig. 44.—Vacuolated anterior ectoderm, in *b* much thickened and pitted. In *a* the vacuoles are confined to the epidermal layer. *a*, G. 1. 8 : iv : '13 (b); *b*, G. 2, 8 : iv : '13 (e).

Fig. 45.—Cells and cell-débris from the anterior end of G. 1, 8:iv:'13 (b). *a*, from the degenerate remains of the brain. The figure includes vacuolated pigmented cells, one with the large pale nucleus of a neuroblast, the other, containing also yolk-granules, probably a spongioblast, a spongioblast without vacuoles but with yolk-granules, fragments of cells with spherules of chromatin from chromatolytic nuclei, and free spherules of chromatin and bits of cytoplasm; *b*, deeply pigmented cells, probably mesodermal; *c*, vacuolated mesodermal elements.

Fig. 46.—G. 2. 8:iv:'13 (a). Group of vacuolated and pigmented neuroblasts, with nerve-fibres. Probably the ganglion of the fifth nerve.

Fig. 47.—G. 1. 8:iv:'13 (b). Section of the abnormal spinal cord and ventrally fused ganglia. In the cord the lumen is pushed dorsally by the extension of the white matter on to the ventral side, where it forms a continuous band. Above this a layer of neuroblasts, with pale nuclei, and then round the lumen the spongioblasts with darker nuclei. There are yolk-granules in both spongioblasts and neuroblasts and a few vacuoles in the neuroblasts. The fibres of the dorsal root are seen on one side (the right) growing in from the cells of the ganglion.

The Sporogony and Systematic Position of the Aggregatidæ.

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With Plate 13.

The very noticeable parasites infesting the alimentary canal of many Cephalopods were recorded by Eberth (**3**) so long ago as 1862. The genus comprising these forms has suffered many changes of nomenclature. In 1875 it was named Benedenia by Schneider (**21**, Note xiii, p. xl) to include B. octopiana from the octopus, and this generic name was employed so late as 1900 by Schaudinn (**20**, p. 203). In Schneider's second paper (**22**) in 1883 the parasite was referred to throughout as Klossia instead of Benedenia, as the author stated that he was convinced that the two genera could not be distinguished. In this Siedlecki followed him in his two papers of 1898 (**23** and **24**) on the parasites of Sepia, which he considered to be identical with those of the octopus, although he found only three or four sporozoites in a sporocyst, whereas Schneider had described eight to fifteen in Klossia octopiana, and Labbé had already given the specific name of K. eberthi (**7**) to the species inhabiting Sepia. In a footnote to his second paper, however, Siedlecki (**24**, p. 800) stated that after seeing Laveran's work on K. helicina he thought the original name of Benedenia should be readopted. It was then discovered that this name had in 1858 been appropriated for a Trematode genus, therefore Blanchard, in 1900, altered it to Légeria. Jacquement (**5**)

in 1903 pointed out that this had been already applied to a polycystid Gregarine, and again changed the name to Légerina. This, however, was never used; for, in the previous year, after Siedlecki had stated that, like the so-called Eugregarines, these parasites did not appear to undergo Schizogony, the genus had been called Eucoccidium by Lühe (**11**). By this generic name the parasites were generally known until, in 1906, the excellent researches of Leger and Duboscq (**8** and **9**) showed that the schizogony, which had been supposed to be absent from the life-history of the genus, took place in the Crab, Portunus, where Frenzel (**4**) had described it in 1885 as a Gregarine under the name of Aggregata; this name is therefore now universally applied to the genus which I hope to prove is undoubtedly Coccidian in character as assumed by Siedlecki. Moroff (**15**) in 1906 claimed to have shown that the fertilisation was of the gregarine type; that is, that the macrogametocyte gave rise before fertilisation to a number of small cells, and that these were the true macrogametes, each of which gave, after fertilisation, a single spore. In 1908, in a later work of great length, Moroff (**16**) admitted that he might have been mistaken in his description of the process of fertilisation, and no longer insisted on these parasites being Gregarines, stating that further investigations were necessary. It was for this reason that, at Prof. Minchin's suggestion, I undertook this study of Aggregata, which has extended over some years. During this time I have examined Cephalopods, chiefly Sepia, from Plymouth, the Solent, and more recently from the Mediterranean in the neighbourhood of Banyuls-sur-Mer. To the authorities and staff, especially to the Director, Prof. Pruvot, of the Laboratoire Arago, I should like to express my thanks for their hospitality and assistance; also to Prof. Minchin, not only for suggesting such an interesting subject for investigation, but for much kind help given me during the years that I have been privileged to work in his Department at the Lister Institute. Since last October the work has been carried on in Oxford, and I am much indebted to

Prof. G. C. Bourne for the kind interest he has shown in the progress of the investigations, and for allowing me the use of a laboratory in his department. My husband, Mr. E. S. Goodrich, has also given me much advice and assistance.

As pointed out above, Aggregata octopiana of the octopus and A. eberthi of Sepia have long been known, but in addition to these, Moroff (**16**, p. 144) claims to have distinguished some eight or nine more species in Octopus and five in Sepia. The distinguishing characteristics given, being chiefly those of size, are not at all conclusive, and, so far as my observations go, I have been unable to corroborate their existence.

According to Léger and Duboscq (**10**) A. eberthi can undergo its schizogony in Portunus arcuatus and P. holsatus as well as in P. depurator (**10**, p. 90 et seq.), but not in P. puber. L. Their attempts to infect other Crustacea, such as Inachus dorsettensis, Carcinus maenas, etc. (**10**, p. 95), failed, although some of these, for instance, Inachus dorsettensis, are sometimes found in nature infected with a species of Aggregata (Smith, **19**). Possibly the natural cephalopod host of the latter is an octopus; it does not, at any rate, seem to be Sepia officinalis. Many experiments such as these appear to me to be necessary before we can hope conclusively to distinguish different species of Aggregata, the possible existence of several of which I am not prepared to deny.

Material and Technique.

It is very essential to have the material quite fresh, and in this connection a method of killing Cephalopods so as to eliminate the difficulty of dealing with the emission of ink may be of interest. This is to slip a loop of fine, though strong string round the head just above the mantle, and suddenly to pull the ends very tightly. In this way the animal is immediately strangled—the general paralysis being too quick to allow time for any ink to be thrown out, and at the same time quantities of blood are left in the heart and may be drawn off to be used as a mounting medium if required.

All Sepia officinalis, whether from Plymouth, the Solent or Banyuls, have been found to be infected: but none of the numerous specimens of Sepia elegans, which occurred in quantities near Banyuls-sur-Mer, had any infection during September, neither had the Eledone and Loligo that were examined.

Both Sepia officinalis and Eledone from Banyuls were almost universally infected with the Cestode Scolex polymorphus, the occurrence of which has already been recorded by Monticelli (**14**) from Naples. In Sepia officinalis the region of the alimentary canal most infected was the spiral stomach between the entrance into the muscular stomach and the longitudinal ridges which run up into the rectum. The latter chiefly contained large cysts, which, being somewhat over one millimetre in diameter, were quite visible to the naked eye. For examination in the fresh state some of the tissue from the spiral stomach was teased out, in sea-water as a general rule, though sometimes intestinal fluid or blood were found to be preferable. Films and portions of the spiral stomach were fixed in Schaudinn's fluid, ordinary corrosive sublimate and acetic acid, or mixtures of the latter with equal quantities of 4 per cent. formalin—this last perhaps giving on the whole the best results. Iron hæmatoxylin was by far the most satisfactory nuclear stain. Ehrlich's and Delafield's hæmatoxylin mixtures were also used, as well as paracarmine, borax carmine and many cytoplasmic stains, such as Lichtgrün and picric acid, eosin or orange G.

Vegetative Stages.

Since Léger and Duboscq (**10**), Moroff (**16**), and others have already given excellent figures of merozoites, trophozoites, and sporozoites, we can begin our study with the adult trophozoite ripe for reproduction. At this stage there is certainly a delicate limiting membrane present—a fact denied by Siedlecki (**24**, p. 804),—also a large vesicular nucleus, containing, as a rule, a single large karyosome. In some

cases the cytoplasm contains large irregular masses, which stain intensely with iron-hæmatoxylin and other chromatin stains. These would seem to be metabolic products of the cytoplasm, such as Comes (**1**) has described in Stenophora, rather than of nuclear origin, and to bear no obvious relation to the processes of reproduction described below. Smaller particles of more uniform size, probably of similar nature, are usually scattered throughout the cytoplasm. The ripe trophozoites vary enormously in size, the longer diameter of some reaching 400 μ, while others may be only about 50 μ; and, since all intermediate dimensions can be found, it does not seem possible to distinguish several species, either in this or subsequent stages, by size alone. Therefore I cannot support Moroff's conclusions on this point (**16**, pp. 146 and 147).

Formation of the Gametes.

While the distinction between males and females becomes very pronounced later on, it is extremely difficult to trace the differences back to the trophozoite stage. However, individuals which are to become females appear, on the whole, less dense, and have their cytoplasm more vacuolated than those destined to be males. The females also as a rule grow to a larger size. After a considerable resting period at the completion of growth the early stages leading up to the formation of the gametes are gone through very rapidly.

In the female there is, first, a migration of the nucleus towards the surface, from which, however, it remains separated by a plug of dense, finely granular protoplasm, which gives off radiations, spreading in all directions through the cytoplasm (fig. 1). The central part of this protoplasmic plug rises up on the surface, forming a cone of attraction, and, after entrance of the microgamete, appears to sink in again, carrying the male chromatin with it (figs. 2 and 3).

In the male the nucleus also approaches the surface and rapidly divides by a peculiar method of mitosis. A centrosome with distinct astral radiations apparently emerges from

the nucleus at the beginning of the process, and undergoes repeated division before the nucleus becomes subdivided. These centrosomes, with their conspicuous asters, spread over the surface of the microgametocyte, giving rise to such polymitotic figures as those given by Moroff (**16**, fig. 50, pl. vi). In the meantime the nucleus becomes irregularly lobed, the lobes growing out in various directions towards the asters. The peripheral lobes eventually become constricted off as separate nuclei, into which the greater part of the chromatin has migrated. At a later stage the nuclei, containing deeply staining chromatic networks, are found evenly distributed over the whole surface. Throughout this process the centrosomes and asters take up a position between the nuclei and the surface of the gametocyte. The peripheral nuclei continue to undergo repeated binary division, preceded in every case by the division of the centrosome and aster. As the nuclei become more numerous the surface at their disposal is increased by the formation of rounded lobes, which gradually become nipped off as separate spheres, covered with developing microgametes (fig. 13). A fully developed male generally consists of several such spheres, sometimes as many as six or seven. The nuclear divisions described above are very similar to those which take place in the formation of the sporoblasts in the zygote, and this fact may have contributed in some measure to Moroff's unfortunate mistake in thinking that these sporoblasts represented the macrogametes and were separately fertilised. It is not clear which process some of his figures are intended to represent; but his figs. 53 and 55 (**16**, pl. vii) appear to me to be stages in the formation of microgametes. The differences to be observed between them will be again referred to (p. 168) after the description of the formation of the sporocytes.

Siedlecki described the nuclear division of the male as taking place by a process of chromidial fragmentation (**24**, p. 815), such as has been described in other Coccidia. This error may, I think, be partly ascribed to the fact that he worked chiefly with films, which he stated (**24**, p. 801) that

he found to be better than sections. I, on the contrary, have found that, owing to the large size and great density, especially of the male gamete, it is practically impossible to make out the very rapid nuclear changes which take place at the beginning of the process without studying an extensive series of sections.

The chromatin of the developing microgametes becomes more and more concentrated until the nucleus is reduced to a small, densely staining mass. This, surrounded by a certain amount of cytoplasm, projects on the surface of the microgametocyte and becomes elongated, carrying the centrosome at its distal extremity. The microgamete now becomes more and more elongated, the nucleus lengthens out, and the stalk connecting it with the residual sphere of cytoplasm becomes drawn out to form the tail region. Two flagella make their appearance at the free end, and the microgamete gradually assumes its fully developed form.

The mature microgametes during life measure about 40 μ in length, which is very much the same as the fixed ones (fig. 11). These lengths do not, of course, include the flagella, which are generally considerably more than half the length of the body of the microgamete. The latter is strap-shaped, with a more or less cork-screw nucleus running through the anterior half. This, at any rate, appears to be the form that the nucleus takes during the phase at which it emerges from the microgametocyst.

It may be of interest to recall that a spiral nucleus has also been recorded in Trypanosoma brucei (Robertson **18**, p. 236) and in the so-called male forms of T. lewisi described and figured by Prowazek (**17**, figs. 35 and 36). In some cases I have observed a faint wavy line extending along the tail, but, except for this, there is no sign of an undulating membrane. In front of the nucleus and at the base of the flagella are situated the granules, which I take to be blepharoplasts. In deeply stained specimens they frequently appear as one large granule. These basal granules are apparently derived from the original centrosome of the

younger stage. During this time the limiting membrane surrounding the male gametocyte becomes considerably thicker. The male individual therefore becomes enclosed in a distinct cyst, which is, however, thinner than that in the female. At an early stage the gametocyte shrinks away from the cyst-wall, becoming separated from it by fluid in which it is suspended, while the microgametes develop on its surface (fig. 13). The cyst is sufficiently resistant to be dissected out of the host tissue, and the microgametes, after the development of their flagella, have been observed actively swimming about inside. Upon the rupture of this cyst they swarm out, but I did not succeed in making them retain their activity for any length of time, although many different media were employed for mounting them, including Sepia, blood and intestinal fluid, as well as ordinary sea-water and saline of varying densities. On this account it was not easy to make out the details of their movement, but flagella could be seen being vigorously lashed in front, and, in addition, the flattened, somewhat expanded tail was used in propulsion.

Fertilisation.

In the fresh state numerous microgametes have been observed crowded round a macrogamete, but only one appears to enter. This it does by the cone of attraction, and on reaching and penetrating the female nucleus its chromatin rounds itself off into granules, as shown in figs. 2 and 3. Moroff's figures (**16**, pl. xi, fig. 91, and text-figs. M_1 and M_2) doubtless represent this stage, although he failed to discover the true meaning, as he was convinced that fertilisation was of the Gregarine type. The macrogamete nucleus has assumed an oval or pear-shaped form by the time the microgamete enters. A network of spindle threads appears running irregularly along the long axis of the nucleus from the point of entrance of the microgamete to the opposite pole. Meanwhile the karyosome undergoes changes. The chromatic substance emerges from the micropyle as a stream of spherules which arrange themselves along the spindle

threads. In some cases strings of granules can be seen inside the karyosome and coming from it; possibly the spindle threads themselves arise in this way. Somewhat similar irregular spindles have been described in other Coccidia; for example, in Coccidium proprium by Siedlecki (**25**) and in Orcheobius herpobdellæ by Kunze (**6**), and no doubt the nuclear fibres figured by Moroff (**16**, pl. iii, fig. 16, and text-fig. N. 2) are of the same nature.

The granules of male chromatin which have become lodged at the superficial end of the nucleus stream inwards, and presumably mingle with the granules of female chromatin on the spindle threads: fertilisation is thus accomplished.

Directly after the entrance of the microgamete a resistant cyst appears round the zygote, which thereupon contracts and comes to lie freely suspended in fluid. A similar contraction has been described as taking place in Coccidium proprium (**25**), but in this species the oocyst is formed quite early, and a micropyle is left for the entrance of the microgamete.

Sporogony.

After fertilisation, upon the break-up of the spindle, the nucleus of the zygote becomes reconstituted, and this synkaryon undergoes mitosis. A centrosome and aster make their appearance on the outer side of the nucleus: they divide, and their daughter-centrosomes and asters separate, accompanied by lobes of the synkaryon, into which passes the chromatin, arranged on spindle fibres in the form of irregular loops (fig. 10). The first few nuclear divisions follow one another in such quick succession that, before the chromatin threads of one spindle have completely separated, the centrosomes have again divided, and the next pair of spindles are being formed approximately at right angles to the preceding one, as shown in fig. 10, where the large asters so generally to be seen are also figured. In this way an extensively branching nucleus is formed, the central region only disappearing at a later stage.

This peculiar form of mitosis in the fertilised female may be considered as intermediate between ordinary mitosis and the polymitosis described above in the male. In the latter the nucleus lags so far behind the centrosomes in its division, and so disturbs the sequence of events, that the connection between the division of the two organs is obscured or lost. Moroff (**16**) has already given numerous beautiful figures of these stages.

After repeated division of the centrosomes and asters large daughter-nuclei with numerous small faintly staining granules are constructed off near the surface (fig. 12). The stage with about twenty to fifty of these is a commonly occurring one, and would seem to indicate a somewhat prolonged resting period. It can generally only be distinguished from the corresponding resting stage in the formation of the microgametes by the larger size and lesser affinity for chromatin stains exhibited by its nuclei. The large early sporoblast nuclei contain, as a rule, small evenly distributed granules of chromatin only, whereas the small early male nuclei have large deeply staining masses, or threads, of chromatin.

This resemblance between the early stages of division in the microgametocyte and those in the zygote is of considerable interest, since it seems to support the view that the two processes correspond to one another, and that not only is the macrogamete of the female homologous with the microgametocyte of the male, but also that the microgamete of the male corresponds to the sporoblast of the female.

Further, whereas in Gregarines the adult female gives rise to small cells (macrogametes) which become separately fertilised and then form the zygotes (sporoblasts), in the Coccidia the adult female gives rise to the small cells (sporoblasts) after fertilisation. Thus the chief difference between the processes of sporogony in the two groups is that fertilisation takes place at an earlier stage in Coccidia than in Gregarines. This could be shown in tabular form as follows, the arrow representing fertilisation:

Coccidia.

♀ trophozoite → macrogamete × n sporoblasts → n sporocysts × mn sporozoites.

♂ trophozoite → microgametocyte × n microgametes.

Gregarines.

♀ trophozoite → macrogametocyte × n macrogametes → n sporocysts × mn sporozoites.

♂ trophozoite → microgametocyte × n microgametes.

Cuénot, in 1901 (**2**, p. 632), remarked that fertilisation was tardy in Gregarines and precocious in Coccidia, but refused to commit himself to a homology of the various steps. The evidence I have given above is, however, in agreement with the theory put forward by Minchin (**12**, pp. 271–274) in 1903 as to the homologies that exist between the gametes of Coccidia and those of Gregarines, namely, that the sporoblasts of Coccidia (formed by the division of the zygote after fertilisation) "may be compared to those of Gregarines by supposing that the process of multiplication, by which the gametocyte of the Coccidia gave rise primitively to a number of female gametes, has not been completely suppressed, but merely deferred until after the process of zygosis" (**12**, p. 272). The same theory is again referred to in 1912 (**13**, p. 354).

During the resting stage in sporoblast formation, as in the same stage of the male, very distinct centrosomes and asters are generally apparent, being situated on pointed processes projecting from the surface. The next stage is marked by the repeated division of these centrosomes and asters, followed by the division of the nuclei. During this time the whole body of the zygote becomes more or less folded, thus increasing the area of the surface on which the nuclei are situated, and giving room for their increasing number. This process corresponds to the breaking up of the microgametocyte into separate spheres at the stage when its nuclei are rapidly increasing in number.

One very usual method of folding in the dividing zygote is for one pole to become invaginated, as in a gastrula; this was noticed by Schneider (**22**, pl. ix, fig. 24). It is clear that when this cup-like body is cut in one direction an arc of cytoplasm surrounded by nuclei will result such as described by Moroff as characteristic of his A. arcuata (**16**, pp. 118 and 146), just as, when cut through a plane at right angles to the former direction, a ring may result.

The effect of this folding and increase of nuclei is to diminish the relative amount of cytoplasm compared to nuclei, so that when the sporoblasts round off there is never any great quantity of residuum, and in most cases none at all. At this stage, therefore, the oocyst is generally crowded with sporoblasts. In these the centrosome and aster persist for some time, forming a projection on the surface (fig. 4), but I have not been able to trace their division preceding nuclear division to form the sporozoites. Before this happens the whole projection sinks down and the nucleus assumes a resting form (fig. 5). On resuming its activity the nucleus divides into two, but though a spindle is formed, no asters are visible. During this first division the sporocyst makes its appearance (fig. 6). In the vast majority of cases only one of these secondary nuclei divides again (fig. 7), and in this way are formed the nuclei of the three sporozoites (figs. 8 and 9) which are normally present. Only occasionally do both of the secondary nuclei divide again, forming four sporozoites. Each sporozoite has at its posterior end an elongated deeply staining nucleus in which no karyosome can be distinguished (fig. 9).

Sometimes streams of sporocysts are to be observed passing along-blood spaces, although, in the Sepia, no tendency can be seen on their part to open in order that auto-infection may take place. The whole oocyst is generally said to pass into the lumen of the Cephalopod's alimentary canal whence it reaches the exterior. So far no definite evidence of the escape of unbroken oocysts has been obtained, and many of them are very deeply embedded in the thick connective tissue

of the wall far from the lining epithelium. Possibly it is by eating the cysts in dead Cephalopods that crabs generally become infected. About the process of schizogony in the crab nothing further need be said, for it has been beautifully described by Leger and Duboscq (**10**).

Systematic Position.

In having this alternation of hosts corresponding to its alternation of generations, the genus Aggregata differs from all other Coccidia whose life-histories are at present known, and on this account would constitute a distinct family, the Aggregatidæ making a third family of Section A of the Eucoccidia in the classifications drawn up by Minchin (**13**, p. 352). The family could not easily find a place in the classification given by Leger (**10**a, p. 86), except that it would belong to the Eimeridea, which corresponds to Minchin's Section A. The subdivisions of Leger's two sections are based on the number of sporozoites in the oocyst, which Minchin (**13**, p. 353) has suggested is not a natural method of classification. This suggestion is supported by the fact that in the Aggregatidæ, now to be included in Coccidia, there is no constancy in the number of sporozoites, A. eberthi having only three or four in each of its numerous sporocysts, while A. octopiana, according to Moroff (**16**), has sixteen, and other species have from eight to twenty-four.

Summary.

(1) The genus Aggregata is undoubtedly to be included among the Coccidia—the fertilisation being not Gregarine in character as described by Moroff (**15** and **16**), but typically Coccidian. In Aggregata eberthi, here described, from Sepia officinalis, the large female gamete is fertilised by the small, active, biflagellate microgamete, and the zygote so formed gives rise to a large number of sporoblasts.

(2) The polymitotic nuclear divisions giving rise to the microgametes are so similar to those giving rise to the sporoblasts that they afford some evidence in favour of the view that these stages are homologous. It is remarkable also that the microgametes further resemble the sporoblasts in being enclosed in a distinct cyst.

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EXPLANATION OF PLATE 13,

Illustrating Mrs. Helen L. M. Pixell-Goodrich's paper on "The Sporogony and Systematic Position of the Aggregatidæ."

[Preparations stained iron-hæmatoxylin and drawn with a 2 mm. lens and compensating ocular 12, giving approximately a magnification of 2000, unless otherwise stated.]

Fig. 1.—Part of a section through a macrogamete ready for fertilisation, with cone of attraction (*co.*) and radiating protoplasmic plug (*pp.*)

separating the nucleus (*n.*) from the surface. Stained Ehrlich's hæmatoxylin. × 800.

Fig. 2.—Fertilisation. The microgamete (*m.*) has just entered, and its chromatin in the form of granules (*ch.*) is passing along the spindle threads (*sp.*). *ca.* Large karyosome also giving off chromatin granules. From a section stained iron-hæmatoxylin and orange G.

Fig. 3.—Complete section through a specimen soon after fertilisation showing nuclear spindle (*sp.*). × 1500.

Fig. 4.—Sporoblast with centrosome and aster.

Fig. 5.—Sporoblast during resting stage.

Fig. 6.—Sporocyst with dividing nucleus. Stained Ehrlich's hæmatoxylin.

Fig. 7.—Sporocyst during its second nuclear division. Stained Ehrlich's hæmatoxylin.

Fig. 8.—Sporocyst with three nuclei.

Fig. 9.—Sporocyst containing three sporozoites with terminal nuclei.

Fig. 10.—Portion of a section of a dividing zygote showing one nuclear division nearly complete and secondary spindles already forming with conspicuous centrosomes (*c.*) and asters. The loops of chromatin granules (*ch.*) are already beginning to arrange themselves on the secondary spindles. Preparation stained with iron-hæmatoxylin and Lichtgrün and picric acid. × 3000.

Fig. 11.—Microgamete with more or less corkscrew nucleus (*n.*) and a blepharoplast (*b.*) at base of each flagellum (*f.*). From a film stained with Delafield's hæmatoxylin. × 1500.

Fig. 12.—Portion of a section of one lobe of a folded and dividing zygote to show developing sporoblasts with large faintly staining nuclei (*n.*) each accompanied by an aster and centrosome (*c.*). From same preparation as fig. 13. × 1000.

Fig. 13.—Section through a microgametocyte showing cyst (*cy.*) containing three spheres surrounded by the nuclei (*n.*) of developing microgametes. The centrosomes (*c.*) and asters are situated on processes of the cytoplasm projecting from the surface. Stained Ehrlich's hæmatoxylin. × 1000.

The Blastocyst and Placenta of the Beaver.

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With Plates 14 to 21 and 6 Text-figures.

Contents.

I. Introduction.

The material upon which this contribution is based was collected by me during the seasons of 1911, 1912, and 1913. In the first season, at the Laurentides National Park in the

province of Quebec, the stage obtained between April 18th and April 27th was that of the submature naked fœtus. In the second season, at the Algonquin Park in the province of Ontario, from May 1st to May 14th, the mature fur-covered fœtus was procured. In the third season, from February 21st to March 11th, in the Lake Edward district between Quebec and Lake St. John, the stages of the early blastocyst and the young placenta were acquired. The result of my first visit to the woods of eastern Canada was published in the second volume of Prof. Spengel's 'Festschrift' (1912). In that paper there will be found the description of a strong extra-placental or periplacental connecting membrane, the "umbilico-uterine ligament," by which the large fœtal sac is suspended from the wall of the gestation sac. This was one of the points that stood in need of further elucidation from new material.

In February, 1913, through the good offices of Profs. J. G. Adami and P. E. Nobbs, of this University, an opportunity for making an effort to secure earlier stages in the development of the beaver was presented to me. To Prof. Adami I am indebted for his kindness in apprising me of the fortunate occasion; while Prof. Nobbs, in the most generous manner, gave me the temporary freedom of a game reservation in which he was interested, together with the use of a hunting-lodge, compactly built of rough-hewn logs, situated on the shore of Lac Gagnon, near Pearl Lake, which is a few miles south of Lake Edward, on the line between the city of Quebec and Lake St. John. I spent eighteen days on this reservation, accompanied by a French Canadian gardien, skilled in woodcraft, named David Fournier, and a French Indian tracker named Joe Moreau. The severity of the season may be judged from the fact that the temperature ranged from $-35°$ Fahr. (February 25th, at 6.30 a.m.) to $+26°$ Fahr. (March 10th, at 1 p.m.).

We succeeded in getting some very young gestation sacs, which were preserved in 10 per cent. formalin. In the summer I brought this material to Europe, and, being

desirous of working upon it without undue delay, I wrote to Prof. Hubrecht, who promptly assured me of a welcome at Utrecht, so that I was able to enjoy the privilege of continuing the research in the Embryological Laboratory of the University of Utrecht for nine weeks, from May 30th to July 31st inclusive. I am deeply grateful to Prof. Hubrecht for his friendship, for the use of his laboratory with all its excellent facilities, for access to the library and to his collection of reprints, and particularly for his criticism upon various questions raised by the preparations, which at the beginning presented puzzling and unique appearances.

The material having been transferred from formalin to alcohol, the several gestation sacs were stained in toto with hæmalaun, serial sections cut, mounted, and counter-stained by Mr. J. G. de Groot, Conservator in the Embryological Laboratory at Utrecht. The preparations have been deposited in the collection of the Institut International d'Embryologie, which was established at Utrecht by Prof. Hubrecht in 1911. Most of the drawings illustrating this paper were made with the assistance of Edinger's "Zeichenapparat." Some of the more finished drawings have been executed by Mr. John Prijs, artist at the Institute, with the kind permission of Prof. Hubrecht.

In addition to the two youngest broods, which were the principal fruit of my third journey, and the only material collected before I left the reservation, later stages were forthcoming by arrangement with the gardien, who forwarded two subsequent consignments to me after my return to Montreal. These included the stage of the half-grown placenta and that of the two-thirds-grown placenta. I made drawings of the more advanced stages in Montreal, but the study of the youngest stages was carried out, as mentioned, in Utrecht.

Finally, I must not omit to acknowledge the assistance in procuring material from the Algonquin Park in 1912, with which I was favoured by Mr. G. W. Bartlett, the Super-

intendent, and by the park rangers stationed at Joe Lake, Messrs. James Bartlett and Mark Robinson.

II. Sex of the Fœtus.

Before entering upon the description of the early stages, reference may be made to the question of the sex of the advanced fœtus. In 1911, of two beavers with young, one contained four fœtuses in the right uterus, the other carried two in the right uterus. There was none in the left horn of the uterus in either case, and all six fœtuses happened to be males. In 1912 I was informed that one gravid beaver had been caught, skinned, and buried several days before my arrival at Joe Lake. When we exhumed the carcase it was evident that the cool sandy loam had kept it perfectly fresh. It yielded five fœtuses, the only fœtal material obtained during the trip. There were three fœtuses in the right horn of the uterus, and these were all males. Of the two remaining fœtuses in the left horn, the one near the oviducal end was a female, the other near the cervical end of the uterus was a male. The male sex of the three right fœtuses thus happens to be consistent with the previous observations, but I am not disposed to lay undue stress upon the coincidence. Still it is well to put such data on record in the presumption that in future years some other observer may similarly rescue trapped beavers from their sandy sepulchre and determine the sex of the unborn young. Out of a total of eleven fœtal beavers whose sex has been determined, ten were males, nine of which were lodged in the right uterus, which has not yielded a female according to present information.

It seems doubtful whether the determination of fœtal sex in a large number of cases would justify the time and expense which would have to be devoted to it. Such data can only be accumulated by recording casual observations of no immediate value in themselves, but likely to be useful for future comparisons. Perhaps it is because occasional determinations seem to be so trivial that there is such a

paucity of references to the curious distribution of the sexes. The records would possibly have an additional interest if the age of the parents could be ascertained, but this is practically impossible in the case of the beaver. The isolated position of the beaver amongst rodents perhaps renders it worthy of special consideration in regard to this matter of the sex of the fœtus which are lodged in the right and left horns of the uterus respectively. As for the apparent preponderance of males, it is known how misleading even impressive numbers may be in regard to the proportions of the sexes of various animals. Thus, in 1911, six females were captured and six fœtal males extracted from two of them. In 1912 beavers were being taken alive in muffled traps and the sex was not always ascertained. My notes contain records of seven females and ten males, including male and female "kittens," two-year-olds and adults. These small numbers, so far as they go, would indicate an approximate equality of the sexes.

With regard to the remarkable multiple embryos of Armadillos, **M. Fernandez** (1909), investigating the case of the "Mulita" (Tatusia hybrida), where the embryos are in packets of about eight (varying in number from seven to twelve) contained within a common chorion and derived from a single ovum, noted that out of nine sets, three were all males, six all females; but as the material belonged to different regions and years it remained doubtful whether the excess of females had any significance. **Newman** and **Patterson** (1909, 1910), working independently about the same time upon the nine-banded Armadillo (Tatusia novemcincta), where the embryos occur as quadruplets, found that out of thirty-eight embryonic vesicles exactly half were male and half female. "All embryos in a vesicle are of the same sex," as was originally recorded for the "Mulita" by **H. von Jhering** in 1885, and only one set is produced at a time.

Newman and **Patterson** (1910) state that it has been known to the natives of Paraguay and of Argentina for over a century that the "Mulita" brings forth at a birth a litter of

young all of one sex. The nine-banded Armadillo does the same; and for this species **Newman** and **Patterson** have found that "there is no correlation between the sex of the embryos and the dextrality or sinistrality of the functional ovary. Out of twenty cases in which the right ovary contained the corpus luteum, the sex of the embryos was male in seven and female in thirteen; while out of thirteen cases in which the left ovary held the corpus luteum, the sex was male eight times and female five. Evidently, then, the position of the functional ovary has no determining influence on sex."

I will conclude this chapter with a couple of miscellaneous notes relating to the beaver. On May 2nd, 1912, a large pregnant female was caught alive by the toes in a muffled trap. On the following day she was taken in a sack to head-quarters at Algonquin Park, and on May 10th gave birth to two kittens about 9 a.m.; at 10.30 a.m. it was noticed that their eyes were open. Towards the end of the day two more were born. The successful rearing of the kittens was ardently hoped for, but was frustrated by their death within two or three days. On May 14th a large male was trapped, measuring 42 in. from tip to tip. It had lost both of its fore-limbs at two previous trappings. The scars of the amputated members had healed up completely. This serious maiming would prevent the beaver from doing its share of work at the lodge and dam, but would not destroy its swimming and feeding powers. It need scarcely be stated that the traps which are set for beaver are concealed as far as possible, and much care is devoted to placing them in the most likely position from the trapper's point of view, so that it says nothing for or against the intelligence of the beaver that after two sad experiences the third proved fatal.

III. The Preplacental Blastocyst.

The most conspicuous feature of the preplacental blastocyst of the beaver is the presence of a deep keel along the length of its embryonic side.

The Rodentia, as was first made known by the pioneer researches of **Bischoff**, show a great diversity in the ontogeny of the fœtal sac, though the end-result is fairly uniform, and the discoplacenta is always mesometric in position. The divergent behaviour of the early blastocyst within the limits of the order involves different methods of amniogenesis. "Schizamnion" is the term used by **R. Bonnet** for the amnion which arises by dehiscence in a solid embryonic knob. He contrasts it with the more familiar "Faltenamnion," which I will translate into pleuramnion.

Whereas Mus and Cavia possess an inverted blastocyst and a schizamnion, Lepus and Sciurus have a plano-convex blastocyst and a pleuramnion; and it may be said that Castor has an everted blastocyst and pleuramnion, in the sense that the embryonic area and adjacent parts project as a sharp-edged keel which floats in the mesometric cavum. The shape of the blastocyst conforms to that of the cavity of the gestation sac or cavum uteri. Reference to Pl. 14, fig. 1, will make this point clear. Here we see the highly characteristic appearance of the walls and cavity of one of the uterine swellings containing a blastocyst in transverse section. There is a round wide portion occupying the antimesometric division and a narrow deep portion in the mesometric division of the gestation sac: the former is the omphaloid cavum, the latter the mesometric or placental cavum.

Lodged securely in the omphaloid cavum is the balloon-shaped omphalon, and projecting freely into the placental cavum is the keel. The longitudinal diameter of the blastocyst is greater than its transverse diameter. Omphalon is a convenient term introduced by **A. J. Resink** (1904) in place of "Dottersack" or "yolk-sac," as applied to the blastocyst of placental mammals.

The keeled blastocyst is attached to the obplacental wall of the omphaloid cavum by means of trophoblastic implantations which substitute themselves in places for the uterine epithelium. There is no constant symmetry in the distribution of the areas of fixation, but a certain degree of regularity may

be detected. Sometimes a single trophoblast cell will take the place of a uterine epithelial cell as shown in Pl. 14, fig. 2, where the implantation takes the form of a festoon-like arrangement. In other places the substitution is more extensive, as in Pl. 14, fig. 3; and sometimes there is a broad arc of adhesion involving the greater part of the dome of the blastocyst, but sparing the mouths of the glands, as is shown in Pl. 14, fig. 4.

The cells which constitute the obplacental trophoblast, surrounding the dome of the blastocyst, are megalokaryocytes[1] (large cells with large nuclei) of two intergrading kinds. This trophoblast, as we shall see hereafter, is engaged in the ingestion and absorption of three visible maternal products: (1) The albuminous fluid secreted by the uterine glands, which appears as a dense coagulum in the cavum uteri; (2) darkly staining granules, which are the lobed nuclei of leucocytes; (3) red blood-corpuscles.

The cells which ingest the leucocytes with their characteristic lobular nuclei may be named chromatophile megalokaryocytes, or more simply, chromatophile trophoblast. This is usually quite free from the uterine wall, the coagulum intervening; and at these places the uterine epithelium remains intact or but slightly altered. The normal megalokaryocytes which ingest maternal erythrocytes are those which attack the uterine epithelium and take its place upon the surface of the mucosa. In the placental or carinal hemisphere of the blastocyst, the megalokaryocytes pass quite gradually into the cubical ectoderm of the keel.

As stated above, there is some regularity in the disposition of the different kinds of trophoblast, and this necessitates the employment of descriptive topographical terms. The semi-diagrammatic fig. 5 on Pl. 14 shows the completest differentiation of regions of the trophoblast and of the mucosa with which it is associated. At the summit of the dome there is an area of trophoblastic implantation marking out the coronal region. On either side of this we have a tract

[1] J. W. Jenkinson (1902).

of free chromatophile trophoblast occupying the pericoronal region of the blastocyst; between this and the uterine epithelium, here intact, there is a narrow pericoronal cavum into which glands open. Beyond the well-marked pericoronal tract there follows a zone which can sometimes be differentiated into two portions: the part next to the pericoronal tract may be distinguished as the equatorial or periomphaloid tract, and the subequatorial festooned implantation may be known, by scientific license, as the pericarinal tract. Between the latter and the keel there extends a nearly horizontal omphalopleuric membrane of very constant formation, the adcarinal membrane. Lastly follows the keel or carinal region, which includes the embryonic shield and extra-embryonic keel.

The gestation sacs which contained preplacental blastocysts were six in number, and were extracted from one female on March 10th, 1913, three in each horn of the uterus. The swellings varied slightly in size, and the differences proved subsequently to be connected with the state of development. The average diameter of the swellings was 10 mm. The lowest swelling in the left horn measured 11 mm., the middle and upper about 10 mm. The lowest right swelling measured 10 mm., the middle right 10 mm., the upper right (i. e. nearest the Fallopian tube) 8 mm.

On March 4th I had obtained two gestation sacs 25 mm. in diameter, and on March 10th four more of similar dimensions. These dates seem to indicate that the typical stage at this part of the season is that of the young established placenta, but that the reproduction of the beaver does not take place with absolute precision between fixed dates, although the pairing season is known to be relatively constant. To the range of fluctuation that exists I owe the fortunate acquisition of the preplacental blastocysts. No two of these blastocysts are exactly alike, the six individuals representing as many substages which exhibit the characteristics of three main phases in the growth of the tridermic blastocyst: (*a*) with solid mesoblast; (*b*) with exocœlom; (*c*) with incipient

notochord. I will now proceed to the more detailed description of the successive substages of the preplacental blastocyst. Three of them are well-defined, the other three are more transitional.

IV. Substage A.

This stage is represented by a series of sections, cut from before backwards relatively to the embryo, transverse to the longitudinal axis of the uterus, numbered VI B in the Utrecht catalogue. It is a critical stage, inasmuch as it affords the key to the comprehension of subsequent stages. The primitive streak occupies nearly the entire axis of the blastodisc, the mesoblast is solid throughout, and there is no notochordal contact between endoderm and medullary plate. It is the smallest of the swellings, having the following sectional dimensions:

	Millimetres.
Total height of gestation sac or uterine swelling, from root of mesometrium . . .	8·50
Total diameter of gestation sac . . .	5·50
Total height of cavum uteri or cavity of gestation sac	4·50
Depth of mesometric groove or placental cavum	3·00
Diameter of ditto	0·25 to 0·50
„ omphaloid cavum . . .	1·00
Thickness of placental mucosa, between root of mesometrium and bottom of placental groove	3·00
Thickness of placental trophospongia or maternal proliferation	1·25
Thickness of periplacental wall or lateral wall of cavum uteri at each side of the placental groove	2·75
Thickness of obplacental wall of gestation sac	1·50

The thickness of the placental mucosa is given in the above table in order to exhibit its contrast with that of the obplacental wall; it includes the layer of circular muscles which

passes across the placental wall of the gestation sac between the root of the mesometrium (in which the main trunks of the uterine vessels occur) and the placental trophospongia. These transverse fibres of the circular muscles in the placental region become interrupted and oblique to a varying extent in later stages. The diameter of the omphaloid cavum is identical with that of the omphaloid portion of the blastocyst, or, in one word, the omphalon, because the trophoblastic wall of the omphalon is implanted upon the inner surface of the uterine mucosa, which becomes denuded of its epithelium at the points and areas of implantation.

The anterior pole of the blastocyst is free, and the embryonic area begins immediately behind the pole. The area itself is keel-shaped, since the embryonic shield of formative epiblast bends round the free edge of the keel. The formative epiblast which is thus exposed at the surface of the blastocyst is distinguished from the peripheral trophoblast by its nuclear constitution, by the fact that the nuclei occur at different levels, and also by the presence of scattered vesicular cells, i.e. embryonic cells in a vesicular phase. The transition from formative epiblast to peripheral trophoblast is singularly abrupt, there being no gradual transition from one to the other at the level where they meet.

The hypoblast dips into the keel, and, at a very short distance from the anterior end, we find in section the solid U-shaped tip of the mesoblast embracing the floor of the hypoblastic groove, with a few cells migrating out from it along the hypoblast (Pl. 14, fig. 6). The free end of the mesoblast has but slight longitudinal extension, since the primitive streak begins immediately behind it, so that we find in section a perfect U-shaped, solid mesoblast, united to the epiblast by the primitive streak. The position of the primitive streak marks the strictly embryonic side of the keel. The peripheral limits of the formative epiblast may be seen clearly under Zeiss oc. 2, obj. A ; the edge of the shield is placed at a much higher level on the embryonic side of the keel than on the other side (Pl. 14, fig. 7).

From this point backwards the primitive streak is continuous, and presents various incidents of growth at different parts of its length. The wedge-like proliferation at Pl. 14, fig. 8, followed by the chink-like cleft in Pl. 14, fig. 9, lies near the anterior end. From the V-shaped mass which proceeds from the primitive streak and embraces the hypoblastic groove a single line of mesoblast cells extends on each side along the hypoblast, about as far as the equatorial region of the blastocyst at this early stage. Later, the mesoblast has a much more restricted peripheral distribution. The juxtaposed walls of the epiblastic keel distad of the primitive streak become closely welded together.

The lateral wings of the mesoblast are in close contact with the hypoblast, but are separated from the epiblast except at the primitive streak. This is especially obvious on the anti-embryonic side of the keel (Pl. 14, fig. 10). The embryonic shield becomes narrower as it is traced backwards, so that its peripheral limits are much lower than in front; on the anti-embryonic side the edge of the formative epiblast reaches the level of the welded walls of the keel, and finally the apex. A small pit appears intermittently at the surface of the primitive streak, but there is no continuous primitive groove (Pl. 14, fig. 11). The mesoblastic wings become unequal as we near the posterior end of the primitive streak, that on the embryonic side being longer than the part which bends round to the anti-embryonic side (Pl. 14, figs. 12 and 13). In this situation it is easy to recognise that the anti-embryonic wall of the keel consists of cubical trophoblast, the embryonic wall of formative epiblast. The keel is thus partly embryonic and partly extra-embryonic in the same transverse section.

Posteriorly the primitive streak contact widens and the distal border of the keel consists of cubical trophoblast only (Pl. 14, fig. 14). In this region we see very clearly a broad primitive streak with mitoses, and no other formative epiblast. One limb of the mesoblast becomes very slender, the other limb dwindles to zero, whilst the bulk becomes massed about

the primitive streak, partly blocking up the cavity of the trophoblastic keel. Shortly behind this region the primitive streak ceases and the mesoblast rapidly diminishes until only a single line of cells remains on one side of the hypoblast as in Pl. 14, fig. 15.

Finally, the mesoblast terminates and the keel becomes didermic. At the same time the two layers are badly folded in the sections owing to a collapse which apparently resulted from rupture of the adcarinal membrane. This fact, added to the great local differences in the character of the epithelial walls of the blastocyst, seems to point to the existence of a considerable amount of elasticity in the inner and outer membranes. In this case the rupture seems to have been due to an extraordinary agglutination of the adcarinal trophoblast on one side to the edge of the placental groove. This adhesion was retained whilst the rest of the blastocyst followed the contraction of the coagulum contained within the omphalon; except for this unusual attachment, the blastocyst would be free at this level. A little farther back the hypoblast withdraws, leaving a trophoblastic keel with its walls welded together distally. Thus we have a trophoblastic keel near the posterior pole of this blastocyst, but not near the anterior pole where the keel is embryonic. The keel formation gradually dwindles away as the posterior pole is reached.

I will postpone the history of the uterine mucosa until the other substages have been dealt with, but a few words are necessary concerning the fixation of the blastocyst. The anterior pole which lies in front of the primitive streak projects freely into the cavum uteri; the posterior pole is fixed with a broad obplacental implantation. Between the free anterior pole and the middle of the blastocyst there is a region corresponding approximately with the middle of the primitive streak where obplacental arcades become established, i.e. intermittent areas of trophoblastic implantation with destruction of uterine epithelium at the points of contact. About the level of the posterior end of the primitive streak, which is about the middle of the blastocyst, the obplacental contact

is given up except at the two pericoronal corners, where a very narrow implantation is retained as in Pl. 14, fig. 13. Between these two angles there extends the free coronal trophoblast, whose cells contain chromatic granules identical with those which occur in the intervening coronal cavum and in the mucosa. I have determined them to be the nuclei of leucocytes. The leucocytes migrate across the uterine epithelium into the cavum uteri and are ingested by the chromatophile megalokaryocytes.

Towards the posterior pole of the blastocyst the active phagocytic attack is resumed, the megalokaryocytes broadly replacing the uterine epithelium, with formation of arcades, skipping the crypt-like mouths of the obplacental glands. In the coronal region there is no visible sign of hypoblast which appears to stop short about the equatorial zone of the blastocyst. The hypoblast comes to an end posteriorly coincidently with the termination of the trophoblastic keel, so that the extreme posterior pole of the blastocyst is monodermic, its wall consisting entirely of flattened cells with distant nuclei, appearing subfusiform in section.

At no stage is the hyploblast conspicuous in the coronal region, but sometimes straggling cells can be found.

V. Substage B.

This is represented by a series of transverse sections cut from before backwards and numbered VI D in the Utrecht Catalogue. It is characterised by the first indication of a linear exocœlom.

	Millimetres.
Height of gestation sac	10·00
Diameter of gestation sac	7·75
Height of cavum uteri	6·00
Depth of placental groove	4·00
Diameter of placental groove near bottom .	0·30
„ „ „ about the centre	0·90
„ omphaloid cavum . . .	2·00

	Millimetres.
Thickness of placental mucosa . . .	3·00
,, placental trophospongia .	about 1·75
,, periplacental wall . . .	3·50
,, obplacental wall . . .	1·10

These measurements, especially as regards the diameter of the omphaloid cavum, show that this substage is intermediate between that described above and that which follows below. The blastocyst covers thirteen slides, with twenty-four sections, 10 μ thick, on each slide; hence the length of the preserved blastocyst is 3·12 mm., its transverse diameter being that of the omphaloid cavum, viz. two millimetres. In front of the blastocyst the omphaloid cavum contains a homogeneous coagulum which has shrunken away from the epithelium upon which it was moulded; in it are scattered cellular débris and chromatic (leucocytic) granules.

The anterior pole of the blastocyst is free; the trophoblast here consists exclusively of megalokaryocytes, and a few hypoblast cells appear against its mesometric wall, not forming a closed hypoblastic sac. The megalokaryocytes exhibit coarsely alveolar cytoplasm and contain a few chromatic granules. The blastocyst appears in section quite free in the centre of the uterine coagulum; its own internal coagulum is reticulated and paler than the surrounding coagulum. Near to the anterior pole the blastocyst is pyriform in section, the narrow portion only being didermic (Pl. 15, fig. 16). Farther back, the blastocyst still appearing free in section, a long keel arises, somewhat folded in the preparation, composed of cubical trophoblast. The hypoblast is now seen to pass uninterruptedly over the cavity of the keel (Pl. 15, fig. 17). About this same region, where the free anterior end of the blastocyst possesses the long trophoblastic keel, as many as nine glandular crypts were counted, opening into the omphaloid cavum.

The plane of the blastocyst represented in fig. 17 shows well the two kinds of trophoblast composing the outer wall, namely, the omphaloid trophoblast with its megalo-

karyocytes, and the carinal trophoblast with its cubical cells. Shortly behind this plane the omphaloid trophoblast begins to attack the uterine epithelium at intervals. Here and there the direct passage of leucocytes with their lobed chromatic nuclei may be exceptionally observed to take place from the mucosa into the coronal trophoblast. As we proceed backwards we come upon conspicuous obplacental attacks by megalokaryocytes between the mouths of the glands, and a broad pericarinal attack on one side only. The pericarinal zone is doubled by hypoblast which does not cover the obplacental half of the omphalon in this plane. The pericarinal adhesion is characterised by the formation of festoons; at the periomphaloid zone, just beyond the pericarinal festoons, there has taken place an ingestion of erythrocytes by the trophoblast. This also occurs in the coronal region, where there arises a broad adhesion bounded on each side by a free belt of pericoronal chromatophile trophoblast with the megalokaryocytes full of granules. The attacking trophoblast abuts, in a manner which is as clear as it is surprising, upon the columnar epithelium of the glandular orifice, two or three trophoblast cells bridging over the gaps as shown in Pl. 14, fig. 4.

Upon approaching the embryonic region we find, in this series, a confused mass of hypoblast and mesoblast collapsed at the foot of the keel, almost defying interpretation, until we come to the region of the primitive streak. There we see an extension of the process which commenced in the preceding stage, namely, the filling up of the keel by a mass of mesoblast derived from the primitive streak. Into this massive carinal mesoblast the hypoblastic groove extends as shown in Pl. 15, fig. 18. In this figure it may be noted that the formative epiblast which lies above the primitive groove is thicker than that which bends round the edge of the keel. This illustrates the stretching of the formative epiblast, which reduces its thickness and causes it to approximate to the condition of the peripheral trophoblast.

Above the massive mesoblast on the anti-embryonic side of

the keel (i.e. the side opposite to the primitive streak) there is an indication of the linear splitting which precedes the formation of the exocœlom. On the embryonic side the mesoblast band is thicker, its nuclei lie at about three levels, and there is no distinct sign of the future splitting. The massive carinal mesoblast persists through some fifty sections of 10 μ. At last it comes to an end, and leaves the elongated apical region of the keel empty. The two mesoblastic bands then bend continuously into one another round the bottom of the hypoblastic groove (Pl. 15, fig. 19). They are generally two cell-layers in thickness with potential cœlom between the layers. More posteriorly there is a little interrupted splitting, giving rise to discontinuous spaces of incipient exocœlom.

Soon the mesoblast ceases and the hypoblast withdraws from the keel. The latter is now in section a very long and narrow trophoblastic process, composed of cubical epiblast, with its opposite walls touching each other in places, hanging freely into the placental groove. This posterior portion of the keel attains a depth of about 0·75 mm. Thus in this case there is a distinct trophoblastic keel-formation both in front and behind. In other words the two poles of the blastocyst are essentially alike; but there is this difference, that the obplacental adhesion is continued nearly to the extremity of the posterior pole.

VI. Substage C.

This was cut from behind forwards, and numbered VIa in the Utrecht Catalogue. It was the first of the set of six early gestation sacs to be sectioned, and the preservation, staining, and mounting left little to be desired. But the series presented the apparent anomaly that whereas there was an exocœlom as well as a quantity of massive mesoblast, there was no obvious embryonic shield. Comparison with the other substages, the recognition of the primitive streak, and the excellent state of preservation showed that the apparent

absence of the formative epiblast which constitutes the embryonic shield was due to the general distension of the blastocyst and the elongation of the keel, whereby the thickened epiblast was reduced to a cubical epithelium.

	Millimetres.
Height of gestation sac (i. e. total height of section, the mesometrium having been trimmed off)	10·00
Diameter of gestation sac	8·50
Height of cavum uteri	5·50
Depth of placental groove	2·50
Diameter of placental groove . .	0·50 to 0·75
„ omphaloid cavum . . .	2·80
Thickness of placental mucosa . .	about 3·00
„ placental trophospongia . .	1·50
„ periplacental wall . . .	3·90
„ obplacental wall . . .	1·35

The anterior didermic extremity of the blastocyst is free, the posterior didermic pole is fixed by an abundant obplacental phagocytic contact with destruction of uterine epithelium. This occurs extensively also in the middle region of the blastocyst. Mitoses occur in the hypoblast and carinal trophoblast, but not in the obplacental trophoblast, where there are signs of amitosis. Here and there, embedded in the substance of the megalokaryocytes, a spherical mass of protoplasm is segregated from the surrounding cytoplasm and contains about a dozen small nuclei arranged in couples. In other cases the small nuclei appear to be budding from a meganucleus. I do not know the precise significance of these nests of small nuclei in the obplacental trophoblast, but they are not to be confused with the megalokaryocytes which are engaged in the ingestion of erythrocytes. Perhaps it is simply a means of adding, by amitosis, to the number of nuclei in the individual megalokaryocytes.

There is an extraordinary luxuriance of maternal capillaries at the denuded obplacental wall of the gestation sac, opening upon the trophoblast. There is no extravasation of blood

into the cavum uteri, but the capillaries actually abut upon the implanted megalokaryocytes and discharge their erythrocytes directly into the trophoblast. Consequently the megalokaryocytes are found commonly charged with half-dissolved blood-corpuscles. As mentioned there is no hæmorrhage; the extravasated erythrocytes are all contained in megalokaryocytes. In the posterior region of this blastocyst the whole of the upper third of the dome is implanted upon the uterine wall. Nevertheless the epithelial crypts at the mouths of the obplacental glands are not attacked by the trophoblast; phagocytic adhesion only occurs between neighbouring crypts, so that the obplacental union is a system of arcades, arching freely over the crypts. These are not like the pericarinal festoons which are miniature arcades not related to crypts.

Near the posterior pole the sections present a typical picture of a blastocyst with a deep trophoblastic keel, the hypoblast passing directly across the cavity without bending into it, precisely as already shown in Pl. 15, fig. 17, with the difference that in the case now under consideration the pole of the blastocyst does not lie freely in the cavum uteri, but is attached by a general obplacental implantation, skipping the mouths of the glands. Proceeding forwards we soon find the hypoblast dipping deeply into the keel, the distal walls of which are agglutinated together. At this level the blastocyst is didermic, there being no mesoblast in sight (Pl. 15, fig. 20).

As we approach the posterior V-shaped extremity of the mesoblast, we observe that the hypoblastic cells become loosened from their epithelial contiguity in the wall of the groove, but they do not escape into the cavity of the trophoblastic keel. This loosening of the carinal hypoblast occurs variously in all the substages; it would naturally be ascribed to defective preservation, but whether this would be correct or not, it certainly denotes a peculiar physiological modification of the hypoblast in this region (Pl. 15, fig. 21). Passing on, the mesoblast dehisces to form a spacious exocœlom, the

main cavity of which is lodged in the keel, while the narrow bands terminate in a solid proliferation or sinus terminalis on each side of the keel (Pl. 15, fig. 22).

About the plane of the posterior end of the mesoblast in the carinal region, the obplacental trophoblast changes its character in the coronal region. The megalokaryocytes are no longer implanted upon the mucosa, but present a free outer surface to the cavum uteri (Pl. 15, figs. 23 and 24). The large, evenly disposed cells contain numerous chromatic granules which they have ingested; similar granules occur in the uterine coagulum, and others are seen traversing the uterine epithelium. The special function of these modified megalokaryocytes is to ingest leucocytes whose nuclei yield and are composed of the granules in question. They occur over a continuous shield-like area of the coronal region of the blastocyst, and spread out forwards as the two pericoronal bands already mentioned (cf. Pl. 14, fig. 5). In some places the chromatophilous cells assume a flattened form as in Pl. 15, fig. 25.

The posterior exocœlom now requires a little extra consideration. It persists through some thirty sections and then gives way to massive tissue in the apical region of the keel, with cubical hypoblast flanked by narrow exocœlom dipping into it. In previous substages we have seen the commencement of the massive tissue of the keel at the posterior end of the primitive streak. In that part of its course where it is occupied by massive mesoblast I propose to designate the keel-formation, which appears like a stalk in section, by the term exostyle. When necessary the posterior exocœlom may be referred to briefly as the post-stylar cœlom. It is not merely post-embryonic in position, because the exostyle itself is post-embryonic.

As we follow the series forwards the exocœlom becomes greatly reduced, almost obsolete, and the massive keel actually adheres by its apex to one wall of the placental groove; this wall happens to be anti-embryonic, i. e. on the side away from the primitive streak. From the tip of the exostyle a cord of

slime is continued towards the bottom of the placental groove. It is noteworthy that where the hypoblastic groove of the omphalon penetrates into the carinal mesoblast, the epithelium retains a cubical character; whereas between this situation and the adcarinal membrane the hypoblast is loose. Another detail to be mentioned is that the uterine epithelium is not specially modified at the point of agglutination of the tip of the exostyle. It can hardly be doubted that the exostyle possesses a highly viscid surface, and the particular adhesion here described does not represent the position of the ultimate placental union (Pl. 16, fig. 26). In this connection it may be called to mind that in his monograph entitled 'Affen Ostindiens' (Wiesbaden, 1891, see p. 201), **E. Selenka** found to his cost that the chorionic ectoderm (trophoblast) of Semnopithecus possesses a high degree of viscidity.

At the level of fig. 26 the chromatophagous layer of trophoblast still forms an uninterrupted calotte over the dome of the omphalon, and the corresponding portion of the uterine epithelium is intact. In the section from which the figure was drawn, two obplacental glands open into the coronal cavum. The granules which have been ingested by the trophoblast are commonly surrounded by what looks like a food-vacuole; this effect may be in part assignable to the bodies of the leucocytes (Pl. 15, fig. 25). A broad peripheral festooned zone of implantation occupies the widest portion of the omphalon, and uterine fluid can circulate through the trophoblastic arcades.

Twenty-six sections of 10 μ intervene between figs. 26 and 27. The nuclei of the exostyle present the appearance of being arranged in vertical rows or sheets. Mitoses are to be observed in the midst of the tissue as well as at the surface. The exostyle is marked off from the rest of the blastocyst by a geniculate bend due to increased resistance of its massive walls. It is penetrated throughout the greater part of its length by a central lumen continuous with the omphalon; the lining of this groove is not distinct from the rest of the stalk-

tissue except at its proximal or omphalad end. At the distal end there is a distinct trophoblastic space forming the cavity of the foot-like extremity which adjoins the uterine wall below the opening of a gland, the neck of which is undergoing occlusion by epithelial mitotic proliferation. Near the bottom of the placental groove the lumina of the glands cease before reaching the surface of the mucosa, the necks being blocked by a mixed tissue composed of its own degenerating elements and proliferating epithelial cells. The cubical trophoblast stops quite sharply at the top of the stalk in the figure. On the right of the figure a piece of the wall of the stalk is torn away and lies near the corresponding uterine wall without any jagged edge; nor is any jagged surface left upon the stalk where the detached piece belonged—a fact that accords with the sheet-like arrangement of the stalk cells mentioned above (Pl. 16, fig. 27).

The central lumen of the stalk (or exostyle) becomes interrupted in places as we trace it forwards, the tissue appearing solid in its central third, with a narrow chink in its distal third (Text-fig. 1). In this figure we see the swollen peripheral edges of the mesoblast, forecasting the sinus terminalis, and on one side only, namely on the embryonic side, a clear narrow exocœlom not extending into the stalk. The cubical trophoblast of the adcarinal wall of the omphalon stops short at the proximal bend of the stalk. A little farther on the stalk is solid throughout its central half, with proximal cavity continuous with the omphalon and a distal linear lumen terminated by a solid apex (Pl. 16, fig. 28).

The chromatophile trophoblast is now present at the sides of the dome, i. e. in the pericoronal region. In the centre of the dome there is a broad phagocytic contact with the denuded mucosa. The columnar uterine epithelium stands out with great prominence on either side of the denuded coronal area, while the broad equatorial zone of the omphalon shows very beautifully the pericarinal festoons (Pl. 14, fig. 5). It is a constant surprise to see normal uterine epithelium contiguous with a belt of implanted megalokaryocytes.

Between Pl. 16, figs. 28 and 29 there are fifty-four sections of 10 μ. The central extension of the umbilical cavity has again become continuous throughout the greater part of the

TEXT-FIG. 1.

Section through mid-region of keel showing nearly solid exostyle. 1. Sinus terminalis. 2. Exocœlom (on embryonic side). 3. Unsplit mesoblast (on anti-embryonic side). 4. Exostyle. 5. Trophoblastic apical cavity.

length of the exostyle. That part of the primordial sinus terminalis which is on the anti-embryonic side of the keel is now at the level of the geniculate bend. As before, the

cubical trophoblast ceases near the proximal end of the stalk. The nature of the cellular membrane which limits the surface of the exostyle is shown very clearly in fig. 29 near the distal end, where it is partly separated from the massive tissue. The solid mesoblast on the embryonic side of the central cavity of the exostyle is thicker than that on the other side. Below each section of the sinus terminalis a narrow exocœlom, very short on the anti-embryonic side, is visible.

On the anti-mesometric side of the cavum uteri the uterine epithelium at this level is complete except for a narrow area in the centre of the coronal region; the variously depressed chromatophile megalokaryocytes are stretched over the dome of the omphalon as far as the pericarinal zone where phagocytic contact is maintained.

The small cœlomic cleft noted on the anti-embryonic side below the sinus terminalis in fig. 29 is a local dehiscence in the mesoblast at that point. The mesoblast on this side becomes thinner, about two layers deep, as compared with six sheets of nuclei on the embryonic side. Great pieces become torn out of the embryonic side of the stalk, apparently owing to their adhesion to that side of the placental groove before preservation; the stalk as a whole is often much lacerated. The uterine muscles had presumably relaxed and so widened the groove after death. The blastocyst becomes quite free from the mucosa except at the two pericarinal angles. After about two dozen sections forwards from fig. 29, the exocœlom on the embryonic side begins to extend well into the stalk, none being present on the anti-embryonic side.

With the extension of the exocœlom into the exostyle on the embryonic side of the central hypoblastic cavity, we begin to recognise the position of the primitive streak at the distal end of the linear exocœlom; and it continues through some twenty sections. Close to the plane of the posterior end of the primitive streak a groove appears at the proximal face of the keel on the embryonic side. This groove persists through about ninety sections, and marks the peripheral

limit of the embryonic shield. At the same level a cœlomic space appears in the apical region of the keel beyond the distal end of the hypoblast. This is the pre-stylar exocœlom (Pl. 16, fig. 30).

We have now followed the massive exostyle throughout its entire extent between the post-stylar and the pre-stylar exocœlomic spaces. We have seen that it is never quite solid, there being always a trace of a linear hypoblastic axis. The position of the primitive streak marks the posterior boundary of the embryonic shield, which at first appeared strangely inconspicuous in this otherwise excellent series. The entire blastocyst is so well extended by the pressure of its internal fluid that the formative epiblast has become stretched, and so has suffered a diminution of thickness. This explanation is supported by the disposition of the nuclei, which are much more crowded on the embryonic than on the anti-embryonic side of the keel. The embryonic ectoderm consists of narrow cubical cells, the nuclei thus occurring in close juxtaposition; on the anti-embryonic side the trophoblastic ectoderm consists of flattened cells with distant nuclei (Pl. 16, fig. 30).

The anti-embryonic section of the primordial sinus terminalis passes definitely into the keel, becoming elongated in section (Pl. 16, fig. 31). Farther forwards it rounds the distal border of the carinal hypoblast, and finally meets its companion, which has meanwhile descended into the keel. In proportion as the two sections of the sinus terminalis approach their confluence, the cœlom dwindles to extinction (Pl. 16, fig. 32). After this the mesoblast ceases.

In front of the primitive streak it becomes difficult to determine with precision the distal limit of the embryonic shield, and its anterior border cannot be defined. In front of the mesoblast there are signs of ectodermal proliferation, especially in the vicinity of the proximal groove, which becomes deeper anteriorly. This is probably a proliferation of the amniogenic trophoblast. The growth of the obplacental trophoblast is accompanied by amitotic nuclear division, that

of the carinal trophoblast is effected by nuclear mitosis (Pl. 16, fig. 33, and Pl. 17, fig. 34).

In this anterior region the keel is didermic. Below the hypoblast there is a long narrow monodermic extension of the keel. The abrupt transition from cubical carinal hypoblast to flattened adcarinal hypoblast, shown on the anti-embryonic side in figs. 32 and 34, is a characteristic feature. It should also be mentioned, though not shown in the figures, that the flattened hypoblast of the adcarinal omphalopleure is separated from the adjacent trophoblast by a well-defined basement membrane, which stains strongly with orange G. In front of the region represented by these figures the proximal groove of the keel soon flattens out; the blastocyst appears free in the cavum uteri, and the anterior pole is reached.

VII. Substage D.

This substage is rather poorly represented by a series of transverse sections cut from behind forwards and numbered VI E in the Utrecht catalogue. It may be considered to be characterised by the initiation of the lateral amniotic folds.

	Millimetres.
Height of gestation sac	11·00
Diameter „ „	8·00
Height of cavum uteri	5·75
Depth of placental groove	2·50
Diameter of placental groove in middle .	0·25
„ „ „ near omphaloid end	0·50
Diameter of omphaloid cavum . . .	3·00
Thickness of placental mucosa . . .	4·00
„ „ trophospongia . .	2·00
„ periplacental wall . . .	4·00
„ obplacental wall . . .	1·00

Both poles of the blastocyst are fixed to the wall of the omphaloid cavum. Towards the posterior pole the adcarinal

omphalopleure was greatly folded, while the folding of the carinal trophoblast indicates the existence of a long keel like that shown in Pl. 15, fig. 17. As in the preceding substage, we first meet the posterior U-shaped solid end of the mesoblast, immediately in front of it the post-stylar cœlom, and then the massive exostylar tissue (Pl. 17, fig. 35).

This series is very useful for the migration of leucocytes from the mucosa, through the uterine epithelium and across the cavum uteri into the chromatophilous megalokaryocytes, as well as for the ingestion of erythrocytes by the phagocytic megalokaryocytes. The mechanism of ingestion of the leucocytes seems to consist of an active immigration, the megalokaryocytes, so long as they retain a free, unattached outer surface, remaining apparently passive. On the other hand the phagocytic activity of the implanted megalokaryocytes is often very obvious. Such cells, when engaged in the ingestion of red blood-corpuscles, commonly possess several large nuclei. The differentiation of the obplacental trophoblast into free leucocytophagous and attached erythrocytophagous cells is a very singular and constant feature of the beaver's preplacental blastocyst.

In front of the region represented in Pl. 17, fig. 35, the carinal epiblast is ruptured, and this has led, over a certain extent of the series, to almost unaccountable confusion of the layers. It can be made out that the mesoblast becomes massed around the hypoblastic groove which dips into the keel as usual and is lined by cubical epithelium. The exocœlom becomes reduced to a linear cleft above the solid exostylar mass, and its walls may be in contact so that no open cœlom appears in the section. The carinal epiblast becomes greatly flattened, and there is an abrupt transition to the peripheral or adcarinal cubical epiblast (Pl. 17, fig. 36).

The rupture of the carinal epiblast noted in preceding sections culminates forwards in a notch at the apex of the keel. If such a section is examined apart from the rest, the bilobed apex of the keel might appear to have a special

bearing, whereas it is really an artefact. After a score of sections from the last one figured, the primitive streak can be recognised on one side of the keel not far from the free distal edge. Its position is approximately in the centre of the thickened formative epiblast which bends round the edge of the keel (Pl. 17, fig. 37). On the left side of the figure the formative epiblast is seen blending with the cubical adcarinal trophoblast; on the right it passes abruptly into what is left of the flattened epiblast of the keel.

The mesoblast continues to be massive on either side of the primitive streak. At the proximal end of the keel on the left side of the figure there is a small space limited externally by cubical mesoderm, internally by columnar mesoderm. I interpret this as part of the pericardial primordium, as will be explained more clearly below. As we pass forwards from this level the massive mesoblast gives way to open cœlom. In the anterior region of the embryonic shield the sections present the general appearance illustrated in Pl. 17, fig. 38. The keel is now embryonic; at its edges are seen incipient amniotic folds. The hypoblastic groove is cut tangentially near its anterior termination, and its carinal part appears separated from the omphaloidean hypoblast. Another part of the pericardial primordium is here seen in the mesoblast distad of the hypoblastic groove.

In front of the embryonic shield the anterior terminal portion of the exocœlom extends characteristically into the proximal half of the keel, leaving the distal half free (Pl. 17, fig. 39). Thus there is a very definite keel-formation near the anterior pole of the blastocyst, well in front of the embryonic shield.

The anterior and posterior trophoblastic portions of the keel with their exocœlomic spaces correspond in position with the ovate areas on the mature fœtal sac which I described (1912, p. 203) as fenestræ pyriformes or umbilico-placental areas. Between the pre-stylar and post-stylar keels occurs the exostyle; between the anterior and posterior pyriform areas occurs the placenta.

VIII. Substage E.

The series is cut transversely from before backwards, and is numbered VI F in the Utrecht catalogue. There is notochordal contact between hypoblast and formative epiblast in front of the primitive streak.

	Millimetres.
Height of gestation sac . . .	about 10·00
Diameter „ „	7·60
Height of cavum uteri	5·00
Depth of placental groove	2·75
Diameter „ „ . . .	0·50 to 0·80
„ omphaloid cavum . . .	3·00
Thickness of placental mucosa . .	about 3·00
„ „ trophospongia . .	1·50
„ periplacental wall . . .	3·40
„ obplacental wall . . .	1·00

The anterior pole of the blastocyst exhibits phagocytic adhesion to the wall of the omphaloid cavum; the posterior pole is free. Beginning from the front end, we find a long keel composed of cubical epiblast with a hypoblastic groove dipping into the proximal part of its cavity. Distally the walls of the keel are agglutinated together so that the cavity is occluded. After about twenty sections of the formation just described, the mesoblast appears with a cœlomic cavity embracing the sides and bottom of the hypoblastic groove, with a long epiblastic keel beyond it. The hypoblastic groove is lined by cubical epithelium with large round nuclei. There is general phagocytic adhesion of the entire obplacental hemisphere in the anterior region.

At length the anterior end of the folded embryonic shield appears on the right side of the keel when the latter points towards the observer under the microscope (Pl. 17, fig. 40). Somewhat before this level is reached the coronal trophoblast begins to show patches of chromatophile cells alternating with phagocytic megalokaryocytes. This leads on to the condition of a continuous coronal chromatophilous calotte

such as has been described in previous substages, the leucocyte granules being extremely abundant. The mesoblast in the region of the embryonic shield is nearly solid, with a linear cleft representing the pericardial primordium.

Proceeding backwards with the sections, the solid mesoblastic bands become separated at a certain spot, and the embryonic hypoblast there comes into apposition with the formative epiblast, its cells at the same time acquiring a typical columnar form. It is the notochordal primordium (Pl. 17, fig. 41). This figure shows the solid peripheral swellings (sinus terminalis) of the mesoblastic bands placed at slightly different levels and a little exocœlom occurring between the swellings and the mass of the bands. The notochordal primordium extends through about a dozen sections of 10 μ, and then it passes into the primitive streak (Pl. 17, figs. 42 and 43). At this level of the blastocyst there is a very extensive megalocytic attack in the coronal region, flanked on either side by a pericoronal chromatophile band; beyond this there is a periomphaloid attack followed by pericarinal festoons — all in the typical manner previously described (cf. Pl. 14, fig. 5).

At the posterior end of the primitive streak the massive exostylar tissue commences and the mesoblastic bands enter into close contiguity with the carinal epiblast over a wide area (Pl. 17, fig. 44). The significance of the exostylar portion of the keel with its massive mesoblast and superjacent trophoblast (carinal epiblast) will be discussed below under "Special Considerations." In the mid-region of the exostyle in this series the keel is damaged in nearly every section in consequence of its having been torn from its viscid adhesion to the uterine wall. The figures are further complicated by the peculiar folding of the keel, which continues for some distance behind the embryonic shield (Pl. 17, fig. 45). Towards the posterior end of the exostyle the fold straightens out and we have the appearance shown in Pl. 17, fig. 46, where the massive tissue is bordered by a simple keel with juxtaposed walls. After this the post-stylar exocœlom opens

out in a typical manner (Pl. 18, fig. 47). Finally, the two sections of the primordial sinus terminalis meet together (Pl. 18, fig. 48), and thereafter the mesoblast ceases. Between the last two figures there are thirty-nine sections of 10 μ.

Behind the posterior limit of the mesoblast we find for some distance a didermic keel with trophoblastic extension (Pl. 18, fig. 49). The balloon-shaped blastocyst is here free from all adhesion to the uterine wall. Farther back, the hypoblast withdraws from the keel (Pl. 18, fig. 50), and eventually the section is exactly similar to that shown in Pl. 15, fig. 17, which relates to the anterior pole of the blastocyst in Substage B. The two poles of the blastocyst, though differing in the extent of their adhesion to the uterine wall, are naturally similar in other respects.

Apart from the broad difference between a free pole of the blastocyst and a fixed pole, there is another more subtle distinction which has been referred to incidentally and may be exemplified once more in this series, namely, the difference between a free and an implanted coronal disc. It may be noted in passing that the term "coronal" is not synonymous with "obplacental." The whole of the wall of the omphaloid cavum to which the omphaloid hemisphere of the blastocyst may be attached is obplacental. At the level of Pl. 17, fig. 44, the coronal region of the blastocyst in transverse section exactly equals the diameter of the field of the microscope when viewed under Zeiss oc. 4, obj. A; throughout this extent the denuded mucosa is lined by a continuous pseudo-epithelial sheet of megalokaryocytes closely attached by their amœboid peripheral ends to the decidual surface, without any arcades. At one edge of this implanted area, a gland opens into the pericoronal cavum at a true epithelial surface; at the other edge the pseudo-epithelium adjoins the true cylindrical epithelium of the pericoronal cavum of that side. The implanted trophoblast does not sink into the mucosa; in general the line of implantation is straight, corresponding to the original basement membrane of the

uterine epithelium. The subepithelial capillaries, at frequent intervals, where they come into contact with the trophoblast, actually open and discharge their red corpuscles directly into individual megalokaryocytes. The obplacental glands almost always open at an epithelial surface subtended by a trophoblastic arcade, but in a few rare instances a gland is seen to open directly upon the trophoblast, into which it discharges a mass of intrusive chromatic granules. At the level of fig. 47, instead of the implanted pseudo-epithelial coronal trophoblast, we find the coronal region of the blastocyst entirely free, composed of chromatophilous megalokaryocytes, as represented diagrammatically in Pl. 15, fig. 24.

IX. Substage F.

This excellent series shows very clearly the embryonic shield with primordia of medullary groove, notochord, pericardium and amniotic fold. It is cut from before backwards and is numbered VI c in the Utrecht catalogue. Both poles of the blastocyst are fixed to the wall of the omphaloid cavum.

	Millimetres.
Height of gestation sac	10·00
Diameter of gestation sac	8·00
Height of cavum uteri	5·00
Depth of placental groove	2·00
Diameter of placental groove . .	0·75 to 2·00
,, omphaloid cavum . . .	3·00
Thickness of placental mucosa . . .	4·00
,, ,, trophospongia . .	2·00
,, periplacental wall .	3·50 on one side, 4·00 on the other.
,, obplacental wall . .	1·00 to 1·35

These measurements, taken in conjunction with Pl. 18, fig. 51, exhibit a placental groove which is considerably wider and shallower than anything that has gone before. This progressive expansion of the cavum uteri accords with

the fact that the blastodisc is farther advanced in development, though the exostyle is still far from its destination on the placental trophospongia. The estimated length of the blastocyst is nearly 4 mm.

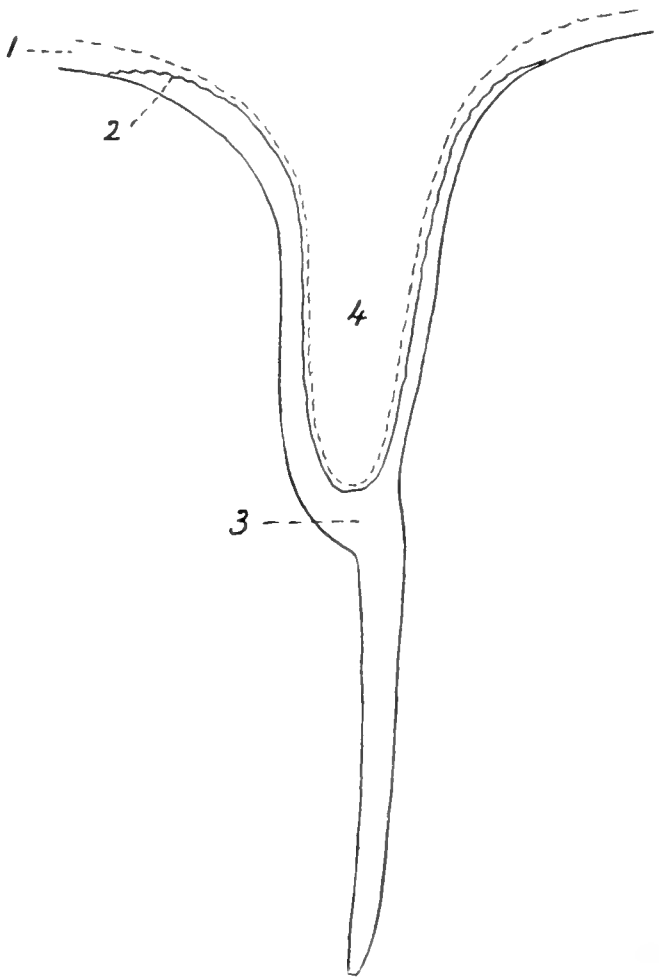
At the anterior pole the blastocyst is monodermic, and is attached by the omphaloid hemisphere in broad arcades. Then the hypoblast appears a short distance removed from the pole; at first it stretches straight across the adcarinal plane and a little further back dips down into the proximal half of the keel, the distal half of which remains monodermic. In previous substages it has been observed that in places, the epithelium which lines the hypoblastic groove, i. e. the carinal hypoblast, tends to break up into rounded cells that lie loosely in the cavity of the groove, but never escape into the distal cavity of the keel. The extent and position of the hypoblastic groove can always be made out, however disconnected its cells may be. This fact is due to the peculiar behaviour of the basement membrane which exists between the adcarinal epiblast and hypoblast.

At a certain point near the mouth of the keel the adcarinal basement membrane can be seen to cross the narrow interval that separates the epiblast from the hypoblast, and to attach itself to the latter, which it accompanies into the keel. The trophoblastic cavity of the keel is thus shut off from the omphalon by a delicate wrinkled membrane. The keel-cavity contains a finely granular coagulum, which differs slightly in its staining properties from the adjacent coagulum in the adcarinal region, so that there is a sharp contrast in the character of the coagulum on either side of this structureless membrane (Text-fig. 2). The membrane is inconspicuous and easily overlooked.

The anterior border of the mesoblast appears on one side of the keel, viz. the embryonic side, as shown by subsequent sections. In this region there is a remarkably deep epiblastic keel extending far beyond hypoblast and mesoblast (Pl. 18, fig. 52). The mesoblast rapidly increases in volume as we trace it backwards, and presently the anterior cœlom opens

out with a deep extension into the keel. Produced beyond this carinal cœlom there is a rather long and somewhat folded epiblastic keel with its walls agglutinated so as to occlude the potential trophoblastic cavity.

TEXT-FIG. 2.

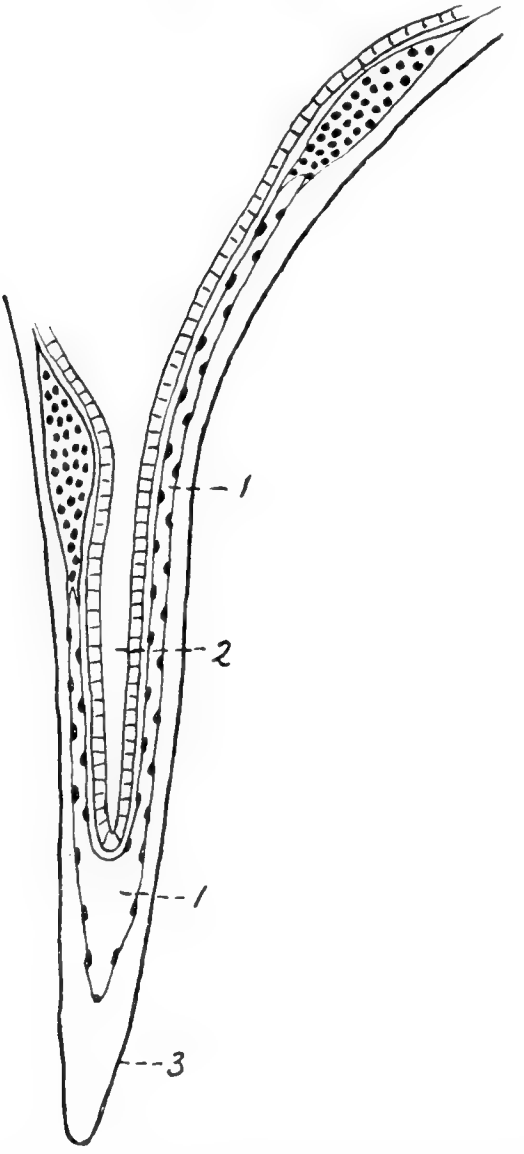


Anterior region of keel, showing relation of basement membrane to carinal hypoblast. 1. Interval between hypoblast and epiblast containing adcarinal coagulum. 2. Basement membrane crossing the interval to join the carinal hypoblast. 3. Trophoblastic cavity of keel. 4. Hypoblastic groove.

The epiblastic keel soon begins to shorten, the carinal cœlom becomes reduced, and the cells of the hypoblastic groove appear as a continuous epithelium on the embryonic side. In the diagram (Text-fig. 3) I have reconstructed the

hypoblast on the anti-embryonic side of the keel, where it is evanescent in the actual section. Between the condition

TEXT-FIG. 3.



Appearance of the keel behind the anterior polar region. The index numbers are placed on the embryonic side of the keel. 1. Exocœlom. 2. Hypoblastic groove. 3. Shortened epiblastic keel.

shown in Pl. 18, fig. 52, and that in Text-fig. 3 there are thirty-nine sections of 10 μ. At the region of the blastocyst which we have now reached the coronal disc is composed

of large chromatophile cells freely subtending the coronal cavum, which is filled with coagulum containing many chromatic granules. Two or three sections after Text-fig. 3, the front part of the embryonic shield begins to be cut tangentially. Its abrupt appearance in section indicates a much sharper definition than in previous substages.

Under the Zeiss microscope, when the keel appears to point towards the observer, the embryonic shield is placed on the right side of the keel. The plate-drawings were reversed under the Edinger apparatus. For descriptive purposes we may regard the edge of the keel as pointing downwards towards the observer, although it really points upwards towards the mesometrium. Above the embryonic shield in the figure (Pl. 18, fig. 53) there is a somatopleuric fold which looks like a lateral amniotic fold and is so interpreted. Below the shield in the figure there is the epiblastic keel into the neck of which the exocœlom of that side is produced, so that the material for the other amniotic fold is in continuity with the keel. Underneath the thick formative epiblast of the shield we find a thick-walled mesodermic saccule, interpreted as the pericardial primordium of the embryonic cœlom. The only cœlom at present existing in the embryo is the pericardial cœlom. Against this the embryonic hypoblast consists of a continuous cubical epithelium, while on the opposite side the hypoblast is evanescent and granular. Below the distal border of the hypoblast a thin sheet of mesoblast intervenes between it and the epiblast (Pl. 18, fig. 53).

Proceeding backwards, the pericardial saccule shortly separates into two moieties. The portion which lies subcentrally under the thickened epiblast shows a characteristic thicker inner wall of columnar cells and a thinner outer wall of cubical cells. Between the two parts of the pericardial cœlom the mesoblast thins out, and so the cubical hypoblast comes nearer to the formative epiblast at this point (Pl. 18, fig. 54). The next figure shows an accentuation of the preceding features, and is introduced to exhibit the

two diverging limbs of the pericardial cœlom which now appear as antimeres (Pl. 18, fig. 55).

As we approach the posterior quarter of the blastodisc, the embryonic shield becomes larger, and a medullary groove with subjacent notochordal plate appears in the position shown in Pl. 19, fig. 56. After another half-score sections we have the structure represented in Pl. 19, fig. 57. The medullary groove and the distal pericardial process have ceased, and the lower part of the keel begins to be occupied by massive mesoblast with a hypoblastic diverticulum extending into it. At the foot of the keel there is an open hollow space containing coagulum; it is possible that it is an incident of growth rather than that it has any special significance, but this may remain an open question. In this figure and the preceding, the sinus terminalis is seen low down on the anti-embryonic side of the keel; on the embryonic side it occurs near the junction of the adcarinal and carinal regions. This unequal behaviour of the parts of the sinus terminalis has been observed in all substages.

At the posterior end of the blastodisc, the primitive streak in this substage is reduced both in extent and definition, and it is evident that it would soon be given up. Its position and the wings of massive mesoblast proceeding from it are shown in Pl. 19, fig. 58. The hypoblastic groove lined by cubical epithelium intruding into the distal mesoblast is conspicuous, and the transition from cubical to flattened epithelium is remarkably abrupt.

We are now rapidly nearing the region of the massive exostyle which always follows behind the primitive streak. In this substage the exostyle exhibits in section a broad distal base with a small trophoblastic cavity and hypoblast penetrating into the massive mesoblast. The epiblast on one side of the keel is peculiarly folded. The distal lumen of the hypoblastic groove is occluded, the occlusion taking place by opposition of the walls accompanied by intense proliferation of the massive mesoblast, whereby the axial hypoblast merges imperceptibly with the surrounding mesoblast, without the

intervention of a basement membrane (Pl. 19, fig. 59). The large clear nuclei of the intrusive tongue of hypoblast contrast with the more darkly stained mesoblastic nuclei in their normal aspect, but they appear to blend with the latter at the sides and apex. On the anti-embryonic side of the keel there is unsplit mesoblast; on the embryonic side an exocœlom whose epithelium is partly cubical. The anti-embryonic part of the sinus terminalis lies low down, midway between the foot of the keel and the position of the sinus on the embryonic side. The massive tissue of the exostyle is concentrated within somewhat narrow bounds, covering about a dozen sections of 10 μ. This concentration and delimitation of the exostyle are what must necessarily precede the initial discoplacental adhesion.

A few sections behind Pl. 19, fig. 59, the axial cavity reappears in the hypoblast and this marks the transition from the exostyle to the post-stylar region. A deep and narrow hypoblastic groove occupies the centre of the mesoblast; above the massive tissue the exocœlom opens out on both sides to form a wide cavity. Below the mesoblast there is a trophoblastic extension of the keel (Pl. 19, fig. 60).

At the region of the blastocyst corresponding approximately with the course of the exostyle, the obplacental wall exhibits the condition of a continuous megalocytic attack accompanied by ingestion of red corpuscles in the coronal area, and a wide pericoronal arcade subtending a pericoronal cavum on each side. As already explained, the term arcade in this connection expresses typically a single bridge of chromatophilous trophoblast with free outer surface washed by the uterine fluid. The particular function of the obplacental arcades, in addition to the ingestion of leucocytes by the chromatophile cells, is to afford spaces for the openings of the persistent obplacental glands.

Posteriorly the two halves of the exocœlom unite to form a common cavity, the post-stylar cœlom, which extends some way into the distal cavity of the keel (Pl. 19, fig. 61). After this the trophoblastic keel lengthens enormously (Pl. 19, fig. 62).

About this region of the blastocyst the sections show demonstratively the ingestion of erythrocytes by the coronal megalokaryocytes and the ingestion of leucocytes with their lobed chromatic nuclei by the pericoronal chromatocytes. In addition to the coronal implantation and the pericoronal cava, there is a typical periomphaloid adhesion and pericarinal festoons.

The two sections of the sinus terminalis descend deeper into the keel, and finally come near to each other at the bottom of the carinal hypoblast as shown in Pl. 19, fig. 63, which is thirty-nine sections behind the preceding figure. At the same time the post-stylar cœlom is reduced, and the trophoblastic extension of the keel is devoid of a continuous axial lumen. In Pl. 19, fig. 64, the two parts of the sinus nearly touch; and fig. 65 shows the sigmoid confluence of the sinus, marking the posterior limit of cœlom and mesoblast.

Next follows the posterior didermic region of the blastocyst. The trophoblastic keel shortens, but the hypoblast retains its carinal extension until near the posterior pole. These features, as well as the relation of the keel to the adcarinal omphalopleure, are portrayed in Pl. 20, fig. 66. Quite at the posterior pole we find in section a round didermic blastocyst free on its mesometric side, adhering intermittently elsewhere.

X. Maternal Trophospongia.

Without undertaking a detailed discussion of the histology of the mucosa, attention may be invited to certain interesting changes which take place in the uterine mucosa of the gestation sac, preliminary to placentation. The perusal of the foregoing pages will doubtless have left a vivid impression of the vital importance to the beaver's blastocyst of its obplacental implantation. One might have expected, from the analogy of the rabbit, that this would involve the degeneration of the obplacental uterine glands. On the contrary, the obplacental glands retain their full func-

tion throughout the period dealt with, and the trophoblast gives free way to their crypt-like openings. It is only in the walls of the mesometric or placental groove, which have not yet entered into very close association with the blastocyst, that the glands have already become atrophic.

The mucosa may be regarded as comprising two classes of tissue: (1) Uterine epithelium and glands; (2) dermatic connective tissue and capillaries. The fixation of the blastocyst resolves itself into two periods: (1) Preplacental period; (2) euplacental period. The size of the gestation sac is determined by two factors: (1) Dermatic proliferation; (2) pressure of blastocyst.

In one of the uterine cornua belonging to a later phase of gestation which will be described below, there was a slightly swollen segment of the uterus, which looked as if it might contain a very young blastocyst. After it had been sectioned, it was found to be in a perfectly healthy condition, but non-gravid. This series is numbered VII x in the Utrecht catalogue. The cavum uteri is a rather narrow lumen bifurcated towards the mesometrium and lined by a high columnar epithelium; opening into it on all sides there is a profusion of glands (Pl. 20, fig. 67). It contains a coagulum with some dark-stained granules diffused throughout it. The lumen is invested outside the epithelium by a narrow uniform zone of intense dermatic proliferation riddled with capillaries; in this zone (not indicated in the figure) the connective-tissue cells are more numerous and closer together than in the deeper parts of the mucosa. On the mesometric side the lumen branches into two grooves, separated by a ridge or dermatic cone covered by epithelium. Numerous large vessels occur between the circular and longitudinal muscles of the mesometric region; the circular muscle-ring is broad and entire, except where penetrated by the vessels.

In contrast with the initial condition of uniform dermatic proliferation and intact glands described above in a non-gravid segment, we find in a gravid gestation sac belonging to the preplacental period that all the mesometric and peri-

placental glands are undergoing necrosis, being met and vanquished by an epithelial proliferation operating centrifugally. The dermatic proliferation acts centripetally. Meanwhile the dermatic proliferation in the future placental region has outstripped the remainder, and now constitutes what has been referred to as the placental trophospongia, the term "trophospongia" having been introduced by **Hubrecht** in 1889 to denote a vascular maternal proliferation. In the beaver such a proliferation takes place all round the cavum uteri, and is at first of uniform thickness.

The epithelial proliferation is a phenomenon of substitution accompanying the necrosis of the glands. It is paralleled by a corresponding phenomenon described by **Hubrecht** (1893) in the case of the shrew (Sorex), where the epithelial proliferation leads to the formation of secondary crypts. In the beaver's gestation sac during the preplacental period, the placental mucosa, towards the bottom of the placental groove, exhibits, sometimes very clearly, radiating bands of necrotic glands, with dark-stained shrunken nuclei (Pl. 20, fig. 68). These glands are to be replaced by an extensive epithelial proliferation, which grows centrifugally into the substance of the placental trophospongia in the form of two lobulate wings, which correspond in their position with the grooves on either side of the dermatic cone in the non-gravid uterus (Pl. 20, fig. 67). Thus the wing-like epithelial proliferations are preceded by necrotic belts. The cells multiply by mitosis. In the niches between the lobes are found subepithelial capillaries (Pl. 20, fig. 69).

In the obplacental region, where the subepithelial capillaries are also excessively abundant, the capillaries are conveyed by the centripetal dermatic proliferation. This happens in the placental region as well, but in addition the centrifugal epithelial proliferation descends to meet the capillaries and embrace them, thereby preparing a nidus for the true placental implantation. At the beginning, the proliferating cells retain their cell-boundaries (Pl. 20, fig. 70). Eventually the proliferation will become syncytial. At the level

of the anterior part of the embryonic shield in substage F, the capillaries which adjoin the inner borders of the wing-like mesometric proliferations are greatly dilated, but there is no trace of any endothelial proliferation (Pl. 20, fig. 71).

At the distal borders of the two principal mesometric proliferations there are to be seen traces of the glands which they have supplanted. There are no signs of glands between the wings; the latter cause the more laterally placed mesometric glands to diverge widely, arching round the mesometric area, appearing healthy in their deep-lying portions, but failing to reach the surface, their neck-portions having been killed. Sometimes a transverse section of a glandular tube is seen, the centripetal half of which is necrotic, the distal half normal. In this way, centripetal glandular degeneration and centrifugal epithelial proliferation take place simultaneously. In the obplacental region the glands persist and the uterine epithelium is largely replaced by trophoblast; in the placental region the glands degenerate and are replaced by epithelial proliferations which are to some extent moulded upon the pre-existing glands. At the sides of the placental groove the necks of the glands sometimes widen out into large ampulliform dilatations with ill-defined walls and wrinkled nuclei; such ampullæ fail to open into the cavum uteri. They may nearly open, but their mouths are blocked, partly by their own degenerate cells and partly by epithelial cells (Pl. 20, fig. 72).

There is not much to add to what has been said already regarding the uterine epithelium. Where the trophoblast is free and the epithelium consequently intact, the character of the latter varies at different points without any regard to symmetry. In one instance my notes record an epithelium so flattened as to become a pavement epithelium, covering the left side only of the omphaloid cavum. The cytoplasm of the uterine epithelium stains dark yellow with orange G, and thus offers a marked contrast to the adjoining trophoblast, whose cytoplasm remains pale. **J. W. Jenkinson** (1902, p. 132) has noted the same distinction in the case of the mouse:

"The cytoplasm [of the trophoblast cells] does not stain intensely with acid stains, and may in this way be readily distinguished from the cytoplasm of the maternal cells." Again, on p. 139, referring to epithelial cells which have been ingested by the trophoblast, he says: "As for the cytoplasm, it stains brilliantly with plasma stains, and offers in this respect a marked contrast to the cytoplasm of the trophoblast by which it has been ingested." This last is true of the erythrocytes ingested by the megalokaryocytes in the beaver.

There are signs that in the beaver the uterine epithelium may not only disappear by direct substitution of the trophoblast in sitû, but that, in a manner not unlike what **P. Nolf** (1896) described in the bat, it may first be reduced to a cubical and then to a pavement epithelium; or finally it may, in places, be shredded off into the cavum uteri.

The migration of leucocytes from the mucosa across the uterine epithelium into the omphaloid cavum and thence into the chromatophilous megalokaryocytes, or, more briefly, chromatocytes, is a phenomenon that requires further comment. Granules staining darkly with hæmalaun, occurring commonly in triads and tetrads, are found deep down in the stroma of the obplacental mucosa. From the deeper zone they scatter through the intermediate stratum and are again met with in great numbers in a subepithelial position, from whence they pass into the epithelium. At first I supposed that they might be derived from the nuclei of the decidual cells, but was unable to find transitional forms. Not only do they traverse the uterine epithelium, but they also pass in smaller quantities into the lumina of the glands, and are then discharged with the glandular secretion into the cavum uteri. In much less numbers are they observed in the capillaries.

In places the obplacental mucosa showed an abundant infiltration of undoubted leucocytes whose lobed nuclei were these same chromatic granules of whose nature I had been uncertain. When they reach the bases of the epithelial cells and penetrate through the latter, their cytoplasm does not

stain so well as it does in the middle of the mucosa, but remains clear so that the granules often appear to be contained in vacuoles. When they arrive in the uterine fluid, which is represented in the sections by a coagulum staining deeply with orange G, the cell-body is often invisible though sometimes quite obvious. The marked tendency which they exhibit to discharge themselves in volleys through the uterine epithelium corresponds with their deep-seated gregarious habit in the mucosa. This infiltration of leucocytes is comparable with that observed by **Nolf** in the epithelioid tissue and hypertrophied venous endothelium of the bat's trophospongia, although their fate is different.

XI. Discoplacental Adhesion.

The stage of the incipient placentation is unfortunately lacking in the material collected by me. In the keel of the preplacental blastocyst four parts with independent destinies may be distinguished: the anterior keel with anterior exocœlom, the embryonic keel, the exostyle, and the posterior keel with post-stylar exocœlom. The first and last will be employed in the formation of the umbilico-placental membranes or fenestræ pyriformes, consisting of diplotrophoblast, i.e. trophoblast doubled by the somatic mesoblast of the exocœlom. The embryo will become folded off, and will sink with its amnion into the exocœlom. The exostyle will furnish the material for the placental labyrinth.

But the chapter recording these events in their actuality cannot yet be written. The groove at the foot of the keel in Pl. 19, fig. 59, is ready to receive the dermatic cone of the maternal trophospongia with its syncytial caps (cf. Pl. 20, figs. 69–71), but we are not at present privileged to witness the act.

XII. The Established Placenta.

The material upon which the following description is based consisted of six spherical gestation sacs about 25 mm.

in diameter, obtained on two separate occasions—March 4th and March 10th, 1913. It was on the latter date that I also obtained the six youngest gestation sacs. In a female beaver, taken on March 4th, there were two spherical swellings, each 25 mm. in diameter, in the left uterus only, and two contiguous corpora lutea in the left ovary. Above the upper swelling there was a smaller one of 12·5 mm., upon which great hopes were set; but it proved barren. The axillary teats of the mother were distinct, but the post-axillary pair was only found after the skin had been removed. The trapper said it was a young female that would have been three years old in the coming spring. In another female, taken on March 10th, there was a single spherical sac in the left uterus, and three in the right. From these data it may be gathered that whilst the beaver produces a litter only once a year in the spring, there is a considerable range of variation with regard to the stages which may be observed upon a given date during the season of reproduction.

I opened one of these sacs in Prof. Hubrecht's presence by slicing away the antimesometric wall with a cartilage knife. We were surprised to find that no liquid exuded when the cavity of the omphalon was exposed; on the contrary it was entirely filled by a translucent coagulum which cut like cheese and showed no tendency to break away. One slice, about 2 mm. thick, was cut through the middle of the gestation sac; the coagulum, stretched from wall to wall like a sheet of gelatine, retained its position exactly, and the cut surfaces remained flat.

In the mesometric half the embryo could be seen through the coagulum lying on one side, its left side being presented towards the omphalon. The embryo lies in the exocœlom between the placenta and the umbilical membrane. The minimum thickness of the uterine wall exceeded 4 mm.; the maximum was nearly 6 mm.; the diameter of the cavum uteri, and consequently of the omphalon that occupied it, was about 12 mm. (Pl. 20, figs. 73 and 74). The placenta, measured in section, had a length of 4·25 mm., and a height

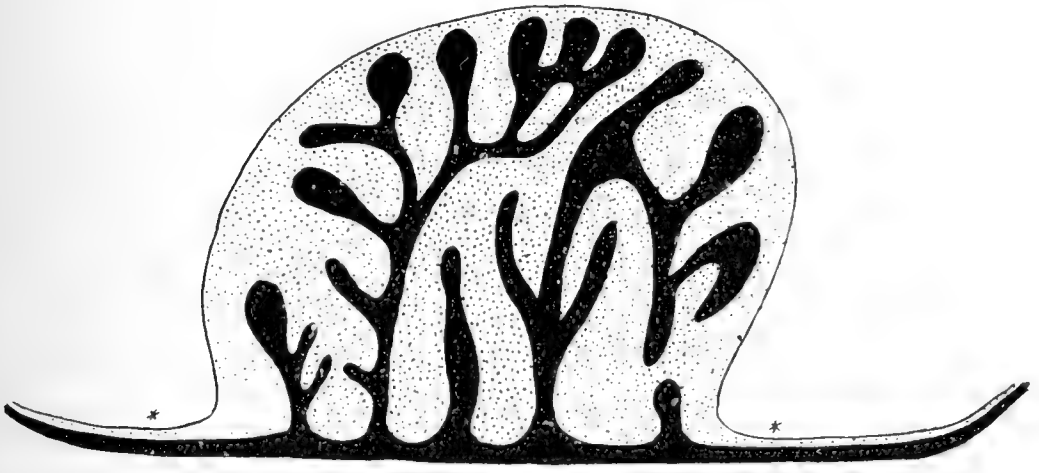
of 2·25 mm. The latter measurement signifies that the somewhat mushroom-shaped placenta projected freely to that extent into the exocœlom. After being reduced to sections the body of the embryo was found to have shrunken rather badly owing to the treatment it had received. When the destination of such early stages is the microtome they should not be cut open previously. Fortunately there was no lack of good material.

In its shape and projection the placenta may be likened to a cushion or a mushroom or a dome. At its edge it overlaps the base of insertion, so that in sections passing tangentially through the margin it appears detached from the uterine wall. Such tangential sections are useful for determining the essential structure of the placental labyrinth. There is a straight trophoblastic base continuous with the rest of the trophoblastic wall of the blastocyst, from which centripetal trabeculæ ascend into the allantoic mesoblast. These trophoblastic trabeculæ are excavated by canals which carry maternal blood, and are what **M. Duval** called sanguimaternal lacunæ in the ectoplacenta. The allantoic mesoblast conveys fœtal capillaries to the placental labyrinth. The allantoic tissue appears in the form of centrifugal villi interlacing with the centripetal trabeculæ, together establishing the placental labyrinth (Text-fig. 4). Neither the trophoblast nor the allantoic mesoblast remains passive in the growing placenta, but they grow in opposite directions, the trophoblast centripetally, the allantoic mesoblast centrifugally.

After passing the point where the placenta is inserted into and united with the uterine wall, the definite basal layer of trophoblast with its sharp demarcation disappears. Wherever the trophoblast touches allantoic tissue it is composed of a cellular layer corresponding to what **E. van Beneden** (1888) called the cytoblast, subsequently lengthened into cytotrophoblast by **J. H. Vernhout** (1894). Towards maternal blood and trophospongia the trophoblast presents a plasmodial layer termed "plasmodiblast" or "plasmoditrophoblast." For the sanguimaternal lacunæ in the trophoblastic trabeculæ

the plasmodiblast furnishes a pseudo-endothelium. The solid buds produced at the growing edge of the placenta consist of cytoblast which ascends into the allantoic tissue. The cytoblastic buds anastomose and so enclose allantoic islands. The cytoblast is the formative layer of the trophoblast; it may be compared with the Malpighian layer of the skin, but instead of giving rise to horny cells on its outer surface, it produces plasmodiblast which is rooted in the vascular trophospongia. The cytoblast itself is rooted in the vascular allantoic tissue;

TEXT-FIG. 4.



Section through growing margin of placenta. Trophoblast black, mesoblast dotted. The asterisks indicate the line where the allantoic and somatic tissues meet; outside the asterisks the double membrane is diplotrophoblast.

it does not give off any buds towards the maternal tissue. The cytoblast is a growing tissue, the plasmodiblast is a feeding organ, and the trophospongia is the nidus supplying the nutriment.

The foregoing aphorisms are indited with special reference to the conditions observed in the beaver, but they derive support from the observations of others upon different animals. In the rabbit, **H. Schoenfeld** (1903), confirming **Maximow**, states that the plasmodiblast is supplied with elements from the cytoblast, and adds that it has no growth of its own; it moves about upon the surface of the cytoblast

as described by **A. Maximow** (1900). In the squirrel (Sciurus), **F. Muller** (1905, p. 560) says that the growth of the placenta does not take place merely by the substitution of fœtal for maternal tissues, but by the progressive centripetal growth of the ectoplacental mass, which thus surrounds the allantoic villi more and more; nevertheless the greater part of the uterine mucosa is supplanted by the placenta, because a continual process of degeneration and resorption of maternal tissue is taking place.

In the bat, **P. Nolf** (1896, p. 610) says that the increase in thickness of the placenta is not the result of peripheral growth at the expense of the maternal tissue. Almost all of its secondary growth is centripetal; the internal or fœtal face of the placenta grows towards and projects into the blastodermic cavity. This is proved by the fact that the vegetative epiblast throughout gestation is continued into the placental cytoblast, not at the level of the internal surface of the placenta, but at the level of its external surface. This conclusion accords, he says, with those deduced by **Duval** for Carnivora, by **Hubrecht** for the shrew, and by **Vernhout** for the mole.

In all these instances the nutritive material for the centripetal growth of the ectoplacental trophoblast is furnished by the maternal trophospongia, which forms a cushion upon which the placenta rests. In the beaver this trophospongia has a twofold origin in a vascular dermatic proliferation and a lobular epithelial proliferation. The latter is now broken up into polygonal blocks by the capillary network, producing an areolated structure in section. Many of the epithelial areolæ have degenerated into syncytia in which numerous dark-stained nuclei are densely aggregated. Sometimes a syncytial mass is extensive and then there appears a curved band of nuclei in the midst of it. The epithelial areolæ, the syncytia, and the aggregations of deeply staining nuclei are very characteristic of the early trophospongia of the beaver's placenta. To these features should be added the presence at certain points of brown granules chiefly surrounding large

degenerating maternal nuclei. In the deeper zone of the maternal trophospongia the cell-islands are not yet syncytial; some are entirely cellular, others are partly syncytial.

There are interesting analogies between events in the preplacental and euplacental periods, as under:

Preplacental period.	Euplacental period.
1. Uterine glands partly normal, partly necrotic.	. Epithelial areolæ partly cellular, partly syncytial.
2. Centripetal dermatic proliferation conveying maternal capillaries.	. Centrifugal allantoic proliferation conveying fœtal capillaries.
3. Centrifugal epithelial proliferation into the dermatic tissue.	. Centripetal ectoplacental proliferation into the allantoic tissue.

At the junction of trophoblast and trophospongia a symplasma is formed. **Schoenfeld** (1902) discusses the use of the terms syncytium and plasmodium which were introduced by Prof. **Haeckel**; and symplasma suggested by Graf **Spee**. A syncytium is immobile, a plasmodium is mobile. In connection with the phenomena of placentation, **Schoenfeld** applies syncytium to maternal formations, plasmodium to fœtal formations. Only when the fœtal plasmodium comes into contact with the maternal syncytium does the latter undergo degeneration and become converted into a symplasma which is defined as a syncytium in retrogression. The symplasma is brought about by the incorporation of maternal protoplasm and nuclei into the substance of the plasmodium. Until the degeneration of the maternal elements is complete, the peripheral zone of the plasmodium is a symplasma containing active fœtal nuclei and passive maternal nuclei. This interpretation involves perhaps a slight extension of the original meaning of the term symplasma. In the dog, **Schoenfeld** described the fusion of decidual cells with the plasmodium, thus converting the latter, for the time being, into a mixed "fœto-maternal plasmodium"; but there can be no harm in calling it a symplasma.[1] Good illustrations of a typical symplasma in the sense here indicated were

[1] To avoid possible ambiguity perhaps the term "symplasmodium" might be preferred.

given by **A. Maximow** (1900, Taf. xxx, figs. 1 and 2) for the rabbit.

At many points the junction of plasmodium and syncytium is rendered conspicuous by the presence of the crowded syncytial nuclei, which contrast in colour, size and shape with the smaller, paler, oval, and more evenly distributed plasmodial nuclei. The conjoint symplasma may be observed to surround superficial capillaries of the trophospongia together with adjoining decidual cells. At such places the plasmodial nuclei may be seen intruding into the syncytium, and no boundary can be drawn between the plasmodial protoplasm and the syncytial protoplasm. The inclusion of maternal capillaries seems to be effected by the tortuous growth of the allantoic tissue which pushes the trophoblast before it into the trophospongia, so that we find in the border zone between trophospongia and placental labyrinth islands of allantoic tissue surrounded by trophoblast, and outside that a mantle of symplasma (Pl. 20, fig. 75). In the diagrammatic figure (Pl. 20, fig. 76), the allantoic villi, with their trophoblastic investment, are seen to be tipped by syncytial groups of nuclei, rendered in black. The characteristic trophospongial islands or areolæ show, in the sections, various grades of syncytiation at different levels, there being no regularity in the distribution of the syncytia throughout the trophospongia, except at the symplasma or zone of contact.

The trophospongial islands, whether syncytial or cellular, are separated by capillaries with normal endothelium. At the base of the trophospongial cushion, between it and the massive dermatic proliferation, there is a basal sinus-like blood-space, to which large capillary vessels pass vertically through the dermatic tissue from the direction of the mesometrium. This arrangement is indicated in Pl. 20, fig. 76. Near each pole of the placenta a large vessel is found receiving its affluents from the sanguimaternal lacunæ in the swollen tips of the trophoblastic trabeculæ; from thence it descends into the mucosa. The two polar vessels possess a proliferated endothelium which they retain until they reach a point deep in the

mucosa, where each of them is continued into a narrow vessel with normal endothelium which passes directly and abruptly into the proliferated walls (Pl. 20, fig. 77). There is an equally abrupt transition from the proliferated endothelium to the plasmodial pseudo-endothelium near the fœtal periphery of the placenta. At one pole the vessel penetrates the placenta near its right margin; at the other pole it enters near the left margin.

The data at my disposal do not permit of a direct comparison of the placental circulation of the beaver with other types which have been investigated. Such comparisons should be made at equivalent stages. The circulation in the early established placenta will necessarily differ in its details from that in the mature finished placenta. As for the latter, excellent figures have been given by **Tafani** of the injected placentæ of various mammals, less successful perhaps as regards the rabbit, but remarkably clear as regards rat, guinea-pig, and bat. The placenta of the guinea-pig possesses its own special features. That of the rat (Mus decumanus), as represented in **Tafani's** tav. v, fig. 2, is more like that of the bat (Vespertilio murinus, tav. vi, fig. 2) than the guinea-pig (Tav. v, fig. 1). In Mus and in Vespertilio there is a central maternal artery penetrating through the middle of the discoplacenta to its fœtal aspect, where it spreads out into the afferent sanguimaternal lacunæ.

To left and right of the placental insertion the areolated trophospongia merges imperceptibly into a marginal zone of hollow crypts, the walls of which are partly necrotic. In the obplacental and periplacental regions of the gestation sac the dermatic cells constitute an epithelioid tissue comparable to Nolf's epithelioid layer in the bat. In the mesometric region the dermatic tissue, though proliferating, still retains a primitive aspect, and does not present an epithelioid mosaic pattern in section.

Median sections through the placenta show that the dermatic tissue in which the vessels are lodged projects like a

cone into the trophospongial cushion (Pl. 21, fig. 78). After leaving the basal trophospongial sinus, which now appears as an arched line between the dermatic axis and the areolated cortical substance, the vessels branch and enter into numerous anastomoses with each other, forming a rete mirabile within the dermatic cone continuous with the trophospongial sinus, which is itself retiform. With a simple lens the trophospongial crescent with its peripheral zone of syncytia, its interstitial meshwork, and its basal sinus, can be seen to perfection following the contour of the dermatic cone with its rete mirabile.

Another series, cut nearly longitudinally through the gestation sac, showed the placenta in a slightly more advanced condition—4·50 mm. in length, 3·75 mm. high. In tangential sections, i. e. such as do not pass through the central trophospongial cone, the allantoic mesoblastic villi penetrate deeply and tortuously into the embryotrophic cap of areolated trophospongia, so that allantoic islands, surrounded by their trophoblastic investment, appear in section amongst the areolæ.

Towards the centre of the placenta, the concentric strata of which it is composed stand out very clearly. Beginning at the fœtal aspect, there is first a narrow zone of allantoic mesoblast containing the superficial allantoic vessels; then the swollen ends of the trabeculæ dilated with the sanguimaternal lacunæ; thirdly, the labyrinth of anastomosing trabeculæ; fourthly, the border zone of symplasma; fifthly, the trophospongial areolæ; sixthly, the dermatic cone. Most of these parts are to be found in figures already referred to, and again in Pl. 21, fig. 79. Since the polar vessels enter the placenta near its right and left margins, neither of them is seen in a median section. Noteworthy is the abrupt transition from the areolated trophospongia to the dermatic tissue.

The position of the embryo in the blastocyst conforms to the rule which applies to those Vertebrates in which there is a great disparity between the animal and vegetative poles, i. e. when an omphalon or a yolk-sac is present. It has been

mentioned above, and the figures show, that the umbilical membrane or area vasculosa stretches across the fœtal sac midway between placenta and obplacenta. The body of the embryo, surrounded by its amnion, lies sideways in the exocœlom between placenta and umbilical membrane, with its right side towards the placenta and its left side towards the omphalon (Pl. 20, fig. 73, and Pl. 21, fig. 79). In the Sauropsida, at the gill-slit or branchiotrematic stage, the embryo also comes to lie with its left side towards the yolk-sac. In the rabbit, at the same stage, the forebody of the embryo by reason of the cervical flexure projects into the umbilical vesicle surrounded by the proamnion, or, more precisely, the proamniotic omphalopleure. In the beaver there is no trace of a proamnion at this stage; and when it is remembered that in the preplacental blastocyst the mesoblast is continuous within the circuit of the sinus terminalis, except at the notochordal contact, and that there exists already an anterior as well as a posterior median extension of the exocœlom, the formation of a proamnion at any stage would seem to be excluded.

In Pl. 21, fig. 79, we see the amniotic membrane stretching between the umbilical membrane and the placenta. In the same figure the opening of the omphalomesenteric duct into the omphalon and that of the allantoic canal into the flattened allantois are indicated, though they do not occur actually on the same section. The allantoic canal communicates with the hypoblastic cloaca, into which the Wolffian ducts now open, and forms the hypoblastic axis of a mesoblastic stalk, accompanied by two arteries and a vein. At the distal end of the stalk the canal widens out as the allantoic sac which is imbedded in the thickness of the mesoblast at the fœtal surface of the placenta, causing no protuberance. This flattened sac has a longitudinal extension of about 0·75 mm. During part of its course from the cloaca to the sac, the lumen of the allantoic canal is occluded by cellular proliferation, so that it becomes solid; this condition has been observed in two series.

In one series (VII A in the Utrecht catalogue) the omphalo-mesenteric duct has a partially occluded lumen; but in another series (V B) the lumen is open, and contains maternal blood-corpuscles, which it conveys from the omphalon to the mid-gut. The presence of red blood-corpuscles in the mid-gut could not easily be attributed to accident, and it appeared at first a mystery how they came to be there. The clue to the mystery was found in the behaviour of the obplacental trophoblast, which at this stage consists of flattened megalo-karyocytes closely applied to the uterine wall; here and there they are greatly distended with erythrocytes, and there is evidence of the transfusion of maternal corpuscles into the omphalon. They pass into this cavity across the obplacental trophoblast, and are in fact to be found scattered in the midst of the coagulum.

I will conclude this chapter with some further details concerning the embryo in order to define the stage of development which it has now reached with greater precision. Its actual age cannot be estimated, and can only be roughly guessed at. If we assume the period of gestation to be one hundred days, then these embryos will certainly fall within the first twenty-five days, and probably within the first fifteen days; the preplacental blastocysts are likely to belong to the first ten days. The relative age is best reckoned according to the size of the gestation sac.

The embryo is now in the gill-slit or branchiotrematic stage; the mouth is open, but there is no anus. In section the mouth-cavity, flattened dorso-ventrally, appears like a pair of gill-slits, but this is entirely deceptive, since the formation is that of the stomodæum with its pituitary diverticulum meeting the blind end of the infundibulum. The four pairs of true gill-pouches just fail to open to the exterior, being closed over externally by a narrow cellular bridge. The auditory sacs are closed; the optic stalk is hollow, the optic cup and choroid fissure are formed, and the lens invagination is still connected with the ectoderm. Suitable sections show a length of the spinal cord flanked by

somites resembling the corresponding figure of a 12 mm. pig embryo in **C. S. Minot's** 'Laboratory Text-book of Embryology,' p. 230, fig. 135. The buds of the fore-limbs are rather more advanced than those of the hind-limbs, which appear as broad crescentic thickenings.

XIII. Intermediate Stages.

Two stages, intermediate between the establishment of the growing placenta and the final period towards the term of gestation when it ceases to grow, were obtained, but have not yet been worked out in section. Their external characters present two points of special interest concerning the early relations of the chorion læve or diplotrophoblast of the umbilico-placental areas, and the appearance of a spherical allantoic vesicle. At the stage described in the preceding chapter, the allantois occurred as a narrow tube communicating proximally with the cloacal region and expanding distally into a flattened sac immersed in the allantoic mesoblast at the surface of the placental labyrinth, not causing any additional protuberance into the exocœlom.

The next older stage is represented by three gestation sacs, one in the right horn of the uterus, two in the left. These were despatched to me in a tin containing 10 per cent. formalin on March 23rd. The swellings were equal, somewhat shrunken, 60 mm. in long diameter. Upon cutting through the antimesometric wall, 2·50 mm. thick, the umbilical membrane (area vasculosa) was seen to be expanded and in contact with the mucosa, though devoid of attachment. Along the courses of the vessels low ridges are developed, which press against the mucosa, so that the impressions of the larger vessels are distinctly visible upon the smooth inner surface. When first opened the mucosa appeared dark reddish and spongy, with white giant-cells showing against the ground colour when viewed under a simple lens.

When the fœtal sac was cut into, the umbilical membrane was found to be adherent to the amnion, from which it could be peeled off. In the mesometric division of the fœtal sac the amniotic membrane bends inwards to its insertion upon the dome of the placenta, and here it becomes free from the umbilical membrane, so that a considerable exocœlomic cavity exists in this region. Thus the baggy amnion hangs loosely in the mesometric exocœlom, but elsewhere adheres by simple contact to the area vasculosa, obliterating the exocœlomic cavity. A linea alba marks its insertion upon the placenta.

By slicing away the wall of the gestation sac, which becomes thicker (5 mm.) towards the mesometrium, and then removing a portion of the vascular membrane, the fœtus, 38 mm. long, is exposed, loosely enveloped by the spacious amnion. On the mesometric side, the area vasculosa ends with a villous rim at the two connecting membranes which surround the aditus of the interutricular segments. In this region the mucosa presents a rugose or convoluted surface, and the folds are continued as opaque whitish buttresses from the mucosa to the connecting membranes, extending as far as the edge of a narrow clear zone below the villous rim of the umbilical membrane. At this stage, therefore, simple dissection suffices to demonstrate the dual composition of the definitive connecting membrane (Pl. 21, fig. 80).

On laying open the whole cavity of the exocœlom the polar areas of the fœtal sac are exposed from the inside. They cover over the aditus of the interutricular segments. The dissection was made shortly after the preservation in formol, and the polar areas (umbilico-placental areas) appeared as clear, non-vascular membranes forming turgid protuberances into the exocœlom near each end of the placenta, each being surrounded by part of the sinus terminalis. The pyriform caudad protuberance was 9 mm. high, 10 mm. long; the convex cephalad protuberance was about 5 mm. high by 11 mm. long (Pl. 21, fig. 81). In another specimen opened several months later the polar areas were flattened

out. In fig. 81 the caudad end of the fœtal sac is also the caudad end of the gestation sac; the head of the embryo is directed towards that segment of the uterus which leads to the Fallopian tube. The cephalad protuberance lies over the opening or aditus of that segment, while the caudad protuberance arches over the posterior opening. The placenta measured 33 mm. in length, 21 mm. in width, and 21 mm. in height. Besides its remarkable form and bold eminence, the most noticeable feature was the absence of an allantoic vesicle. The position of the allantoic sac at the placental surface was indicated by a flat membrane without fluid, situated beyond the centre of distribution of the vessels. In a second specimen from the same tract there was a very small incipient allantoic vesicle with a transparent wall. In both of these instances the head of the fœtus was directed towards the oviduct.

The next intermediate stage is represented by four gestation sacs, one right and three left, sent to me in 10 per cent. formalin on April 6th, having an average length of 81 mm. Soon after the arrival of the material I opened all the sacs by cutting out an obplacental piece from each, and punctured each vascular fœtal sac, collecting the clear fluid that squirted out. The amnion was again closely adherent to the umbilical membrane, so that in puncturing the latter the amnion was also pierced, and the clear fluid, which appeared faintly straw-coloured en masse, was the liquor amnii. In the later stages, when the fœtus has nearly attained its limit of growth, the amnion loses its adhesion to the umbilical membrane, so that the exocœlom then becomes the main cavity of the fœtal sac. At the present stage the amniotic cavity is the main cavity of the fœtal sac, the exocœlom, even to the base of the placenta, being temporarily obliterated.

The wall of the gestation sac has now become reduced to a mean thickness of about 2 mm. In the first specimen examined the head of the fœtus was again directed towards the Fallopian tube. The fœtus as it lies in its amnion has a

length of 73 mm., measured from the crown of the head to the base of the tail. The flattened tail is not bent under the body, but simply curves round against the posterior end of the fœtal sac, extending a little beyond the hind feet; it measured 15 mm. in length. The placenta now has a length of 42 mm.

The most conspicuous difference between this and the preceding stage is the presence of a spherical allantoic vesicle, about 16 mm. in diameter, with thin pellucid wall, projecting boldly into the amniotic cavity. It lies just in front of the centre of distribution of the allantoic vessels into the placental labyrinth (Pl. 21, fig. 82).

The three gestation sacs in the left uterus held their fœtus in the following positions: In the one nearest to the ovary the head of the fœtus is directed towards the cervix uteri; in the middle sac the fœtus lies in the same position, with the head towards the cervix; in the last of the three, i.e. the one nearest to the vagina, the head of the fœtus is directed towards the oviduct, the tail towards the cervix.

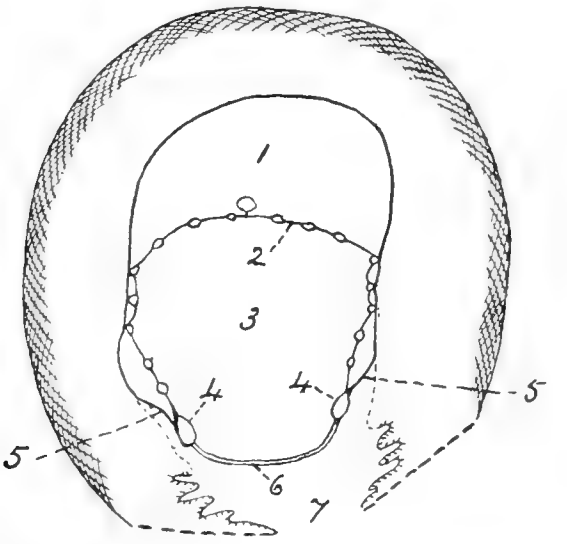
XIV. The Connecting Membrane.

At the early placental stage represented in Pl. 20, fig. 76 and fig. 79, the umbilical membrane is invaginated about halfway into the omphalon and stretches like a diaphragm across the blastocyst, separating the omphalon from the exocœlom. The latter is limited towards the aditus of the gestation sac by a sheet of somatopleure or diplotrophoblast, which constitutes one of the umbilico-placental areas described in my former paper (1912). At the poles of the fœtal sac, beyond the range of the placenta, this membrane stretches free across the cavum uteri within the circuit of the sinus terminalis (Text-fig. 5).

From the outer edge of the sinus terminalis two other membranes arise—the umbilical membrane and the omphalopleure. The short free zone of omphalopleure intervening between the sinus and the uterine wall is the primordial

connecting membrane. It is clearly derived from the adcarinal membrane of the preplacental blastocyst; it is what is left of this membrane after the peripheral encroachment of the primordial area vasculosa has ceased. It continues to grow in later stages, and, by accession of material from the uterine mucosa, becomes converted into the definitive umbilico-uterine connecting membrane. The latter is therefore a composite membrane of dual origin,

TEXT-FIG. 5.



Section through region of one of the aditus of the gestation sac at the early placental stage. 1. Omphalon. 2. Umbilical membrane. 3. Exocœlom. 4. Sinus terminalis. 5. Connecting omphalopleure (representing adcarinal membrane). 6. Diplotrophoblast. 7. Aditus.

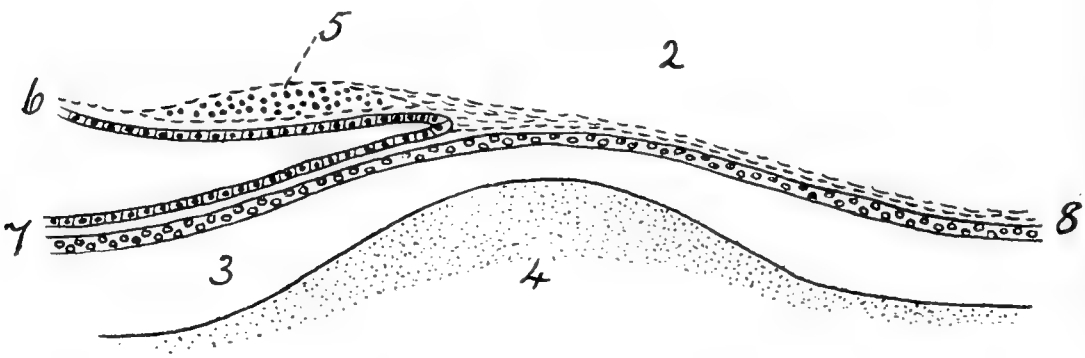
as was indicated in the preceding chapter. This fact is proved by its early history as well as by its histological structure, into the details of which I will forbear to enter at present, although illustrations have been prepared. The manner of junction of splanchnopleure, omphalopleure and somatopleure at the level of the sinus terminalis in the early placental stage is shown in Text-fig. 6.

From the earliest to the latest days of placentation the sinus terminalis is situated at the junction of the three membranes whose primary names have just been given. Their

secondary designations are respectively—umbilical membrane, umbilico-uterine membrane, and umbilico-placental membrane.

The omphalopleure, as a whole, constitutes the inferior (anti-mesometric) wall of the omphalon and of the entire blastocyst. It is present in all its integrity during the early placental period but disappears in later stages, with the exception of the persistent connecting membrane. The origin of the latter is thus explained. It is a secondary product of the primary obplacental implantation of the

Text-fig. 6.



Part of periplacental wall of blastocyst, at the early placental stage, to show the junction of membranes at the sinus terminalis. 1. Cavity of omphalon. 2. Exocœlom. 3. Cavum uteri. 4. Mucosa. 5. Sinus terminalis. 6. Splanchnopleure. 7. Omphalopleure. 8. Somatopleure.

blastocyst, and directly comparable with the adcarinal omphalopleure of the preplacental blastocyst. The umbilical membrane forms the major part of the outer wall of the mature fœtal sac. In my former paper on the beaver (1912, p. 207) I called it the vascular chorion or endochorion of the rodent blastocyst, not being at that time aware of the circumstance that the more suitable name, umbilical membrane, had been applied to it for the rabbit by **E. van Beneden** and **C. Julin** in 1884.

The umbilico-placental membrane is in its origin a non-vascular somatopleure or diplotrophoblast, but in the mature fœtal sac there are intrusive capillaries proceeding into it

from the area vasculosa. They form anastomosing loops, and bear a resemblance to the capillary loops in the chorion læve of the bat's blastocyst as figured by **van Beneden** and **Julin.**

The reason for the existence of the connecting membrane which supplements the placental implantation in the attachment of the fœtal sac to the gestation sac, may perhaps be sought in the semi-aquatic habits of the beaver and in the fact that the female retains her activity, leaving the lodge and swimming under the ice to procure food from the submerged stock of winter-provender, throughout the period of gestation. Were it not for the additional support afforded by the connecting membrane, the narrow deciduous root of the massive placenta might easily be torn asunder. The connecting membrane must relieve the placenta of much of the stress and strain to which it would otherwise be exposed.

The nearest approach to the condition of having a connecting membrane seems to be represented by a temporary formation, which was described in the early gestation sac of Sciurus by **F. Muller** in 1905. It appears that the periplacental mucosa forms a ring-shaped thickening to which the thickened trophoblast adheres before the folding of the amnion. In the following stage this ring-shaped zone of implantation, with the sinus terminalis close to it, continues to extend, leaving the placental part of the cavum uteri still unoccupied, but with numerous crypts opening into it.

In the young gestation sac of Sciurus, when the blastocyst is attached to the obplacental wall, the cavum uteri, as had been previously observed by **Fleischmann**, becomes constricted by a circular periplacental thickening into a smaller mesometric and a larger anti-mesometric portion. The zone of adhesion or omphalo-placental ring ("omphaloiden placentatiering," F. Muller, op. cit., p. 395) occurs at the lip of the mesometric cavum. In this ring an epithelial syncytium is formed, with which the trophoblast becomes intimately united. At length the syncytium is destroyed and

then the plasmodial union is exchanged for simple adhesion. Later still even this is given up. This early periplacental implantation occurs at the level of the sinus terminalis, and is interpreted by **Muller** as a vestigial omphaloid placentation such as had not been described in rodents before. The periplacental connection has nothing to do with the allantoic placenta which forms subsequently. It extends below and thus embraces the openings or aditus of the gestation sac. Its limited centripetal growth and its transitory endurance are its most remarkable features.

There can hardly be a doubt that the temporary omphaloplacental adhesion of Sciurus is comparable with the permanent umbilico-uterine connection of Castor.

XV. Special Considerations.

The two leading characteristics of the preplacental blastocyst of the beaver, the obplacental implantation with differentiation of erythrocytophagous and leucocytophagous megalokaryocytes, and the placental keel, are of specific physiological importance to the growing embryo, but they also have a comparative value which can only be elucidated by a brief discussion. In order that the reader may be orientated with regard to the systematic position of the beaver, a few words of introduction are desirable.

Although the beaver, in structure and habits, is unique amongst the Rodentia, displaying in high degree the qualities of intelligence and adaptability to local conditions, yet it would be wrong to suppose that it is farthest removed from a primitive organisation. On the contrary, in its monotrematous cloaca and pentadactyle limbs, to mention these two external features only, it retains the marks of a very ancient mammalian type. In my former paper (1912) it was suggested that "the beaver occupies a position amongst Rodentia comparable with that attained by man amongst primates." It is well known that man has retained some primitive features in limbs, teeth and digestive tract, as compared with many other

mammals. Just as **Hubrecht's** discovery of the blastocyst of Tarsius with its "Haftstiel" indicated an excessively remote origin for the primate ontogeny, so the blastocyst of the beaver with its exostyle may have an analogous bearing upon rodent ontogeny.

It is generally agreed, under the support of such authorities as **Cope**, **Winge**, and **Tullberg**, that the beaver family is related to the squirrel family, both of these families being associated under **J. F. Brandt's** suborder, Sciuromorpha (1855). **Tullberg** divides the Simplicidentata into two great tribes, Hystricognathi and Sciurognathi. The latter comprises two sub-tribes, Myomorphi and Sciuromorphi; and the latter falls into three sections, Sciuroidei, Castoroidei, and Geomyoidei. **Winge** (quoted by **Tullberg**) held that rodents are to be derived from primitive Mammalia resembling the least specialised Insectivora, from which they diverged by cumulative increase of the gnawing habit.

F. Muller (1905), adopting Haeckel's (1895) generalisations maintained that of all Rodents the Sciuromorpha have diverged least from the ancestral type; and he added that the genus Sciurus in particular occupies the most central position and has preserved the most primitive form. On the other hand, **Max Weber** (1892) regarded the scales on the tail of the beaver as the remains of a primitive scaly covering of the body.

The preplacental blastocyst of Lepus and Sciurus is a plano-convex blastocyst, that of Mus and Cavia is an inverted blastocyst, that of Castor is an everted blastocyst. The task before us is not to decide which of these three is the most primitive type of blastocyst, but to consider which of them offers the readiest comparison with the blastocyst of Tarsius. It may be premised that in one respect the euplacental blastocyst of the beaver is the most primitive known amongst existing Rodents by reason of the persistence of the umbilico-uterine connecting membrane which is a consequence of the periplacental implantation of the trophoblast.

The keel extends from end to end of the elongated balloon-shaped blastocyst, along its superior or mesometric side, dipping into the deep placental groove. One of the fundamental relations of this remarkable formation is so obvious that its importance in establishing the reality of the phenomenon might escape attention—namely, the coincidence in the configuration of the wall of the blastocyst and that of the cavum uteri or cavity of the gestation sac. The keel may thus be accepted at once as a fact, and need not be regarded with suspicion as an artefact.

There are four or five principal structures concerned in the constitution of the keel: the epiblastic keel, the mesoblastic keel, the hypoblastic keel and the embryomic keel, to which may be added the exocœlomic keel. The interpretation and comparison with other forms will hinge upon the massive mesoblastic keel which follows behind the primitive streak. It is convenient to anticipate conclusions to some extent by assuming that the keel represents an ancient or primitive mechanism, and that the exserted mesoblastic keel of the beaver is comparable with the "Haftstiel" of Tarsius, monkeys and man.

History of the "Haftstiel."—So far as I have been able to ascertain, the first use of the term "Haftstiel" as applied to the mammalian blastocyst occurs in **Selenka's** memoir on the opossum (1887, vide his text-fig. C, on p. 136 op. cit.). In this case the main cavity of the blastocyst is the omphalon, into which the allantoic vesicle, surrounded by a narrow exocœlom, hangs freely. In the early Primate blastocyst the main cavity is the exocœlom, into which the reduced omphalon hangs freely. These conflicting relations depend upon the varying degrees of development of the exocœlom, allantois and omphalon respectively. In the opossum the embryo with its allantoic vesicle and exocœlom is suspended from the chorion or wall of the fœtal sac into the main cavity (omphalon) by a hollow exocœlomic stalk. In the Primates the embryo with its umbilical vesicle is sus-

pended from the chorion into the main cavity (exocœlom) by a massive allantoic stalk. There is no massive allantoic mesoderm in the opossum and no hollow allantoic vesicle in man. Just as the term "chorion" is often used to denote the wall of the fœtal sac, irrespective of the constitution of its membranes, so the term "Haftstiel" denotes the mechanism by which the embryo is suspended within the cavity of the fœtal sac.

The real knowledge of the Primate "Haftstiel" dates from **His's** third memoir on the 'Anatomie Menschlicher Embryonen' (1885). I have not seen the original memoir, but the subject is treated very fully by **C. S. Minot** in his contribution entitled "Uterus and Embryo" (1889). **His** made what **Minot** appraised as "the discovery of fundamental importance"—that in the early human blastocyst the allantoic sac appears as a small endodermic tube lying in a posterior prolongation of the body which **His** called the "Bauchstiel," and that at this early stage the allanto-chorionic vessels already run to and branch out upon the chorion. Thus "the allantois is, from the first, continuous with the chorion" (**Milnes Marshall**, 1893).

F. Keibel has stated recently (1913) that the allantoic tube in the human blastocyst is an entirely vestigial structure; he does not say explicitly of what it is a vestige, but the text implies clearly that he considers it to be a vestige of the free allantoic vesicle of Sauropsida. The beaver may help us to another explanation, namely, that it is a vestige of that portion of the omphalon which descends into a keel-shaped "Haftstiel" or exostyle. The latter term can be used in general as an equivalent rendering of "Haftstiel," its etymology being analogous to that of exocœlom, both terms referring to structures that lie outside the embryo.

Selenka's memoir on the "Affen Ostindiens" appeared in 1891. In this monograph it was shown that the characteristics of the monkey's blastocyst are: (1) The early separation of the omphalon ("yolk-sac") from the chorion; it takes no part in the nutrition of the embryo and must be regarded as a

vestigial structure; it is scantily supplied with vessels and floats as a small stalked vesicle in the exocœlom, until by the expansion of the amnion it becomes pressed against the chorion and finally succumbs to resorption.

(2) The spacious exocœlom acts as a reservoir of food-stuffs until the allantoic vessels take over the function of fœtal metabolism.

(3) After the formation of the amnion (the method of which was not observed), the embryo retains its connection with the placental chorion by a solid cord of mesoblast into which a rudimentary allantoic tube penetrates. This massive cord with its vestigial endodermic cavity is the allantoic stalk or "Haftstiel," the vehicle of the placental vessels.

(4) The main cavity of the early blastocyst is the exocœlom. Subsequently the amniotic cavity dilates enormously and the amnion finally fuses with the chorion, so that the cavity of the fœtal sac is then amniotic cavity.

From the above résumé it is obvious that **Selenka's** "Haftstiel" is identical with **His's** "Bauchstiel." As an English equivalent the expression "body-stalk" has been suggested by **C. S. Minot** and adopted by **J. W. Jenkinson**; but if the comparison with the beaver is accepted, the need for a more general term, e.g. exostyle, is indicated.

We must now refer briefly to **Hubrecht's** classical paper in Gegenbaur's 'Festschrift' (1896), entitled "Die Keimblase von Tarsius." The chief characteristics of the Tarsius blastocyst may be summarised:

(1) Rauber's layer, or the trophoblast over the formative epiblast, disappears in the very early stages, shortly after the delamination of the hypoblast.

(2) The exocœlom has a remarkably precocious development and the hypoblastic sac occupies only a small portion of the spacious blastocyst.

(3) The blastodisc is at first excentric. The ectoplacental proliferation of the trophoblast is situated some way behind the blastodisc, not diametrically opposite to it.

(4) Between the ectoplacental proliferation and the posterior

border of the blastodisc (embryonic shield), there extends a solid mesoblastic tract which at the same time forms part of the wall of the closed mesoblastic sac or exocœlom; this solid cord is the "Haftstiel." At this stage there is no mesoderm in the embryo and the region of the embryonic shield is still didermic.

(5) The blastocyst is only attached to the uterine wall by the ectoplacental disc; otherwise it lies free in the uterine lumen.

(6) A complete and close-meshed area vasculosa develops in the wall of the hypoblastic sac or umbilical vesicle, and is filled with blood-corpuscles long before the heart begins to beat; but vascular rudiments arise in the "Haftstiel" before the appearance of the omphaloidean network.

(7) The mesoblast of the "Haftstiel" is at first contiguous with the adjacent trophoblast; but, pari passu with the formation of the amnion, it becomes separated from the trophoblast by insinuation of the exocœlom and so becomes converted into the primordial umbilical cord.

(8) After the "Haftstiel" has become vascularised and enlarged, a tubular outgrowth from the umbilical vesicle penetrates backwards into the connective tissue of the "Haftstiel." This is the allantoic diverticulum of the umbilical vesicle.

From this brief tabulation of **Hubrecht's** discoveries regarding the blastocyst of Tarsius, we gather that although the allantoic tube is a secondary outgrowth, it belongs to the omphalon. There is no question of its being a diverticulum of the hind-gut at its first origin. Again, the "Haftstiel" is directly continuous with the primitive streak, and forms, at the beginning, part of the wall of the blastocyst behind the embryo. In 1889 **Hubrecht**, à propos of Erinaceus, had defined the "Haftstiel" as a caudal mesoblastic cord which grows backwards from the posterior end of the primitive streak in order to promote the early vascularisation of the chorion. To this definition **Resink** (1904) added in italics: "Der

Haftstiel entsteht jedoch als die von Anfang an vorhandene Verbindung des entypierten Keimfeldes mit dem Chorion."

In the preplacental blastocyst of the beaver, the endodermic allantois does not appear as an outgrowth, but is actually a deep hypoblastic groove of the omphalon extending into the keel. It is, therefore, from the first an integral part of the omphalon. The steps by which this hypoblastic groove becomes narrowed down to the allantoic canal with its distal flattened sac cannot be followed in the material at my disposal. It must take place simultaneously with the closure of the digestive tract of the embryo, which is similarly associated with the narrowing down of the omphalo-mesenteric duct.

With reference to the above quotation from **Resink**, a pupil of **Hubrecht**, the important point in my estimation is his insistence upon the primary character of the "Haftstiel," which is not in its essence a secondary formation (cf. **Milnes Marshall**, 1893). The flattened "Haftstiel" of Tarsius may be directly compared with the keel-shaped exostyle of the beaver. Both of these structures are organs of fixation, by means of which the embryo attaches itself to the placental complex. The ectoplacental disc is derived from the proliferation of the exostylar trophoblast. The localisation of the "Haftstiel" in Tarsius and the exostyle in the beaver affords strong confirmation of the view put forward by **Minot** in 1889, that the discoidal placenta is probably a primitive placental type. The chief characteristic of the blastocyst of Castor is the possession of a massive keel-shaped exostyle or "Haftstiel" into which the endodermic allantois extends as a primary groove of the omphalon; and the exostyle itself is directly continuous with the proliferation of the primitive streak. In his useful, though doubtless ephemeral, speculations concerning the origin of the fœtal annexes of Mammalia, **Resink** admitted that he was unable to explain the origin of the allantoic sac. Perhaps the beaver may offer a new point of view from which this problem may be envisaged.

Within the limits of the order of the Rodentia, the conditions preceding the ectoplacental implantation of the beaver's blastocyst are most readily compared with those obtaining in squirrels. According to **Fleischmann** (1893), in Spermophilus, where there is no preplacental keel, the gestation sac is none the less subdivided into an omphaloid cavum and a discoid placental cavum or "Scheibenhöhle." The young blastocyst is attached in the omphaloid cavum, and is so orientated that the embryonic pole (blastodisc) lies over the narrow passage (called "Schlossspalte") connecting the omphaloid cavum with the discoplacental cavum. The latter is comparable with the deep mesometric groove in the gestation sac of the beaver.

The isolated position and high standing of the beaver amongst existing Rodents are primâ facie evidence of an exceedingly remote origin. Other facts of organisation and palæontology are in harmony with such evidence. These circumstances lend peculiar significance to the character of the preplacental blastocyst.

We may now give some attention to the behaviour of the inferior or obplacental hemisphere of the beaver's blastocyst. Without greatly enlarging the scope of the illustrations accompanying this paper, it would be impossible to do justice to the wonderful scenes of substitution of trophoblast for uterine epithelium, which can be witnessed in almost every section. In the preplacental blastocyst of the hedgehog, **Hubrecht** found a phagocytic trophoblast forming a complete trophosphere round the blastocyst. The ectoplacenta is a localised derivative of the trophoblast. **Resink**, however, applied the term "ectoplacenta" to the entire trophoblast. On the present occasion I propose to confine my remarks to Rodentia.

Obplacental ectodermal proliferations were described in the blastocyst of the rabbit by **A. von Kölliker** in 1882. According to **Kölliker** an event of fundamental importance for mammalian embryology was **Rauber's** discovery in 1875

of the so-called "Deckschicht" outside the formative epiblast of the rabbit's blastocyst, showing that the traditional view till then maintained by **Coste**, **Hensen**, and **Kölliker**, that the wall of the monodermic blastocyst had the value of embryonic ectoderm, was wrong. **Kölliker** called **Rauber's** "Deckschicht" the primitive ectoderm of the area embryonalis. Of next importance was **Lieberkühn's** demonstration in 1879 of the origin of the permanent or formative ectoderm of the rabbit out of the inner cell-mass which lies against the primitive ectoderm and splits into two layers—the permanent ectoderm and endoderm. **Kölliker's** primitive ectoderm of the rabbit's blastocyst thus prepared the way for **Hubrecht's** celebrated conception of the trophoblast of the mammalian blastocyst. In the extra-embryonic primitive ectoderm of the rabbit **Kölliker** discovered numerous ectodermal proliferations in the form of elongate, villiform elevations due to local nuclear divisions.

These papillæ were next described by **E. van Beneden** and **C. Julin** in 1884. On opening an eleven-day gestation sac of the rabbit under picrosulphuric acid, by a crucial incision through the antimesometric wall, a liquid escaped and a coagulum was produced. This was the fluid from the blastodermic cavity, which becomes the umbilical or vitelline vesicle or "yolk-sac." The cavity had been opened because the obplacental wall of the blastocyst was intimately united to the uterine mucosa. The union between the wall of the umbilical vesicle and the mucosa is effected by means of the epiblastic buds, which, as the authors stated, **Kölliker** first noticed. These buds arise upon the whole surface of the inferior hemisphere of the blastocyst, commencing about the eighth day. The degeneration of the cells, whose proliferation engenders the buds, begins at the ninth day, and leads to the rapid degeneration of the entire epiblastic membrane, which, at the fourteenth to fifteenth day, detaches in shreds from the mucosa.

M. Duval (1890, pl. iii, fig. 28) described and figured the bilaminar inferior hemisphere of the rabbit's blastocyst at the

tenth day, and observed the plasmodial swellings connected by intervening thin tracts, but he let them degenerate without having effected any useful purpose.

R. Assheton (1895) also mentioned and illustrated ('Quart. Journ. Micr. Sci.,' 37, Pl. 19, fig. 7) the obplacental papillæ of the rabbit. He says the first attachment of the blastocyst takes place between the lower parts of the blastodermic vesicle and the periplacental and obplacental folds of the uterus. It is effected by means of epiblastic papillæ about the eighth day. **Assheton** suggested that the papillæ became wedged into the uterine epithelium by reason of hydrostatic pressure from within the vesicle.

H. Schoenfeld (1902) made the first complete cytological study of the festoon-like adhesions of the preplacental blastocyst of the rabbit on the seventh and eighth days. At seven days the zona pellucida becomes broken on the antimesometric side of the blastocyst and multinucleated ectodermal thickenings have appeared. At 7 days 4 hours the ectodermal buds come into intimate adhesion with the uterine syncytium and push into it with pseudopodium-like processes so as to reach the maternal capillaries, thus fixing the blastocyst to the mucosa in a manner comparable to the rooting of a plant. From the seventh day of gestation until 8 days 4 hours, the blastocyst remains attached to the mucosa on the obplacental side. At 8 days 6 hours numerous plurimitotic figures occur in the epiblast of the obplacenta. At 8 days 22 hours **Minot's** giant-cells appear in the obplacental mucosa. They are of fœtal origin, resulting from retrogressive changes of the obplacental multinucleated epiblastic buds, now embedded in the mucosa. At their origin they are much more voluminous than any elements belonging to the mucosa or submucosa. According to **Maximow**, they reach their maximum development, with a diameter of 100 μ, on the sixteenth to twentieth days.

J. Rejsek (1904) describes the obplacental implantation of the very young blastocyst of the Souslik (Spermophilus citillus). It continues until the blastocyst acquires a

diameter of 2 mm., when it is given up simultaneously with the incipient placentation, which coincides in time with the cervical flexure of the embryo. The obplacental implantation is effected by means of a single multinucleate trophoblastic plasmodial proliferation at the antimesometric pole, sending processes into the mucosa. The number of nuclei increases by amitosis from 22 in a blastocyst of 0·126 mm. to more than 500. This obplacental implantation of the souslik's blastocyst would seem to be a phenomenon sui generis, the initial plasmodial thickening corresponding to a single papilla of the rabbit's blastocyst.

F. Muller (1905) also observed the antimesometric attachment of the preplacental blastocyst of Sciurus (vide his pl. viii, fig. 8).

For the characteristic features of the obplacental implantation of the preplacental blastocyst of the beaver, I must refer the reader to the foregoing text. The obplacental trophoblast is still connected with the uterine wall at the early placental stage, so that the implantation endures for a much longer period than in the rabbit. Eventually, when the invagination of the umbilical membrane is completed and the obplacental omphalopleure has vanished, giant-cells remain in the mucosa, appearing as white specks peppered over the internal surface of the gestation sac, scattered or in groups, not evenly distributed.

In the preplacental stage the trophoblast, at the areas of attachment, is not more than one layer in thickness and its cells remain distinct, though they may have several nuclei. It does not send processes to any depth into the mucosa, but as a rule it has a flat insertion upon the decidual surface, the maternal capillaries pressing towards the trophoblast rather than otherwise. Wherever the uterine epithelium is displaced by the obplacental trophoblast, it disappears without previously forming a syncytium. It is surprising to find the trophoblast planted like a pseudo-epithelium upon the decidual surface, and, at its borders, normal uterine epithelium. This probably means that the uterine epithelium becomes necrotic in

advance of the trophoblastic attack and that the trophoblast does not kill normal epithelium. When the mesometric glands degenerate, they do not form syncytia in the beaver, but the glandular epithelium becomes necrotic. The syncytia in the trophospongia at the early placental stage arise from a special proliferation of uterine epithelium, which takes place by mitosis in the mesometric region at the preplacental stage, before the ectoplacental proliferation of the trophoblast has set in.

XVI. Bibliography.

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EXPLANATION OF PLATES 14 TO 21,

Illustrating Mr. Arthur Willey's paper on "The Blastocyst and Placenta of the Beaver."

[Some of the drawings were reversed under the Edinger apparatus, and others were not reversed. It has not been thought necessary to

mention this in each case. Figures executed by **Mr. John Prijs** are accredited to him under the explanations.]

PLATE 14.

Fig. 1.—Section across middle of gestation sac with blastocyst in sitû. The darker shading round the cavum uteri indicates maternal proliferation. The upper circle surrounds the crypt-like mouth of a gland near which there was a trophoblastic implantation. The lower circle surrounds the keel which projects into the very deep placental groove. (J. Prijs, del.)

Fig. 2.—Portion of pericarinal zone to show isolated intrusions of trophoblast. 1. Hypoblast. 2. Trophoblast. 3. Interstitial substance different from the coagulum within the omphalon. 4. Uterine epithelium. 5. Subepithelial capillary vessel full of red blood-corpuscles. The cytoplasm of the uterine epithelium stains darkly with orange G.; that of the trophoblast lightly.

Fig. 3.—Fragment showing phagocytic attack on the part of megalokaryocytes. 1. Uterine epithelium undergoing destruction. 2. Mucosa. 3. Capillary. 4. Megalokaryocytes. 5. Coagulum in cavum uteri. 6. Portion of gland. (J. Prijs, del.)

Fig. 4.—Portion of coronal region, showing complete substitution of trophoblast for uterine epithelium on each side of the mouth of a gland which retains its epithelium intact and contains groups of leucocytic granules in its cavity. The abruptness of the cellular changes is not exaggerated. Note the flat contact between trophoblast and denuded surface of mucosa. 1. Obplacental gland. 2. Obplacental trophoblast. 3. Flattened trophoblast bridging mouth of gland. 4. Two trophoblast cells charged with maternal erythrocytes.

Fig. 5.—Plan of regions of blastocyst and mucosa about the middle of the gestation sac. The hypoblast is indicated by a broken line (substage C). 1. Coronal region. 2. Pericoronal cavum. 3. Peri-omphaloid zone. 4. Pericarinal festoons. 5. Adcarinal omphalopleure. 6. Keel. 7. Bottom of placental cavum at the mesometric side of gestation sac. 8. Cavity of omphalon. 9. Peripheral limit of mesoblast or sinus terminalis. 10. Obplacental glands. 11. Uterine epithelium. 12. Megalokaryocytes (trophoblast).

Fig. 6 (substage A).—Keel in anterior region of blastocyst. 1. Peripheral trophoblast. 2. Hypoblastic groove. 3. Mesoblast. 4. Formative epiblast rounding edge of keel, showing scattered vesicular growing cells.

Fig. 7.—Same. Near front end of primitive streak. Formative epiblast and mesoblast tinted. 1. Hypoblast doubled by unsplit mesoblast. 2. Primitive streak.

Fig. 8.—Same. Wedge-shaped proliferation of formative epiblast in the primitive streak.

Fig. 9.—Same. A cleft appears in the centre of the primitive streak.

Fig. 10.—Same. Embryonic area 50 μ farther back. Cell outlines are visible in the formative epiblast, but not rendered in the drawing. Mitoses in the primitive streak. The asterisk (*) is placed at the limit of the formative epiblast on that side of the keel; there is a vesicular cell at that point.

Fig. 11.—Same. The peripheral limit of the carinal half of the formative epiblast is nearing the edge of the keel. An intermittent groove is seen upon the surface of the primitive streak.

Fig. 12.—Same. The peripheral limit of the carinal half of the formative epiblast has reached the edge of the keel. The mesoblast band of that side has shortened and become thicker. Another groove is seen at the primitive streak.

Fig. 13.—Same. Plan of cavum uteri with contained blastocyst. Drawn freehand as seen under microscope. The other sections of this series were outlined under Edinger's apparatus with slide reversed. The blastocyst is only attached at the pericoronal angles. The hypoblast is indicated by a broken line. (1) Coronal uterine epithelium. (2) Coronal cavum with coagulum. (3) Leucocyte in coronal cavum. (4) Coronal chromatophile trophoblast. (5) Omphalon. (6) Embryonic shield with primitive streak and mesoblast. (7) Placental groove.

Fig. 14.—Same. Massive tissue at posterior end of primitive streak. This is the commencement of the "haftstiel" or "exostyle" formation.

Fig. 15.—Same. The last of the mesoblast, a thin band on one side of the hypoblastic groove, behind the primitive streak.

PLATE 15.

Fig. 16.—Sub-stage B. Anterior region of blastocyst, lying free. (1) Omphalon. (2) Trophoblast. (3) Hypoblast.

Fig. 17.—Same. Farther back in the anterior region, the blastocyst still lying free. (1) Omphalon. (2) Peripheral trophoblast composed of megalokaryocytes. They are more numerous than in the figure. (3) Hypoblast passing over the cavity of the trophoblastic keel. (4) Keel composed of cubical trophoblast, its walls agglutinated distally. (5) Chromatophile megalokaryocytes. *x*. Peculiar plasmodial effect at the pericarinal region, overlapping the cubical trophoblast. Semi-diagrammatic.

Fig. 18.—Posterior half of embryonic region showing deep groove over the primitive streak. Formative epiblast tinted dark, mesoblast

lighter. The half of the formative epiblast which surrounds the keel is longer and thinner than the portion which lies above the groove in the figure, i.e. it is more stretched out, the proportion being 550 μ to 350 μ. The epithelial change at (*) consists in this, that the nuclei of the peripheral trophoblast lie at one level instead of at different levels, and they are usually stained paler than those of the formative epiblast. There is a potential linear cœlom indicated at the top of the massive carinal mesoblast on the left of the figure.

Fig. 19. Same. Carinal region in the posterior part of the blastocyst showing relations of trophoblast (black), mesoblast (pale), and hypoblast.

Fig. 20.—Substage C. The didermic keel in the posterior region of the blastocyst lying freely between the walls of the placental groove. Subepithelial maternal capillaries indicated in black. (J. Prijs, del.)

Fig. 21.—Same. The tridermic keel in the posterior region. (1) Posterior end of mesoblast. (2) Loose hypoblast.

Fig. 22.—Same. The keel with the posterior exocœlom. The right side of the figure is the embryonic side of the keel. The slide was reversed under Edinger's apparatus. (1) Solid proliferation marking the peripheral limit or sinus terminalis of the mesoblast. (2) Hypoblast (represented by broken line). (3) Posterior exocœlom.

Fig. 23.—Same. Portion of obplacental trophoblast in the coronal region with adjoining part of the cavum uteri and uterine epithelium. (1) Uterine epithelium (nuclei omitted). (2) Coagulum in cavum uteri. (3) Trophoblast. (4) Leucocytes in mucosa crossing uterine epithelium, lodged in uterine coagulum, and entering the trophoblast.

Fig. 24.—Same. Plan of cavum uteri and blastocyst at the level of fig. 23. A band of slime extends from the edge of the keel nearly to the bottom of the placental groove. (1) Coronal epithelium. (2) Coronal cavum. (3) Coronal trophoblast. (4) Omphalon. (5) Keel. (6) Slime.

Fig. 25.—Same. Part of obplacental region showing flattened megalokaryocytes with ingested leucocytic nuclei appearing as dark-stained granules, sometimes surrounded by a vacuole. (1) Uterine epithelium. (2) Coagulum in cavum uteri. (3) Chromatophile trophoblast.

PLATE 16.

Fig. 26.—Same. Section through keel in region of massive exostyle. The cubical epiblast changes its character abruptly, so that the exostyle proper is not covered by a cubical epithelium. (1) Sinus

terminalis on embryonic side. (2) Reduced exocœlom. (3) Hypoblastic groove. (4) Exostyle. (5) Uterine epithelium.

Fig. 27.—Same. Section through exostyle in sitû, showing detached shreds. The piece of uterine wall to the right of the figure is drawn nearer to the keel than it was in reality when seen under the "Zeichenapparat." The position of the nuclei opposite the lower shred seems to indicate traction or stress. (1) Gland opening into placental cavum. (2) Space at apex of keel. (3) Placental cavum. (4) Uterine epithelium on wall of placental cavum. (5) Section of maternal capillary.

Fig. 28.—Same. Section through middle of exostyle, which is here nearly solid throughout, with a linear interrupted cavity towards the apex. (1) Exocœlom. (2) Hypoblastic groove. (3) Cavity of exostyle shut off at this level from (2). (4) Uterine epithelium. (5) Slime at apex of exostyle.

Fig. 29.—Same. Anterior region of exostyle, showing central cavity continuous with the omphalon. (1) Omphalon. (2) Sinus terminalis. (3) Exocœlom on embryonic side of keel. (4) Cavity of exostyle. (5) Uterine epithelium. (6) Loose hypoblast.

Fig. 30.—Same. The keel in the region of the primitive streak and formative epiblast. The asterisks mark the limits of the formative epiblast or embryonic shield. (1) Exocœlom. (2) Primitive streak. (3) Hypoblastic cavity. (4) Sinus terminalis; unsplit mesoblast extends a short way beyond it. (5) Anti-embryonic trophoblast. (6) Anti-embryonic unsplit mesoblast. N. B.—On the embryonic side the sinus terminalis lies at a higher level, out of the range of the drawing.

Fig. 31.—Same. The keel in front of the primitive streak. (1) Peripheral trophoblast. (2) Parts of the cœlom. (3) Proximal groove on embryonic face of keel. (4) Hypoblastic cavity. (5) Anti-embryonic part of the marginal proliferation of mesoblast or sinus terminalis. (6) Epiblastic cavity of keel.

Fig. 32.—Same. The keel at the level of the anterior limit of mesoblast, in front of the cœlom. (1) Sinus terminalis. (2) Hypoblastic cavity. (3) Carinal trophoblast.

Fig. 33.—Same. Proximal groove of the epiblast in front of the mesoblast, showing proliferation with mitoses.

PLATE 17.

Fig. 34.—Same. Keel in front of the mesoblast.

Fig. 35.—Substage D. Section through keel in the post-stylar region cutting the exostyle tragentially. The cœlom appears in two discontinuous cavities, owing to the intrusion of the massive mesoblast.

(Freehand.) (1) Sinus terminalis. (2) Posterior exocœlom. (3) Exostylar mesoblast. (4) Unsplit mesoblast. (5) Hypoblastic groove.

Fig. 36.—Same. The keel in the region of the exostyle. Fifty-five sections of 10 μ intervene between this section and that drawn in fig. 35; in this interval the exostylar mesoblast has been increasing in amount. Note the abrupt passage of cubical trophoblast into the flattened epiblast of the exostyle. The exostyle does not exhibit such a luxuriant development in this series as in others. (1) Sinus terminalis. (2) Exocœlom. (3) Exostyle. (4) Hypoblastic groove. (Freehand.)

Fig. 37.—Same. Section through keel in region of primitive streak. There are twenty-six sections of 10 μ between this and fig. 36. (Freehand.) (1) Exocœlom. (2) Pericardial primordium bounded by cubical epithelium outside, columnar on the inner side. (3) Regular hypoblast co-extensive with the formative epiblast. (4) Primitive streak. (5) Distal limit of formative epiblast, with abrupt epithelial transition.

Fig. 38.—Same. Anterior region of embryonic shield. In the next section the two sections of the cœlom fuse over the carinal hypoblast, which then ends in a tangentially cut mass of cells. (Freehand.) (1) Amniotic folds. (2) Carinal embryonic hypoblast. (3) Pericardial primordium. (4) Omphaloidean hypoblast. (5) Exocœlom. There are thirty-nine sections of 10 μ between this and fig. 37. For position of embryonic shield with reference to the keel-formation, compare Pl. 14, figs. 6 and 7.

Fig. 39.—Same. Anterior end of pre-embryonic cœlom with epiblastic keel-extension. Freehand. (1) Exocœlom. (2) Broken line indicating omphaloidean hypoblast.

Fig. 40.—Substage E. Section through the keel at the level of the anterior region of the embryonic shield. (1) Sinus terminalis. (2) Linear occluded exocœlom. (3) Formative epiblast. (4) Pericardial primordium. (5) Cubical embryonic hypoblast.

Fig. 41.—Same. Keel at level of notochordal primordium (1). The hypoblastic epithelium changes its character on each side of the notochordal contact zone. The asterisk denotes the proximal limit of embryonic shield.

Fig. 42.—Same. Keel at the anterior part of the primitive streak (1). (2) Epiblastic keel.

Fig. 43.—Same. Keel about middle of primitive streak.

Fig. 44.—Same. Post-embryonic keel at the level of the anterior end of the exostyle. (1) Exostyle or "Haftstiel" tissue. For the relations of the surface membrane at such places of close union, compare Pl. 16, fig. 29.

Fig. 45.—Same. Mid-region of exostyle.

Fig. 46.—Same. Posterior end of exostyle. The whole extent of mesoblastic bands is shown. (1) Sinus terminalis. (2) Exocœlom. (3) Hypoblastic groove. (4) Massive mesoblast. (5) Exostyle. (6) Trophoblastic keel.

PLATE 18.

Fig. 47.—Same. Region of post-stylar cœlom. (1) Post-stylar exocœlom. (2) Hypoblastic groove.

Fig. 48.—Same. Posterior termination of mesoblast and exocœlom. The hypoblast has been reconstructed in the drawing. (1) Sinus terminalis. (2) Exocœlom. (3) Uterine epithelium.

Fig. 49.—Same. Didermic keel behind the mesoblast. The flattened epiblast cells graduate into flattened megalokaryocytes (not shown).

Fig. 50.—Same. Towards posterior pole of blastocyst. The hypoblast withdraws from the keel. (1) The last of the carinal hypoblast.

Fig. 51.—Substage F. Section through entire gestation sac with blastocyst in sitû. (J. Prijs, del. (1) Omphalon. (2) Omphaloid cavum; the keel is seen to the left of the index number. (3) Placental groove; to be compared with Pl. 14, fig. 1. (4) Placental trophospongia. (5) Periplacental wall; the fine tubes in the walls are partly glands, partly capillaries. (6) Obplacental wall. (7) Mesometrium.

Fig. 52.—Same. Appearance of the keel in the anterior polar region of the blastocyst. About 90 sections of 10 μ precede this in the series. (1) Commencement of mesoblast on embryonic side. (2) Hypoblastic groove. (3) Trophoblastic cavity. (4) Trophoblastic keel.

Fig. 53.—Same. Keel at level of anterior part of embryonic shield. In an earlier section the pericardium communicated at its upper end with the exocœlom. (1) Sinus terminalis; the exocœlom, real or virtual, extends up to the sinus terminalis on each side, but the solid mesoblast may go a short distance beyond it. (2) Amniotic fold. (3) Embryonic hypoblast. (4) Formative epiblast. (5) Pericardial primordium. (6) Distal exocœlom.

Fig. 54.—Same. The pericardial primordium is separating into two parts. (1) Proximal moiety of pericardium. (2) Distal moiety of same.

Fig. 55.—Same. The pericardium consists of right (proximal) and left (distal) halves. (1) Proximal half of pericardium. (2) Distal half of same. (3) Sinus terminalis.

PLATE 19.

Fig. 56.—Same. Posterior quarter of embryonic shield. (1) The two limbs of the pericardial primordium. (2) Notochordal plate. (3)

Medullary groove. (4) Sinus terminalis. (5) Unsegmented embryonic mesoblast.

Fig. 57.—Same. Near posterior end of embryonic shield. (1) Notochordal plate in front of the primitive streak. (2) Coagulum in hollow at foot of keel.

Fig. 58.—Same. Through the primitive streak. (1) Primitive streak. (2) Dense coagulum in hollow at foot of keel. (3) Hypoblastic groove. (4) Exocœlom.

Fig. 59.—Same. The keel with the massive exostyle. (1) Exocœlom on one side only; the sinus terminalis of this side lies beyond the range of the drawing. (2) Axial hypoblast. (3) Distal trophoblastic cavity. (4) Epiblastic folds. (5) Sinus terminalis. (6) Proliferating mesoblast of exostyle; this is typical "Haftstiel" tissue.

Fig. 60.—Same. Transition from exostylar to post-stylar region. (1) Exocœlom. (2) Hypoblastic groove. (3) Massive mesoblast. (4) Trophoblastic extension of keel.

Fig. 61.—Same. Anterior part of post-stylar region. (1) Sinus terminalis. (2) Hypoblastic groove with cubical epithelium. (3) Post-stylar cœlom. (4) Trophoblastic keel, somewhat folded.

Fig. 62.—Same. Middle of post-stylar region. (1) Post-stylar cœlom. (2) Very long and straight trophoblastic keel. (3) Mucus at edge of keel.

Fig. 63.—Same. Near the posterior end of the post-stylar region. (1) Sinus terminalis. (2) Post-stylar cœlom. (3) Trophoblastic keel with agglutinated walls.

Fig. 64.—Same. The two portions of the sinus terminalis nearly meeting below the hypoblast. (1) Sinus terminalis. (2) Straggling mesoblast cells.

Fig. 65.—Same. Posterior extremity of mesoblast; the sinus terminalis confluent below the hypoblast.

PLATE 20.

Fig. 66.—Same. Posterior didermic region of blastocyst. The cubical carinal hypoblast passes gradually into the flattened adcarinal hypoblast. (1) Adcarinal omphalopleure. (2) Carinal omphalopleure.

Fig. 67.—The lumen of a non-gravid segment of the uterus from a cornu which contained young embryos with established placenta. The pits are the openings of uterine glands. The dermatic cone on the mesometric side is free from glands. (J. Prijs, del.) This is the stage when the connective tissue is uniformly proliferated round the lumen, but this zone containing numerous subepithelial capillaries is not rendered in the drawing.

Fig. 68.—Part of section through the posterior end of a preplacental gestation sac to show the general aspect of the mesometric glands which have become atrophic. They reach the epithelial surface though devoid of a lumen. (J. Prijs, del.)

Fig. 69.—Part of section through a preplacental gestation sac showing epithelial proliferations at the sides and bottom of the placental groove. Between the lobular growths capillaries are lodged. Capillaries are also seen ascending towards the surface. (Freehand.)

Fig. 70.—Detail of epithelial proliferation at bottom of placental groove. A few mitoses are seen.

Fig. 71.—Epithelial mesometric wings (tinted) with dilated capillaries charged with red corpuscles. Substage F. (1) Bottom of placental groove. (2) Dermatic cone. (3) Dilated capillary. (4) Epithelial proliferation.

Fig. 72.—Ampulla of a periplacental gland failing to open into the placental groove. From substage B.

Fig. 73.—Gestation sac opened from the antimesometric side showing embryo in *situ*. (J. Prijs, del.)

Fig. 74.—The same viewed from the side to show how the adjacent segments of the uterus are inserted into the mesometric half of the gestation sac at this stage. (J. Prijs, del.)

Fig. 75.—Detail from the border-zone between trophospongia and placental labyrinth, showing chain of syncytial nuclei (black). (1) Trophoblast. (2) Maternal capillaries containing red corpuscles. (3) Fœtal capillaries containing nucleated red corpuscles and surrounded by allantoic tissue. To right of figure the latter appears as an island surrounded by trophoblast. (4) Symplasma with many syncytial nuclei and a few intrusive plasmodial nuclei (seen to right of figure). (5) Trophospongia. One maternal capillary is included, the other is being included within the trophoblastic labyrinth.

Fig. 76.—Semidiagrammatic section through blastocyst and placenta between one end and the centre of the latter. The hypoblast, indicated by broken line, becomes indistinct in the obplacental region. The height of the blastocyst, measured from the fœtal surface of the placenta to the obplacental wall, was 8 mm. (1) Omphalon filled with dense coagulum, 7 mm. wide. (2) Obplacental wall of blastocyst, applied to obplacental wall of uterus. (3) Umbilical membrane (*area vasculosa*). (4) *Sinus terminalis*. (5) Umbilico-placental membrane (diplotrophoblast). (6) Opening (aditus) of interutricular segment of uterus into gestation sac. (7) Section of embryo in its amnion in the posterior region. (8) Placenta showing diagrammatically radial allantoic villi (tinted) alternating with radial trophoblastic trabeculæ

excavated by sanguimaternal lacunæ. Height of placenta, 3·5 mm. (9) Areolated trophospongia with capillary network and subjacent capillary vessels in the dermatic tissue. (10) Primordial connecting membrane.

Fig. 77.—Section through placenta near one of its poles to show the polar vessel entering it excentrically. (J. Prijs, del.) (1) Polar vessel with proliferated endothelium. (2) Diplotrophoblast. (3) Areolated trophospongia. (4) Sinus terminalis. (5) Placental labyrinth. (6) Allantoic mesoblast at fœtal aspect of placenta.

PLATE 21.

Fig. 78.—Section through middle of placenta, showing the ecto-placental labyrinth resting upon the areolated trophospongia, which is supported by the dermatic cone containing branching and anastomosing vessels. (J. Prijs, del.)

Fig. 79.—Diagram of sagittal section through gestation sac. The amniotic cavity extends between the umbilical membrane and the placenta. (1) Opening of omphalomesenteric duct into the omphalon. (2) Sinus terminalis; the index numbers are in the exocœlom. (3) Amnion. (4) Allantoic canal opening into a flattened allantoic sac. (5) Umbilico-placental diplotrophoblast; the index numbers are at the openings of the gestation sac. (6) Mesometrium. (7) Omphalo-mesenteric vein. (8) Omphalomesenteric artery. (9) Omphalopleuric primordium of connecting membrane.

Fig. 80.—Fœtus of 38 mm. lying in its baggy amnion, exposed by cutting away the wall of the gestation sac and removing part of the umbilical membrane. (1) Cut surface of wall of gestation sac, 5·25 mm. thick behind. (2) Cephalad connecting membrane. (3) Rugose roof of gestation sac. (4) Caudad connecting membrane. (5) Linea alba, marking the insertion of the amniotic membrane upon the placenta. (6) Cut edge of umbilical membrane. Note: At this stage the fœtus already possesses two pairs of mammary pits.

Fig. 81.—Same. The fœtus has been removed, and the amnion, detached from its adhesion to the umbilical membrane, has been cut short and drawn taut, the object being to exhibit the polar umbilico-placental protuberances. (1) Cut edge of amnion. (2) Outer surface of umbilical membrane; the inner surface is shaded. (3) Cut edge of ditto. (4) Linea alba or insertion of amnion upon placenta. (5) Cephalad protuberance. (6) Caudad protuberance.

Fig. 82.—Later stage; fœtal length 73 mm. View of placenta and allantoic vessels from the side after removal of the fœtus. There are three layers of vessels at the surface of the placenta; superficial allan-

toic veins (black); allantoic arteries (clear) in the middle level; maternal vessels (tinted) at the lowest level. The main allantoic vascular trunks are two arteries (clear) and one vein (black). The amnion, together with the vascular area (umbilical membrane), has been cut open and reflected. The vessels of the area show through the amnion, but this is only indicated in the figure at the region of distribution of the omphalomesenteric vessels. (1) Cut edge of amnion together with umbilical membrane. (2) Inner surface of amnion. (3) Allantoic vesicle with vein in the thin wall. (4) Surface of placenta projected into the amnion. (5) Cut surface of umbilical cord. (6) Omphalomesenteric vein. (7) Omphalomesenteric artery.

On Acrossota liposclera, a New Genus and Species of Alcyonarian with Simple Tentacles.

By

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With Plate 22.

The Alcyonarian Colony to be described in the following pages was collected by Prof. A. Willey in the d'Entrecasteaux group of islands, British New Guinea, and sent to me, I am ashamed to say how many years ago, along with a collection of Actiniæ and Zoantheæ. Partly because of the difficulty and uncertainty of identifying spirit preserved actinians, partly because I had turned my attention to another group of animals, I have neglected this collection until, a short time ago, I began to prepare such specimens as were suitable for cutting into sections with a view of identifying and describing them. Among the Zoantheæ I found a colony of small translucent polyps apparently growing in a bunch attached to the fronds of a species of Halimeda. Some of these polyps were partly expanded and exhibited a few simple digitiform tentacles, and this character, together with their general appearance, suggested that they were non-incrusted Zoanthids. On closer examination I found that the polyps arose at intervals from a long, sparsely branched adherent stolon, and that the apparently single colony consisted of several such stolons so closely intertwined that the polyps arising from them were closely

bunched together, giving the deceptive appearance of a compact encrusting colony. And, as I shall describe in detail, I found that the tentacles, though simple finger-shaped structures, are always eight in number, and that the structure of the polyps, in almost all other respects, presents the well-known characteristic Alcyonarian features.

The entire absence of lateral pinnæ on the tentacles of the autozooids of an Alcyonarian has not, to my knowledge, been previously recorded, and as my colony presents some other interesting characters which differentiate it from any described species, I must make a new genus for its reception. It seems necessary also to make the genus the type of a new family, for the possession of simple digitiform tentacles is a unique feature among the Alcyonaria. But, as will appear from what follows, the general characters of the colony and polyps place the new family in the order Stolonifera, in close juxtaposition to the Cornularidæ and Clavulariidæ.

The specimen may be described as follows :

Order—Stolonifera, Hickson.

Family—Acrossotidæ, the tenacles simple, digitiform, without lateral pinnæ.

Genus—Acrossota[1] n. gen.

Zooids borne at intervals on a simple sparingly branched radiciform adherent stolon ; no spicules or calcareous skeleton of any kind ; tentacles digitiform, without lateral pinnæ.

Acrossota liposclera n. sp.

Stolon subcircular where free, flattened where adherent, its cavity formed by a single solenium, but traversed by mesoglœal trabeculæ ; the stolon branched and the branches

[1] *ἀ*, without ; *κροσσωτός*, fringed.

intertwined but not forming a network by anastomosis. Zooids subcylindrical, of various lengths, frequently giving off stolonar outgrowths from their proximal moieties, the distal moiety of each zooid invaginable within the proximal moiety. Tentacles digitiform, completely invaginable. Outer wall of stolon and zooids strengthened by a gelatinoid supporting tissue formed by the ectoderm, and in addition a thin parchment-like external cuticle, the latter forming the chief supporting tissue in the basal parts of the zooids, the gelatinoid tissue more largely developed in the subtentacular region and in the stolon; the walls everywhere relatively thin, and not differentiated to form calices into which the zooids can be withdrawn. Stolon and zooids translucent and colourless in spirit.

Locality, d'Entrecasteaux Group, British New Guinea.

The form and habit of the specimen collected by Prof. Willey are so irregular that they are difficult to define. The central part of the specimen consists of several relatively large zooids, measuring 5 mm. in length and 1·75–2 mm. in diameter and rather closely crowded together. From the bases of these zooids several stolons are given off which creep along the frond of a Halimeda and are closely attached to it at intervals. Where attached the stolons are flattened and tape-like, but in the greater part of their courses they are round and simply twined round their support. The stolons branch and the branches may subdivide several times in an irregular manner, but the branches do not anastomose with one another. The branches and their subdivisions are twined round one another and tangled together, and bear other zooids at irregular intervals. From place to place a branch of the main stolon or a stolonar outgrowth of one of the zooids projects for some distance from the support as a long, free, thin-walled tube, near the end of which a zooid is developed, and from this zooid other stolons are given off, which may in turn bear zooids of various sizes. These free tubular outgrowths are frequently constricted in places, and it is probable that they are eventually separated off from the

parental colony at the points of constriction, and so give rise to new colonies, for the specimen included several short lengths of stolon bearing two, three, four or more zooids, and quite independent of the main colony. But these and the stolons of the main colony were all tangled together and with the Halimeda, and it required some care to disentangle them. Acrossota, therefore, is remarkable, though not unique, among Alcyonaria for producing independent colonies by a form of gemmation. The stolons usually end in blunt, slightly swollen extremities, but sometimes are fixed by one extremity, which is then closely flattened against the surface of support (fig. 1). Evidently each stolon is at first a simple tubular outgrowth of the proximal part of the wall or of the base of a zooid; this outgrowth is lined by endoderm and forms what I have called a "solenium."[1] In Cornularia[2] the stolons are simple solenia, but in Clavularia, Sarcodictyon and other members of the Stolonifera the stolons are compound and contain several solenia. In Acrossota the stolons are not as simple as those of Cornularia, nor so complicated as those of Clavularia. The simple cavity is largely occluded, especially in the older parts of the stolons, by the formation of a spongy-looking tissue within the originally simple cavity. This tissue, as is shown in fig. 3, is formed by numerous fine branching trabeculæ, which pass from wall to wall and anastomose with one another. The trabeculæ are composed of a core of mesoglœa, covered by endoderm, and are more or less flattened in section. Some of the larger trabeculæ contain strings or islets of endoderm cells embedded in the mesoglœa, and the branching of the trabeculæ is apparently brought about by the formation of cavities in these intrusive endoderm cells, but there is no evidence of the formation of definite solenial outgrowths of the endoderm penetrating into the mesoglœa and forming compound stolons. The trabeculæ

[1] G. C. Bourne, "Anthozoa," in Lankester's 'Treatise of Zoology,' Part II, 1900, p. 16.

[2] G. von Koch, "Die Alcyonacea des Golfes von Neapel," 'Mitth. a. d. Zool. Stat. zu Neapel,' ix, 1891, p. 645.

become less numerous in the terminal and most recently formed portions of the stolons, and are absent or very few in number and imperfectly developed in the swollen extremities (fig. 3). They are also scantily developed in the long free outgrowths which give rise to separate colonies. On the other hand, in the flattened adherent portions of the stolons, the trabeculæ tend to fuse together to form laminæ, dividing the lumen of the stolon into several canals, running, on the whole, parallel to the long axis of the stolon.

It is assumed rather than demonstrated that the compound stolons of Clavulariidæ are formed by the union of the walls of a close-meshed reticulum of simple solenia such as is found in Cornularia. It is probable that many band-shaped or flat encrusting stolons are formed in this manner, and all encrusting stolons must be formed in part by the fusion of the walls of radial outgrowths containing solenia from the bases of the zooids, but it is equally probable that a large part of the inosculating channels seen in sections of such stolons have been formed as in Acrossota, by the ingrowth of trabeculæ from the walls of a primitively simple solenium.

The general structure of the zooids, with the exception of the tentacles, conforms to the Alcyonarian type.

The Actinopharynx (as van Beneden[1] has renamed the "Stomodæum" of the Anthozoa) is long, and its upper third is lined by a ciliated epithelium, of which the character is shown in fig. 5. The epithelial cells are elongated and attenuated, their nuclei stain deeply and appear closely crowded together in sections. The cilia take their origin from minute deeply staining granules, which give the free border of the epithelium a striated appearance. In this region there is a distinct sulcus or siphonoglyphe in which the cilia are specially long. But in the lower two thirds of the actinopharynx (fig. 6) the siphonoglyphe dies out; the epithelium is composed of less elongated prismatic ciliated cells, the nuclei are no longer crowded together and stand nearly on

[1] E. van Beneden, "Die Anthozoen d. Plankton Expedition," 'Ergebnisse d. Plankton Expedition des Humboldt-Stiftung,' ii, 1897.

the same level; the cilia are short and of uniform length. In contracted specimens this region of the actinopharynx is thrown into longitudinal folds and its lumen diminished, but the lumen is always considerably larger than in the upper third. At the lower border of the actinopharynx the prismatic ciliated epithelium passes rather abruptly into the endoderm, but is clearly continued down the edges of the two "dorsal" or asulcar mesenteries to form the grooved filament, which in section presents precisely the same characters as those figured by Hickson[1] for Alcyonium. In the grooved filaments the ciliated cells again change their character, becoming small and narrow, with small deeply staining nuclei. The remaining mesenteries have thickened edges covered by endoderm cells, but no distinct filament. As is invariably the case in Alcyonarian zooids, the two "dorsal" asulcar mesenteries bearing the grooved filaments extend much further down in the body of the zooid than the remaining six.

The section represented in fig. 4 shows that in other respects the mesenteries present the usual Alcyonarian features. The mesoglœal thickenings forming the muscle-banners are feebly developed, and only distinguishable in the region of the actinopharynx, but there they can be seen to be borne on the "ventral" or sulcar faces of all eight mesenteries. The peripheral parts of the mesenteries are very thin. Only one of the Zooids that I used for cutting sections was sexually mature. In it testes, in the form of small spherical follicles, were borne on short pedicles near the free edges of all but the dorsal asulcar mesenteries.

The tissues of the specimens I have examined were only moderately well preserved, and the cell elements so minute that I have not been able to work out histological details to my complete satisfaction. The structure of the body-wall in particular has proved very difficult to interpret, and the structure of the various regions into which the body of the zooid may be divided appears to differ in invaginated and

[1] S. J. Hickson, "The Anatomy of Alcyonium digitatum," 'Quart. Journ. Micr. Sci.,' 37, 1895, p. 343.

extended specimens, from which fact I infer that the tissues are very elastic and extensile, capable of being drawn out into an exceedingly thin layer or contracted into thicker sheets, according to the state of contraction of the animal.

From a study of longitudinal and transverse sections of both extended and retracted specimens it appears that the following regions may be recognised : (1) The tentacles and the oral disc. (2) The portion of the body of the zooid immediately below the tentacles. This portion is invaginated in retracted specimens, but in both extended and retracted examples the body-wall in this region is moderately thick, and the tissues, both ectodermic and endodermic, are fairly clearly differentiated. In extended specimens this region is approximately of the same length as the actinopharynx, but in retracted specimens the latter structure is pulled down into the lower part of the cœlenteron and lies below the tube formed by the invaginated distal portion of the body-wall of the Zooid. (3) The middle and basal region of the Zooid, in which the body-wall is extremely thin. (4) The stolons, in which the mesoglœa is thickened, the endoderm fairly conspicuous, but the ectoderm reduced or absorbed in the formation of a gelatinoid supporting tissue.

The first two regions are clearly the seat of the chief physiological activities. The cavities of the tentacles, the walls of the actinopharynx and the central moieties of the mesenteries where attached to the actinopharynx are clothed with a thick highly vacuolated endoderm containing a profusion of zooxanthellæ (fig. 4). The mesoglœa is a thin layer exhibiting a faint striation, but not including any cellular elements. The ectoderm of the tentacles is formed by a layer of small cells, cubical in the extended but more columnar in the invaginated tentacles. The ectodermal muscular fibres are but feebly developed in the tentacles, the circular layer of endodermic muscular fibres being rather better represented, but still feeble. Minute oval refracting bodies can be recognised in the ectoderm of the tentacles, and I interpret them as nematocysts, but I was unable to resolve their structure

with the highest powers of the microscope at my disposal. The ectodermic lining of the actinopharynx has been described already. The ectoderm of the body-wall below the tentacles is illustrated in fig. 7. The continuous layer of cubical cells of the tentacles and oral disc here gives place to what I can only describe as a reticulum of vacuolated protoplasm containing nuclei, but in which the limits of the cell bodies are with difficulty or not at all recognisable. The body-wall is raised into a number of thickened ridges, one of which is shown in fig. 7. In this figure the mesoglœa is seen as a thin but distinct structureless layer, produced on the inner side into small processes to which the muscular fibres of the vacuolated endodermic cells are attached. On the outer side of the mesoglœa is an irregular aggregation of what may be called ectoderm cells, though, as mentioned, the cell outlines are not distinguishable. Some of these abut on the mesoglœa, without, however, forming a continuous layer. Others, more peripherally situated, form a discontinuous lining to the thin darkly staining external cuticle. The intervening space is filled up by a homogeneous more or less vacuolated substance which seems to be formed by the dissolution and conversion into supporting tissue of the ectodermic cell-fusion. The homogeneous supporting substance stains more faintly than the mesoglœa, but is probably of the same or similar chemical composition, for in older and more differentiated parts of the body-wall the ectodermic cell-fusion becomes scantier, the homogeneous substance increases in amount, and eventually fuses with the mesoglœa, and can only be distinguished from it by its staining less deeply. Usually, however, tracts of ectoderm looking like cell ingrowths are included in the homogeneous supporting tissue.

Fig. 8 represents a portion of the extremely thin body-wall of the lower middle and basal part of the zooid. In comparison with fig. 7, it is seen that the endoderm is reduced to a very thin non-vacuolated flattened epithelium. The mesoglœa is a distinct, very thin structureless layer. In one part of the section it seems to consist of two layers, between

which are a few flattened nuclei with remains of cytoplasm surrounding them. The outer layer, however, is nothing more than the deeper part of the homogeneous supporting tissue formed by the ectodermic cell-fusion, and the nuclei between it and the mesoglœa are ectodermic nuclei. Externally may be seen a few nuclei of ectodermic cells which have obviously been used up in the formation of local thickenings of the supporting tissue, and externally is the thin but tough deeply staining cuticle.

In the stolons the endoderm again becomes thicker and vacuolated, but does not contain zooxanthellæ. The external cuticle remains, but the nuclei of the ectodermic cell-fusion have almost entirely disappeared, and the ectoderm appears to have been nearly wholly used up in the formation of supporting tissue, which is now so closely applied to and fused with the mesoglœa as to be scarcely distinguishable from the latter. One would say at first sight that the section shows only endoderm, mesoglœa and cuticle, the ectoderm having disappeared altogether. In this region strings of endoderm-cells make their way into the mass formed by the fusion of mesoglœa and supporting tissue, and these ingrowths extend into the trabeculæ, which are themselves formed as local out-growths of mesoglœa covered by endoderm. This ectodermic gelatinoid supporting tissue of Acrossota is comparable in essential respects to the supporting tissue of Stereosoma celebense as described and figured by Hickson.[1] But there is this difference: that in Stereosoma there is a distinct external layer of cubical ectoderm cells, but no cuticle, whereas in Acrossota the definite external ectodermic epithelium is absent, but there is a distinct and continuous cuticle.

There are no spicules in Acrossota. I have searched for them in teased-up specimens, in sections, and in the residue left after boiling in caustic potash. A felt-work of filamentous algæ enclosing numerous diatoms, sponge spicules, and here

[1] S. J. Hickson, "A Revision of the Alcyonaria Stolonifera," 'Trans. Zool. Soc. Lond.,' xiii, 1895.

and there an alcyonarian spicule, covers the older parts of the stolons and the basal moieties of the older zooids. But all these spicules are adventitious and lie external to, though often closely adherent to, the cuticle.

It may be concluded, from the foregoing description, that Acrossota is an Alcyonarian exhibiting primitive Cornularian characters in the mode of growth and habit of the colony, in the simplicity of its stolons (which, however, are not so simple as those of Cornularia), and in the absence of calcareous spicules, the last character being shared by such clearly primitive forms as Cornularia and Protocaulon molle, and also by Stereosoma celebense, Clavularia reptans, Clavularia celebensis, and the variety of Clavularia australiensis described as variety B by Hickson.[1] The absence of spicules may be regarded as evidence of primitive organisation, but not certain evidence, for the spicules present in variety A appear to have been lost in variety B of Clavularia australiensis. The absence or disappearance of spicules appears to be correlated in the Clavulariidæ with the formation of a supporting tissue derived from vacuolated branched ectoderm cells, and Acrossota shares this feature with Stereosoma and Clavularia australiensis, var. B. The feature peculiar to Acrossota is the absence of tentacular pinnæ, and it is a moot point whether this may be regarded as a primitive character. Stereosoma has but few pinnæ, and those spaced at considerable intervals along the tentacles. Further reduction of such pinnæ might lead to their ultimate disappearance, and the tentacles would then be simple and digitiform. On the other hand, it may be argued that simple tentacles must have preceded pinnate tentacles in phylogeny, and we have in Acrossota an otherwise primitive Alcyonarian with simple tentacles. The evidence seems to point to this being a primitive character, but in the absence of any criterion which shall enable us to distinguish between what is primitive and what is secondarily acquired by degeneration in such structures as these, one cannot be

[1] Ibid.

dogmatic. The one thing certain is that in the possession of simple tentacles Acrossota stands as an exception to a rule otherwise universal for Alcyonarians, and it was this that led me to make as exhaustive a study as I could of its structure.

EXPLANATION OF PLATE 22,

Illustrating Mr. G. C. Bourne's paper "On Acrossota liposclera."

[All the figures refer to Acrossota liposclera nov. gen. et sp.]

Fig. 1.—An independent stolon attached by one of its extremities to a fragment of Halimeda. The stolon bears four zooids, three of which are partially expanded and exhibit the simple tentacles.

Fig. 2.—Two zooids from a specimen stained in hæmalum and mounted in oil of cloves. One of the zooids is expanded, and exhibits the eight simple tentacles; the other is retracted, and shows the invaginated tentacles. *d.m.* Dorsal or asulcar mesenteries.

Fig. 3.—A retracted zooid and part of a stolon from a specimen stained in hæmalum. The body-wall of the zooid and part of the wall of the stolon nearest the observer have been cut away. *d.m.* Dorsal mesenteries. *st.* Stolon. *t.* The invaginated tentacles. *tr.* Trabeculæ in the cavity of the stolon.

Fig. 4.—A transverse section passing through the upper part of the actinopharynx of a retracted zooid, showing the actinopharynx with its sulcus, the eight mesenteries with the muscle banners, the eight invaginated tentacles and the thin external body-wall. The endoderm clothing the actinopharynx, the central moieties of the mesenteries and the tentacles is thick, and contains numerous zooxanthellæ. *aph.* Actinopharynx. *d.m.* "Dorsal" mesenteries. *v.m.* "Ventral" mesenteries. *t.t.* The invaginated tentacles.

Fig. 5.—A section through the upper third of the actinopharynx, highly magnified, showing the elongated ciliated cells, the sulcus or siphonoglyphe, and the vacuolated endoderm. *z.* Zooxanthellæ. Other letters as in fig. 4.

Fig. 6.—A section through the lower part of the actinopharynx, showing the disappearance of the sulcus and the changed character of the ciliated epithelium. Lettering as in the preceding figures.

Fig. 7.—A longitudinal section through part of the subtentacular region of the body-wall, magnified 750. *cu.* The external cuticle. *ec.* Ectodermic cell-fusion with nuclei. *en.* Endoderm. *h. s.* The homogeneous supporting substance formed by the ectoderm. *mg.* Mesoglœa.

Fig. 8.—Part of a longitudinal section through the body-wall of the middle of the zooid, magnified 750. Lettering as in fig. 7.

The Proboscidian System in Nemertines.

By

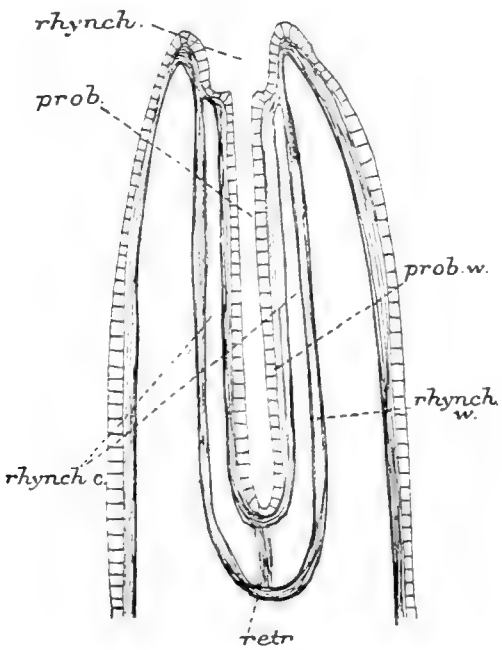
Dr. Gerarda Wÿnhoff,
Utrecht.

With 36 Text-figures.

The proboscis, together with its sheath and the rhynchodæum, are among the most characteristic features in the anatomy of Nemertines, so characteristic even that the system is not absent in any known species, neither of Anopla nor of Enopla. And wherever it is found, the whole system shows the same advanced development of construction; all three organs are completely developed, the proboscis being an introvertible tube, which is fastened to the body-wall at the anterior end of the rhynchocœl, and is connected posteriorly by a retractor muscle to the wall of the sheath. The rhynchodæum is a kind of atrium to the apparatus, through which the proboscis is everted (Text-fig. 1). Both proboscis sheath and proboscis itself possess a muscular wall, and the lumen of the sheath is lined by an endothelium. The cavity of both rhynchodæum and proboscis is lined by continuous epithelium that shows a differentiation of glandular elements in different parts of the system. The proboscis, therefore, consists of three layers—an epithelium, a muscular coat and an endothelial layer; the wall of its sheath of two—an endothelium and a muscular coat. The whole system is imbedded in the body-parenchyma. It has for a long time been assumed that the proboscis had developed out of a retractible part

of the head independently of corresponding structures in other invertebrates. Hubrecht (16) shared this opinion, and tried to explain, by the histological facts shown by the "Challenger" material, that the sheath had developed out of muscular elements in loco, and was an independent structure. However, Salensky (25) had published another hypothesis, comparing the whole proboscidean system of

TEXT-FIG. 1.

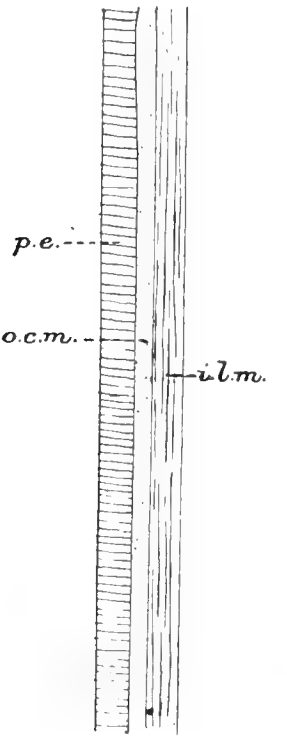


Schema of the proboscidian system in Nemerteans after Salensky (26). *rhynch.* Rhynchodæum or proboscis introvert cavity. *rhynch. c.* Rhyncho-cœlomic cavity. *prob.* Epithelium of the introverted proboscis. *prob. w.* Proboscis wall. *rhynch. w.* Wall of the proboscis cavity (rhynchocœl). *retr.* Retractor muscle of the proboscis. Compare with Text-fig. 36.

Nemerteans with the proboscis of the Rhabdocœlida proboscida. He founded his theory on certain embryological facts denied by Hubrecht (15), who tried to find support for his view in anatomical facts. Bürger (8) looked for a homologue of the whole system in the pharynx and pharyngeal sac of the Polyclads, but neither Bürger nor Hubrecht could gain the agreement of Salensky, who published

two articles in 1909 (**27**) and 1912 (**28**) in which he maintains his views of 1884 (**25**). As Salensky only gives embryological facts, we shall have to look for further judgment to the anatomical data. Wishing to draw my own conclusions as to the value of Salensky's theory, I have compared whatever was known about rhynchodæum, proboscis and proboscis sheath in Nemerteans.

TEXT-FIG. 2.

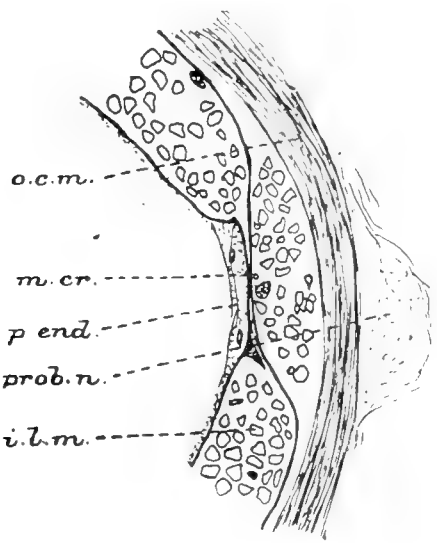


Schema of the proboscis of Palæonemerteans. *p. e.* Proboscis epithelium. *o. c. m.* Outer circular musculature. *i. l. m.* Inner longitudinal musculature.

The first fact that came to light was the great difference in structure between the proboscis of armed and unarmed Nemerteans. For the difference between the two does not consist only in the presence or absence of the so-called stylet; it is shown also in the structure of the muscular coat, and in the differentiation of the tube into different parts. It is even possible in Enopla that the armature has got lost, as seems to be the case with the genus Planktonemertes (**10A**). Mala-

cobdella also does not possess an armed proboscis. The Anopla show a great variety of structure for such a plainly built organ. The tube always consists of two parts, the anterior part sometimes possessing a differentiated region near the insertion to the body-wall. That a difference of epithelial glands exists between these parts is nearly certain; the exact nature of this difference, however, is not sufficiently known. Very often the beginning of the hinder part is

Text-fig. 3.



Section of the proboscis of Carinoma after Bergendal (6, text-fig. 55). *prob.n.* Proboscidian nerve. *p.end.* Proboscidian endothelium. *o.c.m.* Outer circular musculature. *i.l.m.* Inner longitudinal musculature. *m.cr.* Muscle crosses.

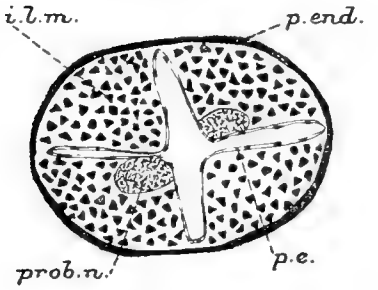
marked by a constriction. The principal seat of the variety of structure, however, is the muscular wall of the proboscis. In all Palæonemerteans the muscular sheath of the proboscis consists of two layers, a circular and a longitudinal, of which the circular layer is situated directly beneath the epithelium (Text-fig. 2).

Exactly this type of proboscis is found in several species of Tubulanus (9), in Procarinina atavia (5), in Carinina (9), Hubrechtia (9) and Hubrechtella (3). The other genera possess a more complicated proboscis, though, along the

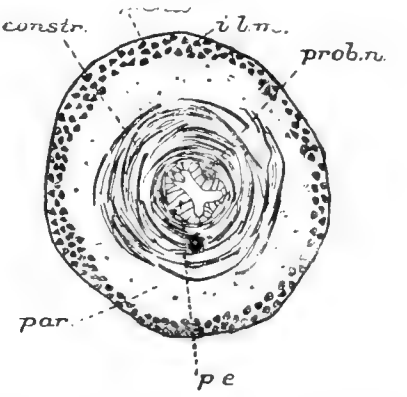
greater part of each, the two muscular layers described above are developed. Moreover, Bergendal described in Carinoma (6) a proboscis in which the circular muscle-fibres show a tendency to divert into the longitudinal layer and build muscular crosses (Text-fig. 3). A circular layer outside the longitudinal fibres is, however, not present. As the tendency to form crosses is also known in the circular fibres of the body-wall, their presence in the proboscis of Carinoma cannot alter our opinion that only two muscular coats are present.

The genera Cephalothrix (33), Cephalotrichella (33) and

TEXT-FIG. 4.



TEXT-FIG. 5.



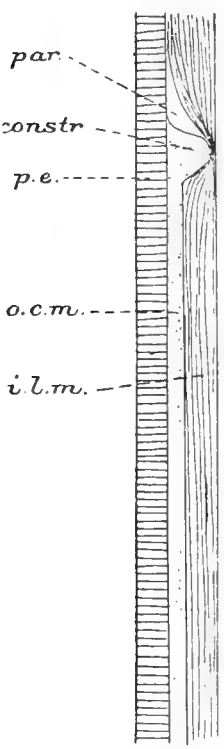
TEXT-FIG. 4.—Proboscidian section of Carinesta anglica, Wÿnhoff. *p. e.* Proboscis epithelium. *i. l. m.*, *prob. n.* and *p. end.* as in Text-fig. 3.

TEXT-FIG. 5.—Proboscidian section of Carinesta orientalis, Punnett. *p.e.*, *prob. n.*, *p. end.* and *i. l. m.* as before. *par.* Parenchyma. *constr.*, Constrictor muscle.

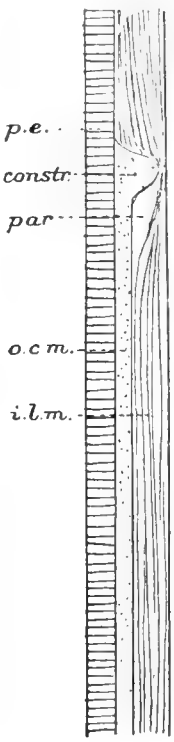
Procephalothrix (33), Callinera (2), Carinesta and Carinomella (12) differ more from the above-mentioned type. In all of them the circular muscle-fibres are wanting in the portion directly behind the insertion of the proboscis (Text-fig. 4). In the family Cephalotrichidæ, as well as in the three other genera, this part is followed by a conspicuous thickening of the circular layer, which acts as a constrictor (Text-fig. 5). At different places in this neighbourhood, in Cephalotrichidæ just before (Text-fig. 6), in Callinera behind (Text-fig. 7), in Carinomella (Text-fig. 8) before and behind the constrictor muscle, the parenchyma is particularly well developed, causing a regres-

sion of the muscular fibres. The longitudinal fibres, however, are present over the whole length of the proboscis (Text-figs. 6 and 7), though often much reduced, as at the places mentioned above. In Carinomella (Text-fig. 8), however, the whole circular musculature has degenerated in the anterior part of the proboscis, and instead of four bundles of muscle-

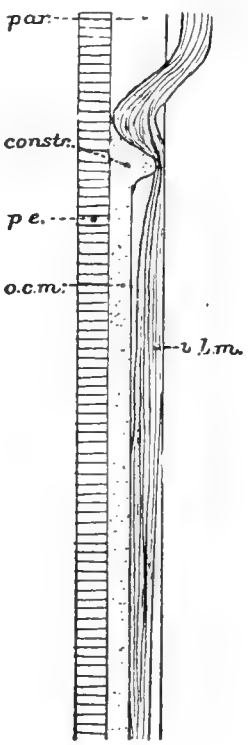
TEXT-FIG. 6.



TEXT-FIG. 7.



TEXT-FIG. 8.



TEXT-FIG. 6.—Schema of the proboscis of Cephalothrix. *pe.*, *par.*, *o. c. m.*, *i. l. m.*, and *constr.* as in Text-figs. 3 and 5.

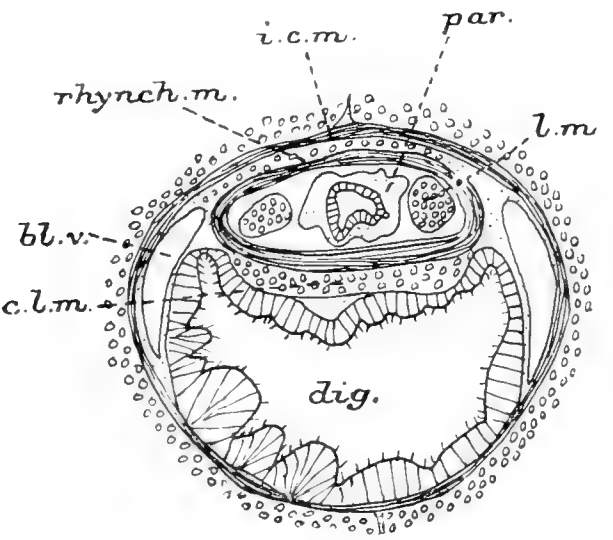
TEXT-FIG. 7.—Schema of the proboscis of Callinera. Letters as in Text-fig. 6.

TEXT-FIG. 8.—Schema of the proboscis of Carinomella. Letters as in Text-fig. 6.

fibres, as in Cephalotrichidæ and Callineridæ (Carinesta [Text-fig. 4] and Callinera), Coe (**12**) found only two longitudinal muscles in the cavity of the sheath, connecting the wall of the sheath with the anterior part of the proboscis (Text-fig. 9). The other parts of the proboscis, however, consist, in all these genera, of the same layers as in Tubulanus (Text-fig. 2); it

seems, therefore, not too presumptuous to say that in all Palæonemertines the muscular coat of the proboscis is composed of two layers, a circular and a longitudinal, the latter being situated beneath the endothelial surface. The deviations from this type, found in the families of Cephalotrichidæ, Callineridæ, and the genus Carinomella, have merely been brought about by the disappearance of the circular fibres in the region directly behind the insertion, accompanied in Carinomella by the egression of the longitudinal fibres.

Text-fig. 9.

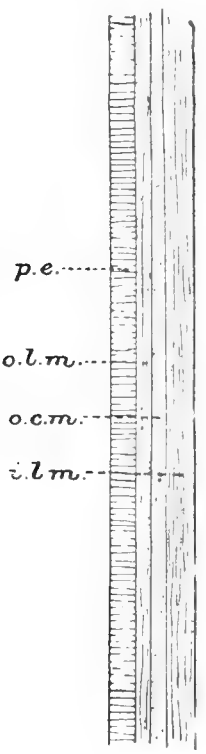


Section through Carinomella after Coe (12), fig. 54. *l. m.* Longitudinal muscle. *rhynch. m.* Rhynchodæal musculature. *c. l. m.*, Central long musculature. *i. c. m.* Inner circular musculature. *bl. v.* Blood-vessel. *dig.* Digestive tract. *par.* Parenchyma.

In Heteronemertea the proboscis shows a much greater variety of structure. A certain number of species possess a proboscis exactly like the Palæonemertea; the outer layer of the ejaculated duct consists of the glandular epithelium, next to it is the circular muscle-layer, limited at the other side by the longitudinal fibres; such are the proboscides in Micrella rufa, Punnett (22), Oxypolia beaumontiana Punnett (22), and in Euborlasia (9). The genera Lineus, Cerebratulus and Micrura, which are so closely connected, that they cannot even be distinguished from each other by anatomical features

alone, show a marked resemblance in their proboscides too. In all three genera species are known with two muscular layers, others possessing a third muscular coat (Tex-figs. 10 and 11). Representative of the Palæo-type are Cerebratulus urticans (J. Müll.) (**9**), and C. greenlandicus Punnett (**23**), Lineus scandinaviensis Punnett (**24**), and L.

Text-fig. 10.



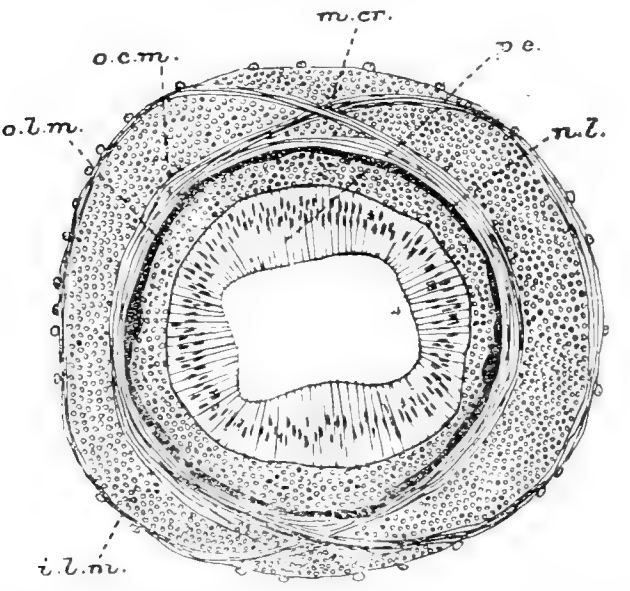
Schema of the proboscis of Heteronemerteans (Cerebratulus marginatus). *o. l. m.* Outer longitudinal musculature. *p. e.* Proboscis epithelium. *o. c. m.* Outer circular muscular layer. *i. l. m.* Inner longitudinal muscular layer.

bilineatus McIntosh (**9**), Micrura varicolor Punnett (**24**), M. bergenicola Punnett (**24**), and M. atra Punnett (**24**). Variations on this scheme are found in Heteronemerteans as in Palæonemertines. Miss Thompson (**30**) described the interesting genus Zygeupolia. The circular muscle-layer fails in the region behind the insertion, exactly as in Cephalotrichidæ and Callineridæ (Text-fig. 12). Other genera and species possess three muscular layers in the proboscis; to this

group belong the Cerebratulus-, Lineus-, and Micrura-species as Cerebratulus marginatus Ren. (9), and C. eisigi Bürger (9), Micrura fasciolata Ehrenberg (9), and Lineus versicolor Bürger (9). Two of these three layers consist of longitudinal fibres and they are separated by the circular muscle-fibres (Text-fig. 10).

The presence of this third layer in the proboscis of Hetero-

Text-fig. 11.



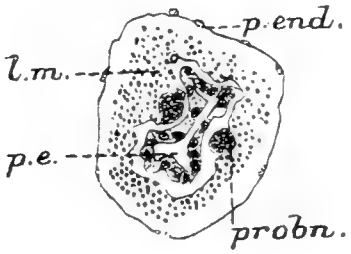
Section of the proboscis of the introverted Cerebratulus marginatus after Bürger (9, taf. 23, fig. 1). *m. cr.* Muscle crosses. *o. c. m.* Outer circular muscular layer. *o. l. m.* Outer longitudinal muscular layer. *i. l. m.* Inner ditto. *n. l.* Nerve layer. *p. l.* Epithelium (outer surface) of the proboscis.

nemerteans is rather striking. The acquisition of a longitudinal muscle-layer between the epidermis and the circular muscle-layer is characteristic of the body-wall in this group of Nemertines. The new layer in the proboscis wall, therefore, being also a longitudinal layer, can immediately be compared with the outer longitudinal layer of the body-wall, and, as its surroundings are exactly the same, it is laid down as part of the outer longitudinal muscle-layer. The circular coat of the proboscis in that case must be the outer circular muscle-layer of the body-wall, and the original longitudinal

layer is part of the inner longitudinal layer of the body-wall. The Palæotype of proboscides consists of the epithelium, the outer circular and inner longitudinal muscle-layers, that also form part of the body-wall. The newly obtained layer of the Heteronemerteans has not yet been acquired by all proboscides; those with the Palæotype have not got it at all, the genus Parapolia Coe (**11**), is in the act of acquiring it (Text-fig. 13). In the hinder part this proboscis shows the Palæotype, in the anterior part the Heterotype.

A peculiarity of the circular muscle-layer of Heteronemertine-proboscides is shown in fig. 11. Some circular

TEXT-FIG. 12.



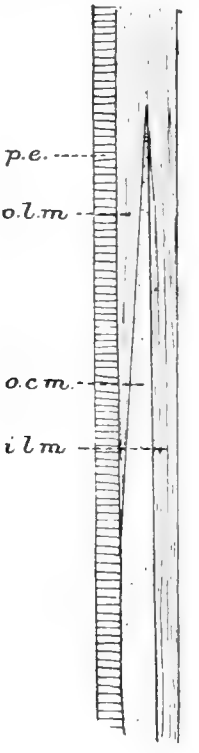
Section of the proboscis of Zygeupolia after C. B. Thompson (**30**).
p. end. Proboscis endothelium. *l. m.* Longitudinal muscles.
p. e. Proboscis epithelium. *probn.* Proboscis nerve.

fibres are seen to divert from their original stratum and to form crosses by entering the inner longitudinal coat, as I shall henceforth call it, conformable to the corresponding layer of the body-wall. These fibres of the muscular crosses continue even outside the longitudinal layer, constituting something like a very thin and very incomplete circular layer just beneath the endothelium. As Zygeupolia has muscular crosses in its proboscis, the thin circular coat beneath the endothelium, described by Miss Thompson (**30**), might be comparable with these circular fibres.

Still another type of proboscis is found in the genera Baseodiscus (**9**) and Joubinia (**9**), at least in two of the three species belonging to this genus. The muscular coat consists again of two layers, one with longitudinal and the other with

circular fibres. Instead of the circular fibres lying beneath the epithelium as in Palæonemertines, the longitudinal muscles are found there (Text-fig. 14). The explanation would have been difficult, for either the layers might have been the inner longitudinal coat with a better-developed new circular layer as described in Lineids and Zygeupolia, or the outer

TEXT-FIG. 13.



Schema of the proboscis of Parapolia. *p. e.* Proboscis epithelium (outer surface). *o. l. m.* Outer longitudinal muscular layer. *o. c. m.* Outer circular ditto. *i. l. m.* Inner longitudinal ditto.

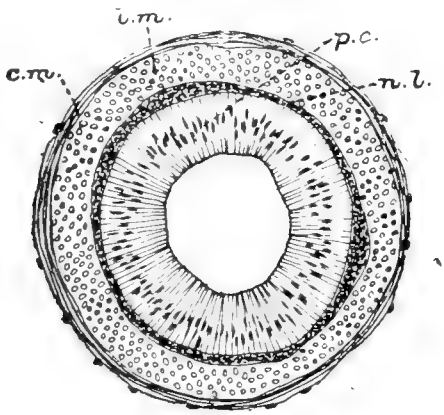
longitudinal and the outer circular muscle-layer, had not a form like Joubinia rubens Coe (**11**) or Oxypolella (**3**) existed. Both species show a different arrangement of the muscular fibres in the two parts of the proboscis (Text-fig. 15). The second part possesses the three muscle-layers characteristic of the body-wall of Heteronemertines. In the anterior part, however, the inner longitudinal fibres are absent, the wall showing the Baseodiscus type. The process of disappear-

ance of these longitudinal fibres, actually seen in Joubinia rubens, culminates in their total absence in J. longirostris and blanca and in Baseodiscus.[1] So if the Palæotype constitutes the first stage in its development, the three-layered muscular coat of Cerebratulus marginatus gives the second stage, Baseodiscus the third.

Putting all genera of Heteronemertines whose proboscis structure is known together in one list, we may find the links between the three above described stages.

Palæotype (Text-fig. 2): Micrella rufa Punnett, Oxy-

TEXT-FIG. 14.



Section through the introverted proboscis of Baseodiscus after Bürger (9, taf. 23, fig. 2). *p. e.* Proboscis epithelium. *n. l.* Nervous layer. *c. m.* Circular musculature. *l. m.* Longitudinal musculature.

polia beaumontiana Punnett, Euborlasia, Cerebratulus urticans (J. Muller) and other Cerebratulus species, Lineus

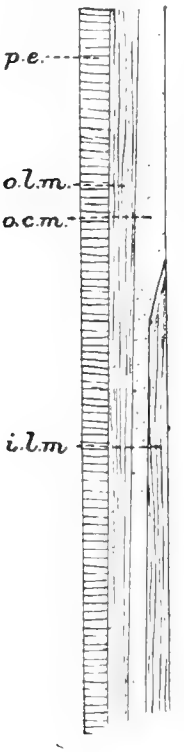
[1] Bergendal (4) described a Valencinia longirostris in which the so-called circular layer shows an interlacing of longitudinal and circular fibres something like the proboscis sheath of Drepanophoridæ. The principal difference between Bergendal and Bürger (9), who gave this peculiarity of the proboscis of Valencinia longirostris in his figure, loc. cit., taf. 23, fig. 9, comes to this: that the circular fibres, according to the first-named author, are arranged in two planes, neither coinciding with the horizontal section of the organ, and that Bürger had not observed this fact. This, however, seems not to be of any importance to our conclusions, since both writers agree as to the circular nature of this musculature. The interlacing of fibres is, as has often been stated

bilineatus McIntosh, and other Lineus species, Micrura atra Punnett and other species; variations on this type, Paralineus (29) and Zygeupolia (Text-fig. 12).

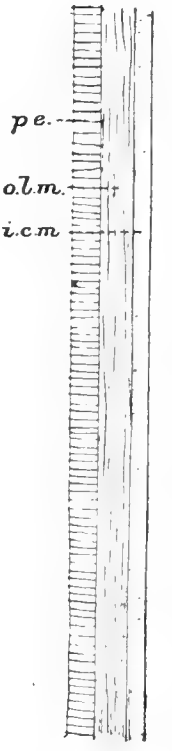
Transition to Heterotype: Parapolia (Text-fig. 13).

Heterotype (Text-fig. 16): Cerebratulus marginatus Renier, and other species, Lineus versicolor Bürger and

TEXT-FIG. 15.



TEXT-FIG. 16.



TEXT-FIG. 15.—Schema of the proboscis of Joubinia rubens. Letters as in Text-fig. 13.

TEXT-FIG. 16.—Schema of the proboscis of Joubinia longirostris and Basiodiscus. Letters as in Text-fig. 13.

related species, Micrura fasciolata Ehrenberg Langia; variety Joubinia longirostris Berg.

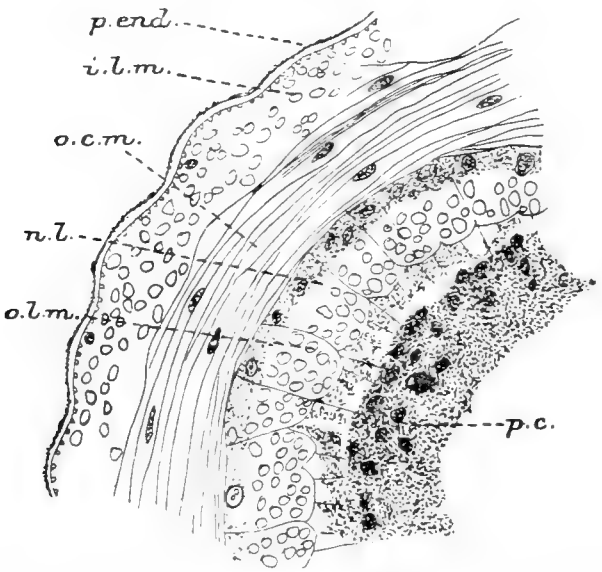
by other investigators, the result of the dissolving of muscular crosses, and seems therefore not to be of any consequence to our explanation. More compromising is the fact that in Baseodiscus the nervous layer, which I have not taken into consideration in this article, but which gives only support to my views, does not lie in the expected place between longitudinal and muscular fibres, but beneath the epithelium, as in Palæonemertines. Is this a reminiscence of a more primitive state?

Transition to Baseodiscus type: Oxypolella and Joubinia rubens (Text-fig. 15).

Baseodiscus type (Text-figs. 14 and 16): Baseodiscus species, Joubinia longirostris and blanca Bürger.

Two genera of Heteronemerteans, Poliopsis and Valencinura have not been mentioned yet. As regards Poliopsis this is due to our ignorance about its proboscis; in all the specimens which I know, it has been thrown off before capture. As

TEXT-FIG. 17.



Section through the introverted proboscis of Valencinura bahusiensis after Bergendal (4, taf. 1, fig. 15). *p. end.* Proboscis endothelium. *p. c.* Proboscis epithelium. *n. l.* Nerve layer. Other letters as in Text-fig. 13.

regards Valencinura its proboscis has been described accurately by Bergendal (4). The anterior two parts of this organ possess a muscular coat very much like the anterior part of Parapolia's proboscis. The third part, however, lacks all circular musculature. From the description given by Bergendal I cannot possibly make out whether the longitudinal fibres of this last part are the continuation of the outer or of the inner longitudinal layer. Two figures are given of the region in which the circular musculature is getting thinner before it disappears.

The order in which different coats are arranged is: Epi-

thelium, outer longitudinal layer, nervous layer, circular muscles, inner longitudinal muscle layer and endothelium with endothelial circular fibres (Text-fig. 17). The next section that is figured, however, gives: Epithelium, circular fibres, nervous layer, longitudinal muscle layer and endothelial circular fibres (Text-fig. 18). Probably, therefore, both outer circular and outer longitudinal muscle-fibres have disappeared, or, as in Parapolia, the outer longitudinal muscle-fibres have not yet been developed in the hinder part of the proboscis, and the outer circular muscular layer has disappeared, as it does in

TEXT-FIG. 18.

Section through the proboscis of Valencinura bahusiensis after Bergendal (4, taf. 1, fig. 14). *e.m.* Outer longitudinal muscles. *e. p.* Proboscis epithelium. Other letters as in Text-fig. 17.

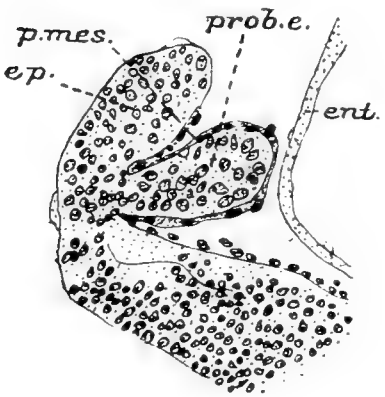
the other Nemertines. The place of Valencinura in our list should then be next to Parapolia; the nervous layer in changing places with the circular muscle-fibres, however, makes one cautious as to this supposition.

The facts taught by comparative anatomy, therefore, lead us to the conclusion that the proboscis of the Anopla is a structure of the body-wall in which both epithelium and muscular coat have taken part. We shall now have to consider whether all muscle-layers or only part of them helped to form the proboscis.

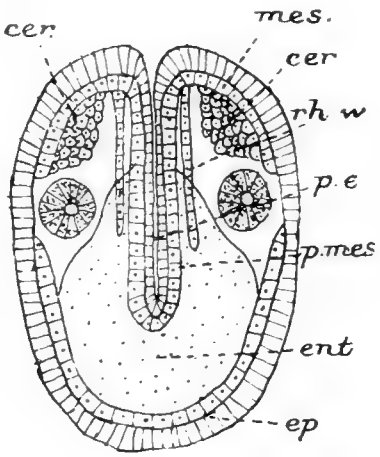
The body-wall of Anopla is, as Miss Thompson (30) tried to make certain, composed of four different muscle-layers. Of these all Palæonemerteans, with the exception of Carinoma,

only possess three, namely, the outer circular, the inner longitudinal and the inner circular muscle coat. In Heteronemerteans, as in Carinoma, an outer longitudinal layer of fibres has been developed. In the proboscides of Palæonemerteans we have found traces of no more than two of the three layers of the body-wall. Of the third or inner circular muscle-layer I have not been able to detect any traces in the proboscis of any Heteronemertean either, endothelial circular muscle-fibres being of an entirely different nature. Here embryology comes in to tell us where to look for the missing part.

TEXT-FIG. 19.



TEXT-FIG. 20.



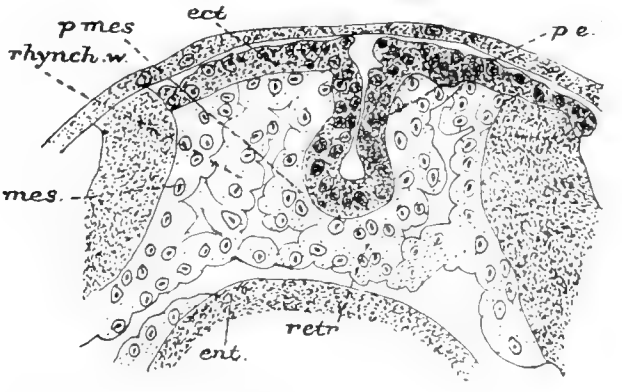
TEXT-FIG. 19.—Longitudinal section through an embryo of Prosorochmus after Salensky (28. taf. 3, fig. 27 A). *p.mes.* Proboscidian mesoblast. *ent.* Entoblast. *prob. e.* Proboscidian ectoblast. *ep.* Epiblast.

TEXT-FIG. 20.—Schema of an embryo of Lineus obscurus after Hubrecht (15, fig. 101). *rh. w.* Rhynchocœlomic wall. *mes.* Mesoderm. *cer.* Cerebral ganglia. *p. e.* Proboscis epithelium. Other letters as in Text-fig. 19.

A description of the development of the proboscis and its sheath is given by Salensky (25, 26, 27, 28), by Hubrecht (15), Bürger (7), Lebedinsky (18, 19, 20), and Arnold (1). Of these authors, Hubrecht, Bürger and Arnold studied the embryology of Lineus ruber, Salensky (26, 28) Pilidium gyrans (probably the larva of Cerebratulus marginatus) and Pilidium pyramidale. The Hoplonomertea subject to these investigations were Prosorochmus viviparus (Salensky,

25, 27), Prostoma vermiculum (Lebedinsky, 19, 20), and Drephanophorus spectabilis (Lebedinsky, 18, 20). All these investigations are essentially in agreement with each other as regards our subject, with the exception of Hubrecht's. This author, like the others, described the origin of the proboscis as an invagination of the ectoderm lying between, or, according to other authors, being part of, the two ectoblastic discs which give rise to the epidermis of the adult worm. All authors agree also as to the fact that this ectoblastic invagination, never becoming separated from the epiblast,

TEXT-FIG. 21.

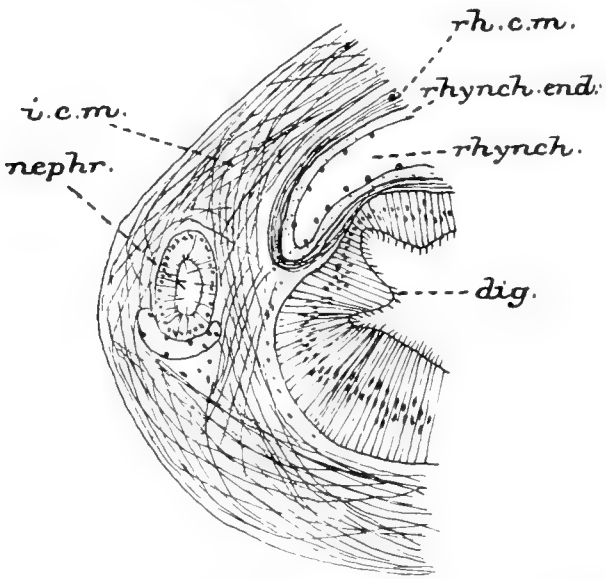


Section through the proboscidian system of an embryo of Lineus after Arnold (1, taf. i, fig. 18). *retr.* Retractor muscle. *ect.* Ectoblast. *ent.* Entoblast. Other letters as in Text-fig. 20.

gets its own mesoblastic investment, which is ectodermic in its origin (Text-fig. 19). At a later stage the ectodermic invagination of the proboscis has got two mesoblast layers separated from one another by a space. Hubrecht (15) considered that this second layer had grown out from the mesoblastic layer of the body-wall, cutting off part of the body-cavity, or, according to Hubrecht, part of the archocœl (Text-fig. 20). Later investigations, however, have shown that Hubrecht made a mistake. Bürger (7) studied Hubrecht's sections, and came to conclusions in perfect accordance with those of Salensky and all later investigators. The second mesoblastic layer takes its origin from the first one by delamination. Lebedinsky has described the presence of two "urmesoblasts" at the hinder

border of both layers. Two mesoblastic sacs take in this way the ectoblastic proboscis between them; when these sacs reach each other behind the proboscis, the retractor muscle is formed at the place where the walls fuse (Text-fig. 21). The inner layer gives rise to the muscular layers of the proboscis-wall; the outer to the wall of the sheath. Originally, however, these two structures belong together, their separation being brought about by a splitting of the mesoblast.

Text-fig. 22.



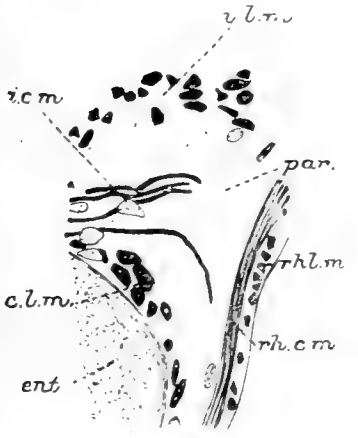
Section through Carinina grata after Bürger (9, pl. xi, fig. 3). *rhynch.* Rhynchocœlomic cavity. *rh.c.m.* Rhynchocœlomic circular musculature. *rhynch. end.* Endothelium of the rhynchocœl. *dig.* Epithelium of the digestive cavity. *i.c.m.* Inner circular muscle layer. *nephr.* Nephridium.

Here we have found the evidence we looked for on p. 4. The proboscis has revealed itself as a part of the body-wall, of which the innermost layer of the muscular coat, the inner circular musculature, is absent. Embryology teaches that the proboscis sheath belongs to the proboscis, as both take their origin from the same tissue. We must, therefore, look for the missing part in the rhynchocœlomic wall. If the splitting took place between longitudinal and inner circular muscle-layer, the sheath must consist of a circular layer alone; if the longitudinal layer was the seat of these changes,

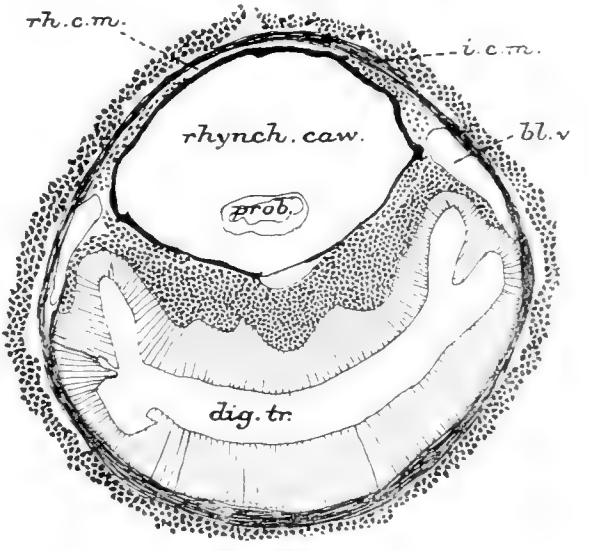
the wall of the rhynchocœlom should have an inner longitudinal and an outer circular muscular coat. The following facts anatomy discloses:

Procarinina possesses a proboscis-sheath, consisting of nothing but a very thin layer of circular fibres (**5**); in Carinina nothing but circular fibres are found (Text-fig. 22). Tubulanus linearis has a rhynchocœlomic wall, as Procarinina (**9**); in Tubulanus polymorphus inside this layer

TEXT-FIG. 23.



TEXT-FIG. 24.



TEXT-FIG. 23.—Part of a section through Procephalothrix linearis. *rh.c.m.* Rhynchocœlomic circular musculature. *rh.l.m.* Rhynchocœlomic longitudinal musculature. *c.l.m.* Central long. musculature. *i.l.m.* Inner, and *c.l.m.* central longitudinal muscles. *par.* Parenchyma. *ent.* Entoblast.

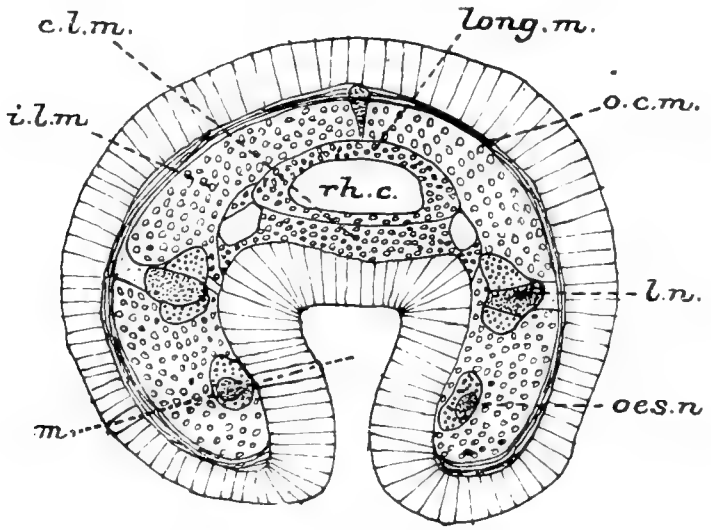
TEXT-FIG. 24.—Section through Carinesta anglica. *rhynch. caw.* Rhyncocœlic cavity. *rh.c.m.* Rhyncocœlomic musculature. *i.c.m.* Inner circular muscles. *prob.* Proboscis. *bl.v.* Blood-vessels. *dig. tr.* Digestive tract.

one row of minute longitudinal muscle-fibres can be found. Cephalotrichidæ (Text-fig. 23) agree with Tubulanus polymorphus, and so does Hubrechtia; but the Callineridæ (**2**) do not possess any longitudinal muscle-fibres inside the circular layer of the proboscis-sheath (Text-fig. 24).

The body parenchyma, in which the proboscidian system is imbedded, develops, as a rule, longitudinal fibres in the

Palæonemerteans. A localisation of these fibres is known to develop in several cases as the longitudinal septum between sheath and intestine, but also, as I have tried to demonstrate (**31**), as a longitudinal muscle-layer around intestine and proboscis-sheath, or as longitudinal muscle-bundles around the latter. In Procarinina these fibres have no connection at all with the sheath; neither have they in Carinina, in which a septum has developed. They seem to fail in Hubrechtia,

Text-fig. 25.



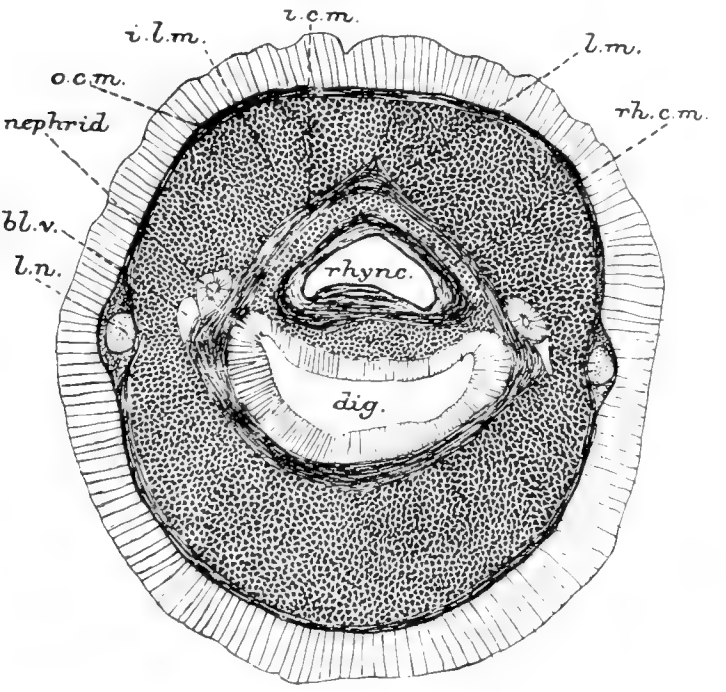
Section through Cephalotrichella after Bürger (**9**, taf. 11, fig. 14). *rh. c.* Rhynchocœlomic cavity. *long. m.* Outer rhynchocœlomic longitudinal musculature. *l. n.* Lateral nerves. *œs. n.* Œsophageal nerves. *m.* Mouth.

but are present in Carinina in the septum, as in some Cephalotrichidæ. In Callinera and some Tubulanidæ longitudinal fibres are present between the inner circular muscle coat of the body-wall and the rhynchocœlom, without building a distinct layer, as is the case in Cephalotrichella signata (Text-fig. 25), Carinomella (Text-fig. 9), and Carinesta (Text-fig. 26).

In all these cases they are regarded as being a new acquisition of the proboscis-sheath, developed out of the central longitudinal musculature. A strong support to this opinion is given in the behaviour of this musculature in the

family Cephalotrichidæ (**31**). Even different species of one genus as the two so nearly related species, Procephalothrix filiformis and P. aliena, show the development of a longitudinal musculature in the first and an ordinary septum in the other (**31**, **33**). The Tubulanidæ show the same differences in one genus.

TEXT-FIG. 26.



Section through Carinesta anglica. *rhync.* Rhynchodæum *rh. c. m.* Circular muscles of same. *l. m.* Longitudinal muscles of same. *dig.* Digestive canal. *nephrid.* Nephridium. *o. c. m.* Outer circular muscular layer of body. *i. c. m.* Inner ditto. *i. l. m.* Inner longitudinal ditto. *bl. v.* Blood-vessel. *l. n.* Lateral nerve.

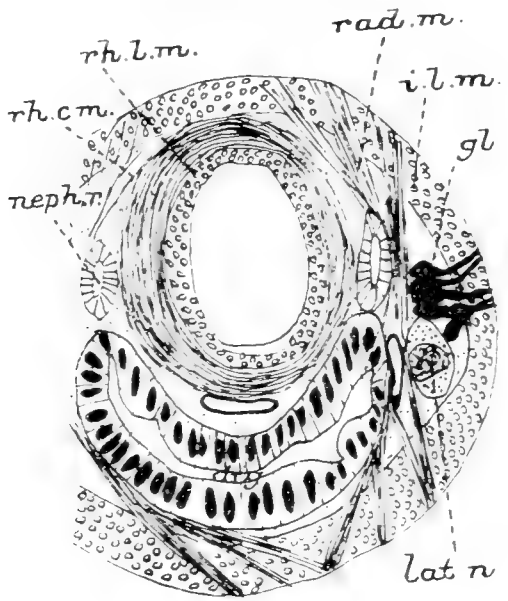
Therefore, if a longitudinal muscle-layer is developed outside the circular musculature of the proboscis-sheath, we must regard it as a secondary one, that has not been present ab initio, and not as an inherent part of this organ.

In Heteronemertini the two layers are noted. The circular muscle-layer is always present, an inner longitudinal coat has been recorded for Euborlasia, several Lineus and Cerebratulus

species, Joubinia longirostris, Micrura and Langia, Valencinura, Parapolia, Zygeupolia, etc.

A secondary longitudinal muscle-layer does not seem to be frequent; as far as I know it is not present in any Heteronemertean. Therefore we may conclude that as a rule the proboscidian sheath of the Nemertea anopla consists of two layers, an inner longitudinal layer, which may be absent

Text-fig. 27.



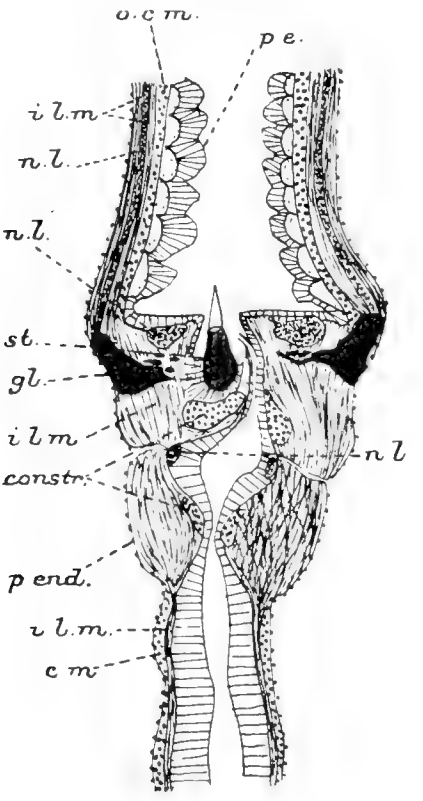
Section through Emplectonema gracile after Bürger (9, pl. xv, fig. 27). *rad. m.* Dorso-ventral musculature. *gl.* Glands. Other letters as in other figures.

(as in some Palæonemerteans), and has sometimes the thickness of one layer of fibres, and an outer circular muscle-coat.

If ever theory were in accordance with the facts it is in this case. We were led to suppose that the proboscis was an inverted part of the body-wall by anatomical facts. One layer, however, failed, and embryology taught us that we might find it in the proboscis sheath, the two structures belonging together and being one in ontogeny. The missing part is, as a matter of fact, found in the proboscis sheath, and this consists of the two layers we knew a priori it must consist of, if the supposition were right. We conclude,

therefore, that in Nemertea anopla the proboscis and its sheath have phylogenetically the same origin, both being part of an inverted portion of the body-wall; that the two structures became separated by a rent in the muscular coat, which took place in the inner longitudinal or between the two inner muscle-layers. The proboscis has, at least, three layers,

TEXT-FIG. 28.



Longitudinal section through the proboscis of Prosorochmus after Bürger (9, pl. xxiii, fig. 14). *st.* Stylet. *gl.* Glands. *constr.* Constrictor. *c. m.* Circular muscle layer. Other letters as in previous figures.

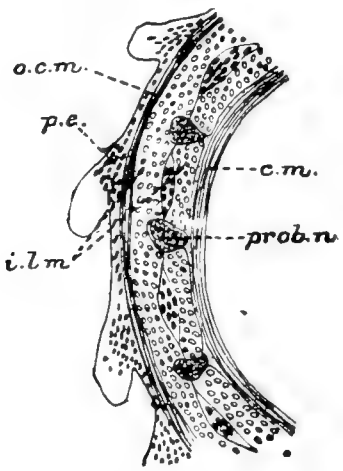
if reduction does not take place: the epithelium, the outer circular and the inner longitudinal muscle-layer, an outer longitudinal layer not necessarily developing, if it is present in the body-wall. The proboscis sheath has two or one muscular coat, an inner longitudinal layer, if present, part of the inner layer of the body-wall, and an outer circular muscle layer, the inner circular muscle-coat of the body-wall.

I have purposely not confused these facts and conclusions in Anopla with those in armed Nemerteans, for the development of the armature in Hoplonemertea has so specialised this organ, that we must expect great deviations from the original conditions.

The genus Malacobdella also lives under such unnatural circumstances for a Nemertine that all kinds of anomalies may be expected.

Of the three original muscle-layers of the body-wall, the

TEXT-FIG. 29.



Section through the proboscis of Amphiporus pulcher after Bürger (**9**, taf. 23, fig. 3). Letters as in other figures.

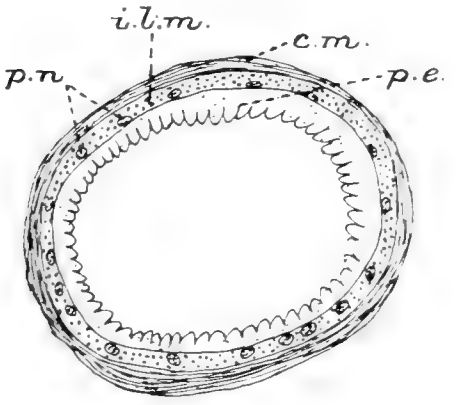
inner circular fibres, as such, are never present; we have to look for them, as has been demonstrated by Miss Thompson (**30**), in the dorso-ventral musculature of the body (Text-fig. 27). The outer longitudinal muscle-layer of Heteronemertini has nowhere developed, and we can recognise the other longitudinal layer by the nervous system being imbedded in it. Therefore we shall have to look for three muscular layers in the proboscidian system: a circular and a longitudinal layer, the latter possibly giving notice of its nature by the seat of the nerves, both in the proboscis, and probably remnants of the same longitudinal layer, and a circular layer in the wall of the sheath.

Now let us examine the facts. Malacobdella has a proboscis

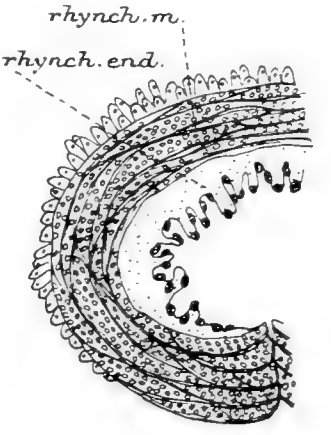
as described above, an epithelium with a circular muscle-layer underneath, and the longitudinal layer divided into two parts by a nervous layer. The endothelium, which lines both proboscis and sheath, is in the latter structure followed by a circular muscle-layer, as the presence of longitudinal muscle-fibres between them seems to be doubtful (9). These facts are in perfect accordance with those in Anopla.

In Hoplonemertea some diversity exists as to the structure of the sheath. In all species, however, the wall of the proboscis has developed in a very similar way. As a rule we can

TEXT-FIG. 30.



TEXT-FIG. 31.



TEXT-FIG. 30.—Section through the hinder part of the proboscis of Amphiporus marmoratus after Bürger (9, pl. xxiii, fig. 18). *p. n.* Proboscis nerves. Other letters as in previous figures.

TEXT-FIG. 31.—Section through the rhynchocœlomic wall of Drepanophorus after Bürger (9, taf. 23, fig. 37). Letters as before.

divide the proboscis into three parts (Text-fig. 28), the middle part being the seat of the characteristic structures. Of these parts the first region has a wall consisting of three muscle-layers, two of which are circular coats separated by the only longitudinal coat in which the nervous layer is present (Text-fig. 29). The longitudinal coat, therefore, must be regarded as the inner longitudinal layer of the body-wall, and naturally one looks upon the circular layer between epithelium and longitudinal fibres as the outer circular muscle-coat. The other circular layer is present throughout the whole length

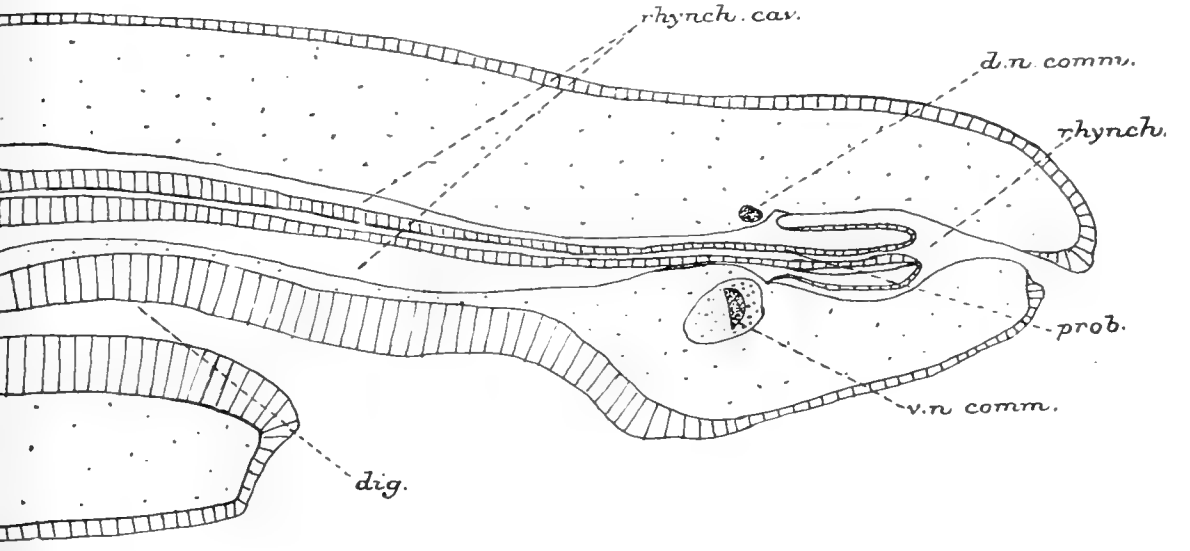
of the proboscis, as is the longitudinal layer. The outer circular muscle-layer, however, has absolutely disappeared in the hind part of the proboscis (Text-fig. 30), and is found in the middle part in the shape of two distinct sphincters (Text-fig. 28). One might feel inclined to regard the second circular layer as the inner circular coat of the body. This, however, is not in accordance with the embryological facts, that taught us to regard the sheath as part of the body-wall. Moreover, this sheath has a musculature exactly like that of Anopla. In all Hoplonemertea two muscular layers are present—an inner longitudinal layer often feebly developed, and an outer circular layer (Text-fig. 29). There is no reason not to regard them as identical with the same layers in Anopla, and therefore the inner circular muscle-coat of the body-wall is represented by this circular layer of the sheath, the longitudinal fibres being part of the inner longitudinal musculature. The family Drepanohporidæ, however, has a differently constructed sheath, in which, as in the hinder part of the same structure in Carinoma, longitudinal and circular fibres have become interlaced (Text-fig. 31).

We have already discussed a similar process in connection with the proboscis of Joubinia longirostris. As two kinds of fibres are present, and in all related genera these fibres are situated in the way stated above, we might regard their arrangement in Drepanophorus as a complication of that stage, which cannot disturb our view of the facts.

So homologues of all layers of the body-wall are found in Hoplonemertea in exactly the same place as in Bdellonemertea and in Anopla; the second circular layer of the proboscis, however, cannot be homologised with any part of it, neither do we know any such layer in Anopla. However, two cases are known, in which a tendency seems to exist to form a new circular layer underneath the endothelium; Bergendal (**6**) described the presence of circular fibres at that position, caused by the existence of faint muscle crosses (Text-fig. 3). C. B. Thompson (**30**) showed the presence of a layer of endothelial muscle-fibres. Though both are probably of a

totally different nature, we may look for an analogy between the development of this second circular muscle-layer in the proboscis of Hoplonemertea and the above described structures. Moreover, the Hoplonemertea have such a highly developed proboscis with a stylet, reserve stylets, the presence of the whole middle part (Text-fig. 28) with its glands and ejaculatory duct, etc., that the obtaining of a new muscle-layer seems nothing compared with the development of the

TEXT-FIG. 32.



Schema of a longitudinal section through Cerebratulus marginatus after Bürger (**9**, pl. xxi, fig. 1). *d. n. comm.* Dorsal nerve commissure. *v. n. comm.* Ventral nerve commissure. *rhynch.* Rhynchodæmu. *dig.* Digestive tract. *prob.* Proboscis. *rhynch. cav.* Cavity of the rhynchocœl.

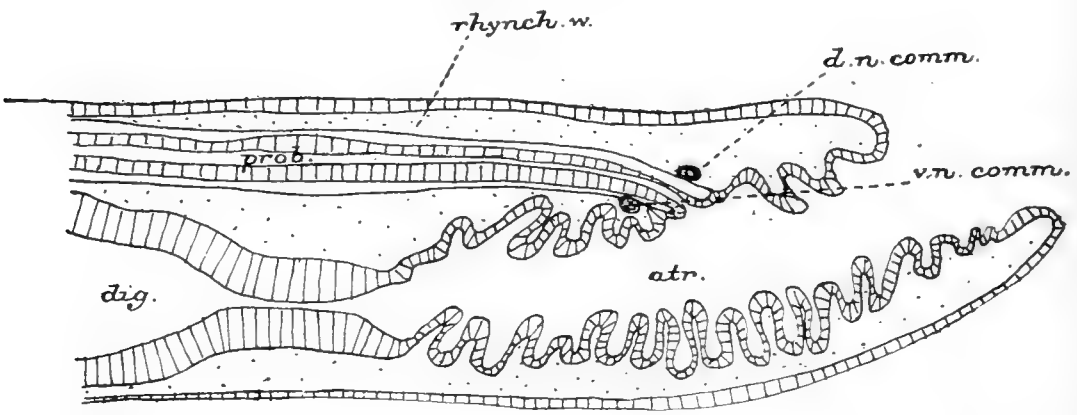
armature. And if we regard this layer as an acquisition of the proboscis itself, the facts found in Enopla are in perfect accordance with those in Anopla.

After all, it seems to me that the structure of the proboscis in all Nemerteans together with the structure of the rhynchocœlom can only lead to this conclusion, that they both took their origin from the body-wall, a separation being brought about in the musculature, which, as in embryology, caused the development of a proboscis and of a sheath.

Proboscides have been described in many invertebrates;

they are known in Cestodes, in Turbellaria, in Hirudinea, in Acanthocephala, in Sipunculids and Priapulids, in Echiurids and in Kinorhyncha, and in Nemertines. There can be no doubt that these proboscides are not homologous with one another. In Kinorhyncha, Priapulids and Sipunculids the so-called proboscis consists of the anterior part of the body, bearing the mouth at its top. The body-cavity extends into this part, and muscles extend from this region of the body-wall to the wall of the trunk, causing the invagination of the anterior part as a prolongation of the pharynx.

TEXT-FIG. 33.



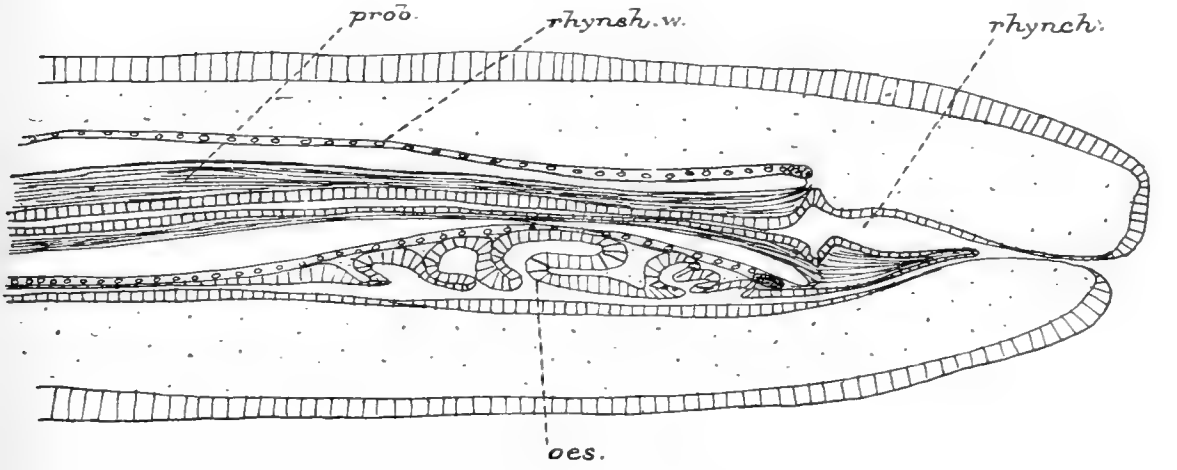
Schema of a longitudinal section through Malacobdella grossa after Bürger (**9**, pl. xviii, fig. 2). *atr.* Atrium. *rhynch. w.* Rhynchocœlomic wall. *d. n. comm.* Dorsal nerve commissure. *v. n. comm.* Ventral ditto. *dig.* Digestive tract.

In Hirudineæ the so-called proboscis is quite another structure. The wall of the pharynx of Rhynchobdellida shows a protrusible part, which bears the opening of the intestine at its extremity when erected, in the manner of the pharynx of Turbellaria. Now, Bürger (**8**) suggested that the pharynx of Turbellaria is homologous with the proboscidean system of Nemertea. Both structures, therefore, must be considered more closely in this connection.

Bürger was brought to this conclusion by certain very characteristic features in Enopla. For, though in all Anopla the proboscis is extruded through a separate proboscis-pore situated in front of the brain (Text-fig. 32), and the mouth is

always found behind the cephalic ganglia, digestive and proboscidean systems having no connection whatever with each other, they are closely connected in all Enopla (Text-figs. 33 and 34), with the exception of Drepanophoridæ. The genus Malacobdella, in many features so widely different from all Hoplonomertea, shares this character, though the way in which digestive tract and proboscidian system are connected is quite exceptional in Malacobdella (Text-fig. 33). The mouth is situated at the anterior extremity of the head, being

TEXT-FIG. 34.



Schema of a longitudinal section through Nemertopsis peronea after Bürger (**9**, pl. xv, fig. 1). *rhynch.* Rhynchodæum. *rhynch. w.* Rhynchocœlomic wall. *prob.* Proboscis muscles. *oes.* œsophagus.

wide and slit-like as in many Heteronemerteans, and giving entrance to the large œsophagus which lies underneath the cerebral ganglia, and into which the intestine proper opens. The rhynchodæum, which, in fact, is so small that as such it does not seem to exist, opens into the dorsal wall of the œsophagus, the anterior part of the latter being called atrium because of this fact.

In Hoplonomertea exactly the reverse of this condition is found (Text-fig. 34); here the proboscis-pore opens to the exterior, giving entrance to a well-developed rhynchodæum, in the hinder part of which the proboscis is inserted. Through

an opening in the ventral wall of the rhynchodæum the œsophagus gets an open connection with the rhynchodæum. Bürger took the rhynchodæum to be the homologue of the atrium of Malacobdella, and declared the proboscidian system to be a kind of appendix of the digestive organs. As both œsophagus and rhynchodæum originate by invagination of the ectoblast, lined by the mesoblastic layer which gives rise to the muscular coat of these organs, Bürger's hypothesis seems to be not impossible.

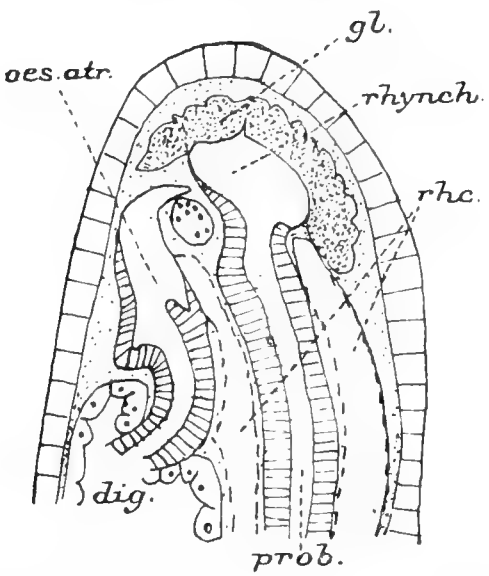
Bürger (**8**), who stands for the Turbellarian relationships of Nemertea, compared the intestine of both classes, and found a pharynx in the first and a proboscidian system in the other class without any homologues. He then suggested that they were themselves homologous, both being derived from the epiblast and the mesoblast underneath. The pharyngeal sac of Turbellaria should be the homologue of the atrium s. rhynchodæum in Nemertea. The great difference between the two organs, however, consists in their different relation to the œsophagus, which passes right through the pharynx, and has no direct connection with the proboscis; for Bürger suggested, as we have done, that a splitting must have taken place in the muscular coat of the pharynx, giving rise to a rhynchocœlom. This seems altogether very plausible, but how is the emancipation of the pharynx from the digestive tract to be explained? Bürger says: "Denken wir den Pharynx nicht in den Vorderdarm gestülpt, sondern über oder vor der Anlage des Vorderdarms in das Parenchym gewachsen und dann einen Spalt im Mesoderm des so verschobenen Pharynx entstanden, dieses in zwei Blätter teilend, so bekommen wir den Pharynx in einer vor oder über dem Vorderdarm befindlichen Höhle mit mesodermaler Wandung zu liegen, welche dem Rhynchocölom homolog sein würde, der Pharynx selber aber verhielte sich vollstandig wie der Nemertinenrüssel." The way in which the œsophagus must attain a direct communication with the pharyngeal sac is rather absurd: "Es ergiebt sich ohne Weiteres, dass wir in dem Pharynx ein Zuviel haben, denn nur seine Tasche, die,

nachdem wir den Pharynx exstirpiert haben, direct mit dem Darm communiciert, entspricht dem Nemertinen vorderdarm." Even if Bürger did not suppose this mode of arriving at the described situation to have really taken place, as I am sure he never did, I cannot see in what other way the pharynx could be emancipated from the digestive tract. It is quite impossible to picture the way in which these changes had to take place without any such lesion, and on this ground alone the theory of Bürger seems to lose vitality. But there is more. Bürger gives three reasons to support his views. The first one we have already given above. The second support is found in embryology : "Der Nemertinen-rüssel entsteht stets aus einer Ectodermeinstülpung, die mit einem diese umgebenden Mesodermwulste verschmilzt. Die Anlage des Rüssels erfolgt bei den Metanemertinen am selben Orte wie die des Vorderdarms und mit ihr gemeinschaftlich." The similarity of histological structure of the two parts is the third one. As to the latter, the histological resemblance of pharynx and proboscis consists only in the presence of an epithelium and the muscular layers of the body-wall, and therefore the same statement as before, that both are structures of the body-wall, must be repeated. Real histological resemblance there cannot possibly be, as the epithelium of the proboscis in Nemertines shows a great variety of elements in different parts and different species. That the muscular tissue of Nemertines and Turbellaria agree in structure is too well known to have any proving importance in this case, for the proboscis musculature is built up in exactly the same way as the muscles of the body-wall. Bürger's third reason, therefore, is not at all convincing. The embryological facts, which follow in the second place, have since been contradicted by Lebedinsky and Salensky. In the first place, Bürger compares the invagination of the proboscis-ectoderm with the extirpation of the pharynx-ectoderm; then the pharyngeal sac is the primarily planned organ, the pharynx developing afterwards at the bottom. In Nemertines the proboscis is the organ that appears first,

a later invagination of ectoderm giving rise to the rhynchodæum, the homologue of the pharyngeal sac.

But even if one takes into consideration the plain embryological facts in Nemertines, it seems difficult to come to the same conclusions as Bürger. For in what way do Nemerteans get a mouth? In Anopla the blastopore sometimes, after becoming closed, is transferred to the inside of the animal by the invagination of the œsophagus. In Enopla the facts are

Text-fig. 35.



Longitudinal section through Prosorochmus after Salensky (**27**, fig. 8). *œs. atr.* Œsophageal atrium. *rhynch.* Rhynchodæum. *rh. c.* Rhynchocœlomic cavity. *gl.* Glands. *dig.* Digestive canal. *prob.* Cavity of inverted proboscis. *rhc.* Rhyncocœl.

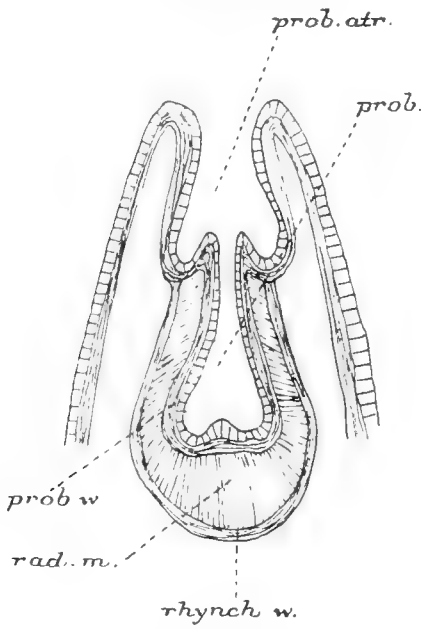
less simple. Drepanophorus, the genus in which œsophagus and rhynchodæum open separately, shows no connection at all between the two systems, not even in embryology; for here the blastopore is closed, the narrow entodermic part giving rise to the blind gut by being removed forward. The primary ectodermic œsophagus invaginates near the proboscidian system, but perfectly separate. The place where œsophagus and entoderm communicate in Drepanophorus has, therefore, nothing to do with the same spot in Hetero-

nemerteans. In all other Hoplonemertea the primary œsophagus originates in exactly the same way ; the mouth closes afterwards, and the primary œsophagus gets a new opening to the exterior through the rhynchodæum. Lebedinsky described in Tetrastemma vermiculus how the rhynchodæal epithelium grows towards the œsophagus, a bifurcation of the cavity being produced. Salensky (**27**), however, is of opinion that in Prosorochmus the communication is brought about by the œsophagus, which has produced through histological differentiation an atrium of its own (Text-fig. 35). The difference of opinion between these two authors does not matter in the least for our decision, for it is evident that the conditions in Drepanophorus are primitive, and that the facts on which Bürger based his theory are not primitive at all, as the Drepanophorus stage is passed through in ontogeny. This fact, the difference between the communication in Malacobdella and Hoplonemertea, the impossibility of a pharynx developing into a proboscis, the difference of origin between the two organs, the differences in the connection between pharyngeal sac and pharynx at one side, proboscis and rhynchodæum at the other, the insufficiency of the grounds on which Bürger bases his theory, make me very sceptical as to its value. In fact, I cannot find any other point of resemblance between the two organs than that they are derivatives of what the Germans call die Hautmuskelschlauch.

There are, however, other organs in Invertebrates, even in Platyhelminthes, which have a similar origin, and we shall have to look for comparison between them. For example, the proboscis of Echiurids is a structure of the body-wall—at least it consists of an epidermis and muscles only ; moreover it is situated in front of the mouth, and is known to be very elastic. As, however, this proboscis is supposed to be the præoral lobe of the larva and cannot be retracted into the body, it does not give any special indication of affinity to the same structure in Nemerteans. In Acanthocephala (**14**) a proboscis is found, which can only partly be retracted into the body. The proximal part is surrounded by a sheath of

muscles, the circular fibres of which act as protractors; the retractor muscle, which retracts proboscis and sheath, the two parts being continuous, is inserted at the hind end of the sheath, and extends through the body-cavity to the body-wall. Though some agreement in these structures in Echinorhynchus and Nemertea cannot be denied, I do not suppose there is any relationship between them. The proboscidian system of Acanthocephali is entodermic in origin and only secon-

TEXT-FIG. 36.



Schema of the proboscis of Macrorhynchus croceus after von Graff (13, pl. x, fig. 12). *prob. atr.* Proboscidian atrium. *prob.* Cavity of the introverted proboscis. *prob. w.* Proboscidian wall. *rad. m.* Radial musculature. *rhynch. w.* Rhynchocœlomic wall (Muskelzapfen). Compare with Text-fig. 1.

darily gets its epidermis. This fact alone seems to exclude all possibility of homology with the proboscis of Nemerteans.

Coming to the consideration of two other structures, both in Platyhelminthes, the proboscides of Tetrarhynchus and of Proboscida, the four proboscides of the characteristic genera of Cestodes superficially show a great resemblance to those of Nemerteans. The proboscis is an introvert,

being retracted by a muscle-bundle at the bottom of the tube just as in Nemertines. A muscular sheath is present, separated from the proboscis by a cavity filled with a liquid. Histologically, however, a great difference seems to exist. For instance, the proboscis itself has no muscles at all, if I understand Pintner (**21**) right; the wall of the sheath is differently constructed at its anterior and posterior part, a muscular sheath only existing in the hinder part, the anterior being a derivative of the ectoderm. Moreover the proboscides of these Cestods are paired, those of Nemertines being unpaired.

Much more striking is the likeness to the proboscis of Turbellaria proboscida. Salensky (**25** and **26**) was the first to compare them, and though he already proclaimed them homologues in 1884, nobody seemed to agree with his opinion. Hubrecht (**16**) raged against it, and afterwards Bürger (**8**) gave a new theory. Salensky, however, was not convinced, and tried to give fresh support to his hypothesis in his articles of 1909 and 1912 (**27**, **28**).

The proboscis, which is found in certain genera of Rhabdocœlida, is localised at the anterior end of the head. Von Graff (**13**) described the organ in this way: "Der Probisciden rüssel ist nichts weiter als eine bleibend gewordene Einstülpung des Vorderendes, wie wir sie vorübergehend bei Mesostomum rostratum entstehen sehen." It consists of two parts, the proboscis and a kind of atrium, through which the proboscis is everted (Text-fig. 36). The cavity of both is lined by the somewhat changed epiderm. The wall of the atrium has, like the body-wall, a muscular coat, which is the direct continuation of the muscular layers of the body-wall. At the base of this sac or atrium the proboscis is inserted. Here the muscular coat is broken up into two layers; the inner layer continues along the epidermis, the other one forms the outer lining of the proboscis. A thick layer of radially placed muscle-fibres fills the space between the two parts of the muscular coat of the body-wall. In Nemertines (Text-fig. 1) we find the same arrangement: an atrium to the proboscis, being part of the

body-wall, that has grown inward and is called rhynchodæum. The continuation of the epithelium is found in the epithelium of the proboscis. The muscular coat of the body-wall is split, and has given rise to the muscular wall of the proboscis and of the sheath. The cavity of the rhynchocœl is even traversed by a band of radially placed muscle-fibres, the retractor muscle. So Salensky (**26**) proposed the following homologies.

Rhabdocèles proboscifères.	Némertiens.
1. Poche de la trompe.	1. Vestibule de la trompe.
2. Epithélium de la trompe.	2. Epithélium de la trompe.
3. Couche interne de la calotte musculaire (Muskelzapfen v. Graff).	3. Couche musculaire de la trompe.
4. Couche externe de la calotte musculaire.	4. Parois de la gaîne de la trompe.
5. Muscles radiaires de la calotte.	5. Bride musculaire.

When von Graff (**13**) described the structure of the proboscis of Macrorhynchus and other Rhabdocœlida the origin of the proboscis and sheath in Nemertines was not known. He could not find any homologue of the sheath in his genera, and on this ground denied any relationship between the proboscides in these two classes of Platyhelminths. Another ground for denying it was found in the division of the proboscis of Nemertines into two parts, the posterior glandula part not being present in Turbellaria proboscida. This ground, however, seems not to be very important; the proboscis of Rhabdocœlida represents rather a simple stage of organisation in comparison with the same structure in Nemertines. It does not seem to be necessary to go into such details of structure, especially not since we know what a great variety of epithelial structure is to be found in different genera of Nemertea.

Salensky looked for support for this theory to embryology. And certainly the fact that the muscular coats of rhynchocœlomic and proboscidian walls take origin out of the same

layer of mesoblastic elements, a splitting giving rise to the cavity of the sheath, is highly in favour of his views. It was exactly on this point that Hubrecht did not agree with him; therefore Hubrecht, who, moreover, claimed the Annelida-relations of Nemertea, could not possibly follow Salensky. As, however, later investigations proved that Salensky was right, this reason for disagreeing with his theory gave way. But Hubrecht gave other reasons. In the '"Challenger" Report' he wrote, p. 104: "Salensky would probably not have made his startling hypothesis above alluded to, based on ontogenetical observations of a scission in the proboscidian wall, by which (1) a muscular proboscidian sheath surrounding the proboscis becomes separated from, and independent of, the musculature of the proboscis itself, and (2) an isolated cœlome —the proboscidian cavity—is originated, if he had been as well acquainted with the comparative anatomy of the animals about which he writes as he is with certain details of their ontogeny." The anatomical facts, that tell against Salensky, Hubrecht summarises in this way (loc. cit., p. 103): "There can hardly be any doubt, when we take into consideration all the morphological data at our disposal, that the muscles composing the proboscidian sheath gradually took their origin by the increase and modification of pre-existing muscular elements which belonged to the body-wall and to the body-parenchyma before the proboscis, modified from a tactile organ, as it appears to have primitively been, had yet become evolved, through the growth inwards of the anterior tip of the body, into an aggressive weapon with stylet or nematocysts, etc." Since this was written many facts have been disclosed as to the anatomy of the proboscidian system, and we have seen that they lead us to the conclusion, that the sheath is part of the body-wall, and has not originated in loco out of muscular fibres in the parenchyma of the body. The central parenchyma does not possess any circular muscle-fibres. Wherever other than longitudinal fibres have been found in it they have been demonstrated to be part of one of the layers of the body-wall, as a rule the inner circular

muscle-layer. Longitudinal muscle-fibres may be present in the central connective tissue, and we have already seen that in some cases they are arranged around the proboscis sheath so as to form a secondary longitudinal coat. This, however, is not the rule, not even in Anopla, where a central longitudinal musculature is developed in the majority of genera. Our conclusion is exactly the reverse of the above statement of Hubrecht; the facts described in the first part of this article seem not to allow the denial of our conclusion. Both on anatomical and embryological grounds we must declare the muscular coats and walls of the proboscidian system of Nemertines to belong together ab origine; the facts put forward by Hubrecht against the theory of Salensky have been refuted by later embryological investigations. I hope to have given a new support to Salensky's ingenious theory by this comparative study of the anatomy of the proboscidian system. The fact, stated by Hubrecht, that "we find the shorter proboscides and the less significant proboscidian sheaths among the more primitive genera of Nemertea," is, though mentioned by him as telling against the Turbellarian relations, in perfect accordance with, if not in favour of, Salensky's theory.

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Observations on the Gametogenesis of Grantia compressa.

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With Plates 23 to 26.

Contents.

(A) General Introduction.

The problem of the gametogenesis of Sponges is one which has attracted a good deal of attention from time to time, but it can hardly be said that our knowledge of the subject is as yet by any means in a satisfactory condition. As far back as 1851 Huxley described the occurrence of supposed spermatozoa in Tethya, and in 1854 Carter did the same for Spongilla, but Haeckel is probably right in supposing that both these authors were in reality describing flagellate collared cells, a mistake which, owing to the difficulty of detecting the collar in certain conditions, it is very easy to make. Haeckel, indeed, claims for Lieberkühn the original discovery in 1856 of the sexual differentiation of sponges and of the ova and spermatozoa (in Spongilla).

Haeckel points out the great difficulty in finding the spermatozoa of sponges, a difficulty which has been, and doubtless still is, experienced by many spongologists, and which arises chiefly, no doubt, from their extremely minute size, the fact that they occur scattered throughout the sponge and not segregated in definite gonads, and their liability to confusion with other tissue elements. The ova, on the other hand, in their later stages of growth, are readily recognisable by their large size, and, at certain periods, their conspicuous vesicular nuclei, although these again are not collected in definite ovaries, but occur scattered through the sponge-tissues.

The spermatozoa of calcareous sponges were discovered by Haeckel himself in 1871, and at about the same time by Eimer. In his well-known monograph, "Die Kalkschwämme" (1872) the former author gives an account of all that was previously known of the germ-cells of sponges, and describes his own observations and conclusions with regard to the Calcarea. He finds reasons for deriving the spermatozoa from collared cells of the so-called entoderm (gastral layer), and gives several figures of sperm-morulæ lying amongst the ordinary collared cells in the gastral layer of several species.

With regard to the ova he remarks that the question of the origin and of the original position of the egg-cells is the most difficult and darkest part in the histology of calcareous sponges, an opinion with which those who have studied the question will hardly feel inclined to quarrel. Haeckel himself at first regarded the ova as originating in the "exoderm," in which he, of course, includes everything but the "entoderm," but his more mature conclusion is that they really originate in the "entoderm." He says (1872, p. 159): "Einzelne Geisselzellen des Entoderms vergrössern sich, ziehen ihren schwingenden Geisselfortsatz ein, und entwickeln sich direct durch Aufblähung des Kernes und bedeutende Volums-Zunahme des Protoplasma zu Eizellen."

Since Haeckel wrote, opinion as to the origin of the germ-cells in sponges has changed, and though very few writers have recorded any detailed observations, it is generally held, in accordance with the teachings of F. E. Schulze and others, that both ova and spermatozoa arise from amœboid wandering cells in the mesoglœa, which, of course, is part of Haeckel's "exoderm." No one, since Haeckel's time, appears to have seen the sperm-morulæ lying amongst the collared cells in the walls of the flagellate chambers, and no one has traced the stages by which collared cells might be supposed to have been converted into spermatogonia. Haeckel himself seems to have been by no means satisfied that his own observations on the subject were conclusive, and Poléjaeff, writing ten years later (1882), evidently regarded them with grave suspicion.

Nevertheless, I believe that Haeckel was essentially right in maintaining that oögonia and spermatogonia both arise from collared cells. It is, at any rate, quite certain that bodies resembling spermatogonia are frequently to be found in small morula-like clusters amongst the collared cells in the walls of the flagellate chambers of Grantia compressa, very much as figured by Haeckel for other Calcarea, except that I have never observed the tails of the spermatozoa in this situation, and, indeed, have only seen the sperm-morulæ

in properly prepared, stained sections. Between the original collared cell and the mature germ-cell of either sex amœboid stages intervene, which are frequently to be found in the mesoglœa, at any rate in the case of the oögonia. It is an easy matter to trace the growth of the amœboid oögonia into mature ova, but the question is—Whence come the earliest amœboid stages ?

The modern answer to this question is that they are derived from primitive amœboid cells ("archæocytes"), but I cannot help suspecting that this answer is merely an echo of Weismann's well-known views as to the early segregation of the germ-cells and the continuity of the germ-plasm. It depends, so far as I am aware, not upon direct observation, but upon a process of reasoning by exclusion. The germ-cells are supposed to remain over after the somatic cells have been subtracted from the sum total of cells derived from the segmenting ovum.

I believe that this particular theory of the origin of the germ-cells in sponges originated with Dr. Otto Maas (1894), who sums up his conclusions as follows (p. 35) :

" (1) Wir können hier eine directe Abstammung der Keimzellen der einen Generation vom Ei nachweisen, indem durch Subtraction aller somatischen specialisierten Elemente schliesslich eine Anzahl indifferent gebliebener Elemente übrig ist, die Urgeschlechtszellen.

" (2) Der Hauptunterschied zwischen den somatischen und den Geschlechtszellen zeigt sich vom Anfang wie später im Kern, und zwar in der Quantität und Anordnung des Chromatins."

Anyone who has studied the gametogenesis of sponges knows how greatly the condition of the chromatin varies in different stages, and I do not believe it is possible to indicate any nuclear character by which primordial germ-cells can be definitely distinguished from somatic cells, at any rate in the present state of our knowledge. The alleged distinction between the two groups of cells is a purely theoretical one. Indeed, the latest writer on the subject, Max Jörgensen (1910), has already come to the conclusion that the distinction which Maas endeavours

to draw between germ-cells and somatic cells cannot be maintained in sponges, and "dass es sehr wohl zur Bildung von Geschlechtszellen aus somatischen Zellen kommen kann, wie man dies ja bei dem primitiven Charakter der Schwämme von vornherein erwarten würde" (p. 170). This author derives the oögonia of Sycon from ordinary "mesoderm" cells rather than from primitive undifferentiated amœbocytes (archæocytes), though he thinks that the latter may also give rise to oögonia. He does not deal with the spermatogenesis.

My own attention was first particularly directed towards the problem of the origin of the germ-cells in calcareous sponges under the following circumstances. Some years ago Prof. Herdman invited me to write a memoir on the common British species, Grantia compressa. In the course of this work it became evident that although the ova of this sponge were easily recognisable in the maternal tissues in various stages of growth, and also embryos in various stages of development, no one had ever been able to find the spermatozoa, though they were searched for repeatedly by Mr. Carter (1875, p. 25). For the sake of completeness it appeared highly desirable that this gap in our knowledge should be filled.

In April, 1912, accordingly, I paid a visit to the laboratory of the Marine Biological Association at Plymouth, and although I was unable to find spermatozoa in the living specimens, of which I examined a large number, I preserved material which on subsequent investigation furnished the clue to the solution of the problem. I found that the sponge is hermaphrodite, producing both male and female germ-cells simultaneously, but that the spermatozoa are produced in comparatively small numbers, the minute sperm-morulæ being scattered here and there, enclosed in spermatocysts, between the collared cells of the chamber walls, and also occurring free in the flagellate chambers. The spermatogonia in these sperm-morulæ are extremely minute, and I am unable to say anything with regard to their mode of division. Apparently they are sometimes transferred as sperm-morulæ to the inhalant canals of the

same or of another individual, where they break up into spermatozoa, but it is probable that they may sometimes break up into spermatozoa before leaving the parent sponge. The evidence on these points is, however, curiously scanty, and I conclude that the spermatozoa are rarely, if ever, liberated in large numbers.

With regard to the process of oögenesis, on the other hand, I found a number of very interesting stages, and with the exception of the second maturation spindle (described by Max Jörgensen in Sycon), am able to give a fairly complete account. My observations agree in many respects with those recorded by Jörgensen on the oögenesis of Sycon, but I am able to add a good deal to his account, and in some respects my interpretations are different. Though I have repeatedly observed mitotic figures of various types I have always found the chromosomes very small and ill-defined, and I cannot pretend to give such precise descriptions of the mitotic phenomena as Jörgensen has done. It is very difficult to understand his account of these phenomena, or to harmonise it with currently accepted views, but I must leave to specialists in cytology the detailed criticism of his work in this respect. I may perhaps say, however, that many of his figures appear to me to be very diagrammatic.

One of the most remarkable phenomena observed during my investigations is the nutrition of the growing oöcyte by means of phagocytic nurse-cells. Very extensive phagocytosis has also been observed in the case of certain large amœbocytes, which seem to devour the young germ-cells in some cases in a wholesale fashion.

Though well aware that I am not able to give by any means a complete account of the history of the germ-cells in Grantia compressa, I hope that the following pages may not only help to fill a conspicuous gap in our knowledge of this common British sponge, but also throw some new light on the difficult problem of gametogenesis in sponges generally.

I must take this opportunity of thanking my friends at the Plymouth Laboratory, especially Dr. Allen and Mr. Orton,

for their hospitality and assistance during my visit. I am also greatly indebted to the University of London for the use of their table at the Laboratory.

(B) Material and Methods of Investigation.

My observations at the Plymouth Laboratory, made from April 10th to April 22nd, 1912, were mainly directed towards the discovery of the spermatozoa of Grantia compressa. The sponge may be obtained in large numbers close to the Laboratory, at low water, and I also received a number of fresh specimens from Drake's Island and Rum Bay. They varied greatly in size, from quite small to as much as 80 mm. in height by 18 mm. in breadth (a specimen from Rum Bay). A large number were microscopically examined in the living condition, either by teasing or by means of hand sections, or by pipetting out the contents of the central gastral cavity, but my search for living spermatozoa was fruitless.

I preserved a considerable number of specimens, however, for future examination, and the results recorded in the present paper are based almost entirely upon the study of these by means of paraffin sections.

The material that turned out satisfactorily was fixed either in strong Flemming's solution, or in a mixture of Flemming, formol and sea-water. In the former case it was graded up, after washing, to 70 per cent. alcohol; in the latter it was preserved in formol sea-water.

The sections were, for the most part, cut of a thickness of 5 μ and stained on the slide. I found that iron-brazilin gave excellent results, but iron-hæmatoxylin was also used.

For staining in bulk borax-carmine or paracarmine was employed, the latter being sometimes followed on the slide by picro-indigo-carmine, but without much effect.

I desire to express my thanks to my skilful laboratory assistant, Mr. Charles Biddolph, for the care which he has taken in preparing the sections.

Although many specimens were preserved, only five have

actually been used for the purposes of the present investigation, numbered in my notes 11, 21, 22, 23 and 24 respectively.

It will save repetition if I give particulars concerning these specimens at once, and of the mode of treatment of the material.

No. 11.—A very large specimen from Rum Bay, 80 mm. high by 18 mm. in breadth; brought in about mid-day (April 15th) and examined the same afternoon (about 1 o'clock). When examined a vigorous stream was coming out from the main vent. A section of the living sponge from near the base showed large ova, but no embryos were seen. A section near the vent showed smaller germ-cells. The specimen was cut in half lengthwise, and half fixed in strong Flemming's solution (in the dark) for half an hour, then washed for an hour or more in tap-water and graded through 30 per cent. and 50 per cent. to 70 per cent. alcohol.

No. 21.—A rather small specimen, about 14 mm. high, collected about mid-day on April 18th, and examined the same afternoon. There was an active current issuing from the vent, bringing with it a quantity of fine yellowish-grey sediment, which collected at the bottom of the glass dish. In hand section numerous large rounded cells were seen free in and projecting into the flagellate chambers, apparently actively moving, but probably only owing to the movements of the flagella of the collared cells. Fixed in strong Flemming's solution and preserved in 70 per cent. alcohol.

No. 22.—A moderate-sized specimen, about 25 mm. high by 18 mm. broad; collected about mid-day on April 18th and examined the same aftrnoon. Fixed entire in Flemming and sea-water formol[1] for about a quarter of an hour, then washed in formol and sea-water and preserved in same.

No. 23.—A moderate-sized specimen, collected on April 18th and examined and fixed on the 19th, having been kept in the circulation of the aquarium overnight. Stream coming from osculum when examined. Found no amœbocytes or

[1] Take 10 c.c. commercial formaldehyde in 90 c.c. sea-water and add 20 c.c. strong Flemming's solution.

other cells in water pipetted from gastral cavity. On examination of living section found very numerous rounded amœbocytes with coarse granules, and a few with pseudopodia, in the chambers, some, if not most, attached to walls by short peduncles. Half of specimen fixed in strong Flemming's solution for about an hour, washed and graded up to 70 per cent. alcohol. Another part fixed in absolute alcohol.

No. 24.—A good-sized specimen from Rum Bay, examined and preserved on April 22nd, after having been kept in the aquarium circulation since April 15th. The collared cells were found to be still active and did not show the characteristic signs of suffocation. Bulk of specimen fixed in Flemming and sea-water formol.

(c) The Breeding Season and Life-Cycle.

There is strong, indeed, I think conclusive, reason for believing that Grantia compressa is an annual sponge, growing rapidly during the winter and spring and breaking up and perishing in the autumn, after producing numerous embryos. I have already given particulars as to the sizes of some of the specimens met with at Plymouth in April, 1912, and my colleague, Mr. R. W. H. Row, tells me that about the middle of August, 1913, when he visited Plymouth, they were already breaking up, and it was difficult to obtain a good specimen of any considerable size—indeed, most of them had apparently already disintegrated.

The breeding season at Plymouth would seem to begin in the first half of April; germ-cells are then being produced in enormous numbers, but comparatively few embryos are found. At least that was my experience in 1912.

Previously, in 1911, I had observed mature embryos being shot out of the osculum of a specimen which I examined in the laboratory in the first week of June. I also find plenty of advanced embryos, along with germ-cells in various stages of growth, in a specimen collected for me by Mr. Row about the middle of August, 1913. The germ-cells (ova), however, are nothing like so abundant as in material taken in April.

It seems, therefore, that the breeding season lasts throughout practically the whole of the spring and summer.

According to Mr. Orton, who kindly allows me to make use of information about to be published in the 'Journal of the Marine Biological Association' (1914), there are really two breeding seasons at Plymouth for Grantia compressa. In June embryos are discharged from large specimens (which subsequently disintegrate). These embryos develop into individuals which, while still very small, produce numerous embryos in October. Mr. Orton has also obtained data supporting the view that the same specimen may breed twice during its life-history—once in late autumn and again in the following summer.

(D) The Distribution of the Germ-Cells in the Sponge.

As a result of my observations I think I have been able to establish the fact that Grantia compressa, unlike certain non-calcareous sponges, such as Oscarella lobularis (Schulze, 1877), is hermaphrodite, producing male and female gametes simultaneously. In the case of Sycon raphanus, Poléjaeff, as far back as 1882, came to the same conclusion, but considered that that sponge afforded an example of incomplete sexual separation, some individuals being predominantly male and others predominantly female. The former were extraordinarily rare, but produced an immense quantity of spermatozoa, as well as numerous ova. The latter produced very few, or even (apparently) no spermatazoa, but a large number of eggs.

It is quite possible that the same relations may exist in Grantia compressa, but if so I have never been fortunate enough to find the predominantly male individuals. All that I have examined appear to be predominantly female, the sperm-morulæ occurring only in comparatively small numbers.

Poléjaeff derives both the sperm-morulæ and the ova (in Sycon) from ordinary amœboid wandering cells, and figures

them scattered in the mesoglœa, apparently without any special arrangement.

Görich (1903) also accepts the usual views as to the origin of both male and female germ-cells from amœbocytes, but he states that in Sycon raphanus ova are produced in the lower two thirds and sperm-cells in the upper third of the sponge. He has not, however, followed the spermatogenesis beyond its earliest stages. The only generalisation that I can make about the distribution of the germ-cells in Grantia compressa is that the younger parts of the growing sponge, towards the osculum, only contain immature germ-cells, exactly as might be expected.

It may be admitted that in Grantia compressa also the germ-cells, both male and female, can be traced back to amœboid wandering cells, but, according to my own observations, these amœbocytes can, in their turn, be traced back to collared cells of the gastral epithelium lining the flagellate chambers. Presumably any collared cell may become directly transformed into a primary oögonium or spermatogonium, losing its collar and flagellum and becoming amœboid. The evidence for this statement will be presented in the next section.

Having become amœboid, the young germ-cells are free to wander about. The primary oögonia first migrate into the mesoglœa from the gastral epithelium, and later on, when fully grown, they migrate back into the chambers, where they undergo repeated division and give rise to small oöcytes. The young oöcytes remain, feeding and growing, for some time in the chambers; then they migrate once more into the mesoglœa, where they undergo enormous growth, followed by maturation and fertilisation.

The migrations of the spermatogonia appear to be of a less extensive character. I have reason to believe that they may migrate into the mesoglœa and there become provided with their cover-cells or spermatocysts, but they appear to spend most of their existence in, or attached to, the walls of the fla ellate chambers.

It is easy to observe, in hand-cut sections of living specimens in the early part of the breeding season, that the flagellate chambers contain large numbers of amœbocytes hanging, as it were, from their walls. These are, for the most part, germ-cells of both sexes in various stages of growth, although other amœbocytes may also occur in the chambers.

There is thus no localisation of the germ-cells in Grantia compressa, nothing that can be spoken of as gonads, neither ovaries nor testes. Just before undergoing maturation, however, the relatively enormous ova withdraw their pseudopodia and round off, each one taking up a definite position behind the gastral epithelium of an adjacent chamber, and causing the layer of collared cells to bulge out into the chamber. Here fertilisation and the earlier stages of development take place, the embryo becoming surrounded by an endothelial capsule, derived from the mesoglœa, during the latter process. Finally the ciliated amphiblastula breaks through the layer of collared cells into the chamber cavity, and is discharged into the sea through the central gastral cavity and vent.

The sperm-morulæ are also discharged into the flagellate chambers, and doubtless find their way out through the vent. I have found them, not only in the chambers, but also adhering to the outer surface of the sponge and in the inhalant canals, though, except in the chambers themselves, only in very small numbers. I have also found some evidence of their breaking up into spermatozoa in an inhalant canal.

There can be little doubt that fertilisation is effected by spermatozoa which enter the sponge (perhaps as sperm-morulæ) through the dermal pores with the inflowing stream of water, but whether these spermatozoa are derived from the same sponge as the eggs which they fertilise, or from another individual, would seem to be a matter of pure chance.

In a paper on the "Anatomy of Grantia labyrinthica, etc.," published in 1891, I expressed the opinion that the ova migrated through the walls of the inhalant canals and were

fertilised while suspended in the inflowing stream of water, subsequently migrating back to undergo their development in the mesoglœa. I certainly did observe amœbocytes suspended in this position, but I now realise that they were far too small to be mature ova, and must unreservedly withdraw my interpretation of the observation. It is evident that maturation and fertilisation (in Grantia compressa) both take place after the ovum has taken up its definitive position in the mesoglœa behind the gastral epithelium. Possibly the spermatozoon has to penetrate a thin layer of dermal epithelium and mesoglœa in order to reach the ovum, or perhaps the presence of the enormous ovum causes some rupture in the wall of an adjacent inhalant canal. It is impossible to say exactly what takes place.

One more point may be mentioned in this section, and that is the tendency of particular stages of gametogenesis, or at any rate of oögenesis, to occur in large numbers in certain specimens, or in certain parts of the sponge, while more or less completely absent from others. Instances of this phenomenon will be given in the following pages; it seems to indicate that the oögonia are produced in successive crops which go through their developmental stages synchronously.

(E) The Relations between the Different Tissue Elements.

There can be no doubt that the tissues of sponges are far less definite and less permanent than those of typical Metazoa. Without going back to the old view that the sponge is nothing more than a colony of Protozoa, which seems to be negatived by the degree of histological differentiation that they exhibit and by the facts of sponge embryology, we may safely say that the individual cells of which the sponge is composed often exhibit a remarkable power of changing their relative positions and also a high degree of polymorphism. Thus it will be remembered that Minchin (1898) has shown that the cells (scleroblasts) which secrete the triradiate spicule-systems in calcareous sponges migrate into the mesoglœa from the

dermal epithelium ("ectoderm"), and that the porocytes in Leucosolenia, when the sponge contracts, migrate through the gastral epithelium and fill up the central cavity (Minchin, 1900). It is also well known that the epithelial cells of the so-called ectoderm are highly contractile and capable of great change of shape, and Maas has shown (1900) that in the developing Sycon the epithelial cells lining the central gastral cavity are derived by immigration from the dermal epithelium on the outer surface of the sponge.

In the case of Grantia compressa it is hardly possible to speak of permanent tissues at all. According to my observations any of the constituent cells of the sponge may become amœboid and wander off to some new situation. This may very easily be demonstrated for the collared cells by examining teased preparations of the living sponge in sea-water, when the collared cells can be seen putting out long, hyaline, finger-shaped pseudopodia in an extremely characteristic manner.[1] The fact that the collar may still be present along with the pseudopodia, as shown in fig. 1, affords unmistakable proof of the origin of these amœboid cells in teased preparations. In stained sections we sometimes see something of the same kind, and fig. 2 represents a collared cell sinking into the mesoglœa from between its fellows of the gastral epithelium. In this case pseudopodia and flagellum are seen to be present simultaneously but the collar is not visible. In fig. 3 an amœbocyte, probably derived from a collared cell, but apparently without collar, flagellum and pseudopodia, is seen lying in the mesoglœa behind the gastral epithelium.

Appearances such as are represented in figs. 4, 6 and 7 also indicate very clearly that the cells of the so-called ectoderm are not only contractile, but may become converted into amœbocytes and wander off into the mesoglœa. In fig. 4 is shown one of the epithelial cells lining the central gastral cavity in the contracted or "flask-shaped" condition (*a*), and in the subjacent mesoglœa a typical amœbocyte (*b*). Fig. 6 shows how such amœbocytes may be directly derived from

[1] Compare Carter (1875, p. 22) for a similar observation.

epithelial cells which have migrated inwards, the identity of the two being clearly indicated by the presence of the numerous darkly stained granules that characterised the epithelial cells of the central gastral cavity, at any rate in this specimen. Fig. 7 shows a similar relation between the much less granular epithelial cells and amœbocytes around an inhalant canal.

The mesoglœa of Grantia compressa contains, of course, a large number of amœbocytes, and there is no need to suppose that all of them are merely amœboid phases of either collared or pavement epithelial cells (compare figs. 58–61). Sometimes small amœbocytes may appear to form connective-tissue networks of stellate cells, but I doubt very much if they really do so, at any rate more than temporarily, and even in this condition they bear such a close resemblance to the epithelial cells lining the inhalant canals that I entirely fail to see how they can be distinguished cytologically.

Under these circumstances it is, of course, quite impossible to say what cells of the adult sponge are derived from each of the cell-groups recognisable in the larva. It also seems quite inadequate to say that the germ-cells are derived from wandering cells in the mesoglœa.

The one constant and characteristic feature about sponge histology is, of course, the collared cell, and that is only constant in the sense that its typical form is that which possesses collar and flagellum. The sponge is, after all, not very much more highly advanced in organisation than a colony of choano-flagellate Protozoa. In such a colony we should certainly, I think, expect the germ-cells to be derived from collared cells, either directly or indirectly through an amœboid phase, and in a later section I hope to be able to show that this is how they actually originate in sponges.

There is one feature about the collared-cells which appears to have attracted but little attention from sponge histologists but which deserves notice in this connection. I refer to the accumulation in them of what appear to be granules of reserve food-material. I have observed these as a very constant

feature in sections stained in a variety of ways, as polygonal bodies scattered more or less abundantly in the cytoplasm (figs. 2, 3, 8, etc.). They vary much in size and in the intensity with which they stain. In sections of material (spec. 23) fixed in absolute alcohol and stained with borax-carmine followed by picro-indigo carmine they are distinctly recognisable, and stain a pale, greyish colour. In sections of Flemming material without further staining they can easily be detected, though only very lightly stained.

They are quite distinct in Flemming material stained with iron-brazilin, but, I think, more so after counter-staining with picro-indigo carmine, when they appear of a dark grey colour (spec. 11). They are hardly affected by nuclear stains and are evidently not chromidial in nature. The depth to which they stain exhibits a curious variation in some cases, as will be seen by reference to fig. 8, from a section of material (spec. 21) fixed in strong Flemming and stained with paracarmine and picro-indigo carmine, where variation in this respect is visible even in one and the same cell. They are usually most abundant in the lower part of the cell (figs. 3, 8).

These observations on their staining reactions appear to me to be quite in harmony with my view that the bodies in question are "reserve granules." The question next arises, Are such granules characteristic of the collared cells, or do they occur also in the other tissue-elements? We have already had occasion to notice the presence of numerous granules in the epithelial cells lining the central gastral cavity and in the amœbocytes derived from these (fig. 6). These granules, it will be observed, are of a different character from those now under consideration, being smaller and more highly refractive. My observations lead me to believe, however, that granules similar to those in the collared cells may also occur in the epithelial cells both of the gastral surface and of the inhalant canal system, though perhaps less abundantly. They certainly occur in many of the amœbocytes, and I had at first hoped that their presence might have served as a means of distinguishing those amœbocytes that originate from collared

cells from those that do not. I fear, however, that this hope must be largely abandoned, though the granules in question may perhaps serve as supplementary evidence of origin in certain cases.

Another point to be noticed in connection with the collared cells concerns the condition of the nucleus. Usually in my sections this appears to be darkly and almost uniformly stained, but frequently it exhibits a distinctly reticulate character, with small, scattered, chromatin granules at the nodes of the reticulum, while intermediate conditions occur between the two extremes. As all conditions may occur close together in the same preparation it is difficult to account for the differences, but such variations have to be borne in mind in considering the origin of the germ-cells as indicated by nuclear characters.

Miss Muriel Robertson and Prof. Minchin (1910) have given an account of the division of the collared cells in Clathrina coriacea, and Miss Robertson (1911) has dealt with the corresponding phenomena in Grantia compressa and Sycon sp. Though figuring the collared cells in detail, neither of these authors refer to the "reserve granules," which I find to be such a constant feature in Grantia. This is perhaps due in part to the fact that their preparations were stained especially with a view to demonstrating the phenomena of mitosis. They figure much the same variations in the appearance of the nuclei of the collared cells as I have seen, but I cannot agree with their views as to the general occurrence of a single, relatively large karyosome (nucleolus). I have myself only occasionally seen such a body in these nuclei and do not attribute any special importance to it.

Alike in Clathrina, Sycon and Grantia the division of the collared cells was found by Robertson and Minchin to take place longitudinally and to be accompanied by a typical mitosis. In Clathrina the number of chromosomes was found to be "about sixteen," but in Grantia and Sycon the chromosomes are said to be "not very distinct," and the number is not given. Miss Robertson's figures, however, especially

fig. 12 on Pl. 19, suggest eight or ten as the number rather than sixteen. I have myself hardly ever observed mitosis in the collared cells, but my fig. 9 represents a probable case, and it will be seen that so far as my very limited observations go they agree with those of Miss Robertson.

I do not, of course, profess to give a complete account of the collared cells in this place; much more might be said about them, but the only thing needful here is to emphasise those points which have a direct bearing upon the problem of the origin and maturation of the germ-cells.

(f) Oögenesis.

(a) Historical.

By far the most complete account that has yet been given of the oögenesis in any sponge is contained in Dr. Max Jörgensen's memoir (1910). As this account refers to a type (Sycon) closely related to Grantia compressa, I propose to give a brief review of Jörgensen's results before passing on to describe my own.

As already noticed, this author derives the primary oögonia from so-called mesoderm cells, either resting stellate connective-tissue cells, or amœbocytes, between which he considers that no essential difference exists. The mesoderm cells multiply mitotically and become converted into oögonia of the first order. These increase in size and presently wander into the flagellate chambers, where they undergo mitosis and divide into oögonia of the second order. The latter divide again into oöcytes. The two oögonial divisions are said to be "atypical," but quite similar to one another. During the mitosis eight chromosomes make their appearance "in Form von Tetraden"; these are believed to be formed by fusion of several segments of a segmented spireme. Each "tetrad" is figured as dividing into two tetradiform daughter-chromosomes on the spindle.

Although the division of the oögonia frequently occurs in

the flagellate chamber, the author evidently regards this as a more or less accidental circumstance, which takes place only if the öogonia have not got room enough to divide in the mesoglœa. I think myself that the migration of the oögonia into the chambers before dividing must have a deeper significance than this.

After their divisions are over the daughter-cells of the oögonia wander back into the mesoglœa as young oöcytes. Here they undergo a short resting stage, and then they pass through the prophases of what must apparently be regarded as the mitosis belonging to the first maturation division. A long spireme is formed which arranges itself in a characteristic "bouquet" form, and even shows a contraction phase similar to synapsis, though this may be due to the action of reagents; at the same time "chromidia" appear in the cytoplasm, probably ejected from the nucleus. A large nucleolus is likewise present, but this is also a characteristic feature of the oögonia. Some of these young oöcytes now degenerate, possibly furnishing nutrient cells for the older oöcytes. In others the spireme breaks up again into chromatin granules, and the oöcyte continues its growth.

In the later stages the oöcyte increases enormously in size and puts out long, branching pseudopodia. When the growth of the cytoplasm has reached its completion the nucleus enters upon the so-called "critical stage," in which the nucleolus has completely disappeared and the nucleus is almost entirely devoid of chromatin. Apparently the chromatin has migrated through the nuclear membrane into the surrounding cytoplasm, where it is represented by "chromidia." Chromatin-granules and nucleolus now reappear in the nucleus, which becomes very large and vesicular. Then a second diminution in the amount of chromatin takes place, this time apparently effected by solution or absorption within the nucleus itself and not by extrusion into the cytoplasm. The nucleus, with its diminished quantity of chromatin arranged once more in the form of tetrads, approaches the surface of the oöcyte, which has in the meantime rounded

itself off. The nuclear membrane now disappears, and the eight tetradiform chromosomes, now much diminished in size, arrange themselves on the equator of the first maturation spindle. Each chromosome divides into two daughter tetradiform chromosomes and the mitosis is completed in the ordinary way. Then the first polar body, containing eight of the daughter-chromosomes ("tetrads"), is twisted off from the surface of the oöcyte in a very characteristic manner. A second maturation division now takes place and eight chromatin elements finally remain in the fertilised egg, eight having passed out into the second polar body, but the formation of the second polar body was not fully observed.

It is difficult to understand completely the author's views as to the manner in which the reduction of the chromosomes is carried out, and, indeed, although he discusses the problem at some length, he does not profess to have come to any certain conclusions: "Leider ist mein Objekt zu klein und ungünstig, um diese wichtige Frage sicher zu entscheiden." At any rate he evidently considers that the somatic number of chromosomes is sixteen and the reduced number eight, for he finds sixteen in the segmentation nuclei of the embryo. The most curious thing appears to be that the number is already reduced to eight in the oögonial mitoses, but it is suggested that this may be a pseudo-reduction. Possibly it is connected with the tetrad formation which is supposed to take place at this stage.

The author describes an interesting process of nutrition of the growing oöcyte by ingestion of nutrient cells. I have observed a somewhat similar phenomenon myself in the case of Grantia and shall discuss it at length later on.

As regards the fertilisation of the ovum and the subsequent segmentation stages the most interesting feature appears to be the splitting up of the pronuclei and segmentation nuclei into karyomeres, a phenomenon which I have also observed in Grantia.

(b) Origin and Growth of the Primary Oögonia.

Before proceeding to describe the origin and growth of the primary oögonia as observed in Grantia compressa it may be well to point out the great difficulties that arise with regard to the problem of seriation in the earlier stages of oögenesis. These difficulties are accentuated by the absence of definite localisation of the germ-cells and the consequent mingling of different stages in the mesoglœa or in the chambers, so that it is often impossible to be certain even to which generation a particular cell belongs. We can only fit the different observed stages together in what seems to be the most probable order on the sometimes scanty evidence available.

In the later stages of growth of the oöcyte there is less difficulty, because the size of the cell not only shows that it is an oöcyte, but indicates at the same time its place in the series.

Inasmuch as amœboid cells of various sizes frequently immigrate into the chambers and come to lie between the collared cells, the mere fact of the occurrence of a young germ-cell in such a position affords no conclusive evidence that it has been derived from a collared cell. The only way in which such an origin can be demonstrated, as it seems to me, is by finding cells which exhibit the characters of young germ-cells while still retaining the collar or flagellum, or both, of the collared cells. It must be admitted that it is not often that such intermediate forms are met with, and it is obvious that they can only be hoped for in very carefully prepared sections. I have, however, seen a few such cases, which seem to me to place my conclusions almost beyond doubt. Figs. 10, 11, and 12 are all taken from sections of specimen 21, stained with paracarmine and picro-indigo carmine. In Fig. 10 two collared cells are represented side by side. In both the collar and flagellum are clearly visible, and in one the body of the cell is already considerably enlarged, apparently by the accumulation of reserve material. This enlarged collared cell I take to be a primordial germ-cell, but

whether it would have turned into an oögonium or a spermatogonium cannot be decided. The appearance of a vacuole around an unusually large granule of reserve material, however, suggests to my mind the latter.[1]

Figs. 11 and 12 represents a later stage, in which the body of the cell is greatly distended with reserve granules similar to those which occur in the ordinary collared cells, and the remains of collar and flagellum are, unless I am mistaken in my interpretation of the appearances, still visible. The nucleus has retreated to about the middle of the cell and a distinct nucleolus or karyosome has appeared. The whole cell projects conspicuously beyond its neighbours into the cavity of the flagellate chamber, and there is a clear indication of pseudopodium formation represented in fig. 12, the pseudopodium being formed by a drawing out of the proximal end of the cell between the adjacent collared cells. (An apparent pseudopodium shown in fig. 11 may belong to another cell not seen in the section.) In fig. 11 it will be seen that the young oögonium lies actually next to the exhalant aperture of the chamber, and the one represented in fig. 12 also lies close to an exhalant aperture, but I have not sufficient evidence to show whether or not there in any constancy in this position.

The primary oögonia appear to leave the layer of collared cells very soon after their origin and migrate into the mesoglœa (fig. 13). The collar and flagellum completely disappear and pseudopodia are put out (fig. 14). The nucleus at first appears uniformly stained except for the large nucleolus, but presently small granules of chromatin appear scattered between the nucleolus and the nuclear membrane, though the nucleolus appears to be surrounded by a narrow ring free from granules (fig. 15). The reserve granules are still abundant and easily recognisable in the cytoplasm.

The primary oögonium now appears to round itself off more or less completely before entering upon mitosis, as shown in

[1] Compare the account of the origin of the primary spermatogonia, later on.

fig. 16. In specimen 22 the mesoglœa between the chambers is crowded with oögonia in this condition, and their numbers suggest that it may be a resting state. It will be seen that the cytoplasm is only slightly granular, but may still contain reserve material in the form of polygonal granules. The nucleus is faintly reticulate, and there is a very large and conspicuous nucleolus and a thin nuclear membrane.

(c) Multiplication of the Oögonia.

Having reached the stage represented in fig. 16 the oögonia begin to prepare for division, which is effected by mitosis. During the progress of this mitosis they migrate through the layer of collared cells into the flagellate chambers (fig. 21), where the cell-division actually takes place. Although the earlier stages of the mitosis are found in the mesoglœa (figs. 17, 18), I have never seen the actual division taking place except in the chambers themselves, and cannot therefore agree with Jörgensen that the oögonia only migrate into the chambers when they are short of room in the mesoglœa. This view is not in harmony with the fact that the fertilised eggs, which are many times larger than the oögonia, manage to find room for their development in the mesoglœa by pushing out the gastral epithelium without rupturing it. I therefore think that there must be some special reason for the migration of the oögonia into the chambers. I would suggest that it may enable the young oöcytes to find abundant nutriment in the chambers in the first instance, and subsequently to distribute themselves more readily throughout the sponge by creeping along inside the walls of the chambers and re-entering the mesoglœa at various points.

Jörgensen is of opinion that there are two generations of oögonia in Sycon, and the large number of oöcytes present seems to indicate that there must be at least two oögonial divisions in Grantia. Further evidence of this is to be found in the small size of the youngest oöcytes as compared with the daughter-cells formed by division of the primary oögonia (compare figs. 29 and 26).

Before proceeding to describe the mitosis of the primary oögonium it is necessary to say a few words with regard to the character of the chromatin substance in the nucleus. The growing oögonium contains, as we have seen, a large spherical nucleolus or karyosome (fig. 14, etc.), which stains very darkly, and in its later stages at any rate minute granules of chromatin may also appear in the nucleoplasm (fig. 15). During the prophases of mitosis all the darkly staining granules of chromatin disappear from the nucleus, while the nucleolus may be cast forth from the oögonium altogether (figs. 17, 18). There remains behind a quantity of more lightly staining chromatin which forms the spireme, and, subsequently, the chromosomes. It is, I think, impossible to avoid the conclusion that there are here two totally different kinds of chromatin, for which we may accept the terms "trophochromatin" and "idiochromatin" respectively, the former being concerned in the metabolism and growth of the cell and the latter in the processes of reproduction. The trophochromatin is represented chiefly by the nucleolus. Small granules of chromatin are possibly cast out into the cytoplasm as chromidia, for there is some evidence that chromidia may be found at this stage, but they are nothing like so conspicuous as they are in the oöcyte. In the latter the chromidia are, of course, supposed to be concerned in yolk-formation, which has hardly commenced in the oögonium. The large nucleolus appears to be bodily cast forth from the oögonium during mitosis (fig. 18). It can, therefore, hardly be supposed to be directly concerned in yolk-formation at this stage. It may possibly be of a different nature from the chromidia, and represent a mass of waste products accumulated in the nucleus during the growth and metabolism of the oögonium. In the young oöcyte, however, as we shall see shortly, the nucleolus definitely gives rise to chromidia ("yolk nucleus"), while in the maturing oöcyte it appears to undergo degeneration and absorption in the cytoplasm.

Jörgensen gives an interesting discussion on the nature and behaviour of the chromatin in the oöcytes of Sycon, to which

I must refer the reader, but he appears to have paid very little attention to this question so far as the oögonia are concerned, and does not seem to have observed the bodily ejection of the nucleolus.

We may also say a few words here with regard to the formation of the chromosomes. Jörgensen figures both spireme and chromosomes as being stained perfectly black—as black, in fact, as the chromidia. His material, like mine, was fixed in Flemming's solution, and he used iron-hæmatoxylin for staining (controlled by borax carmine and saffranin preparations). My own preparations were for the most part stained with iron-brazilin, but I have also used iron-hæmatoxylin. There is, of course, a good deal of variation in the results obtained by either of these methods, but my experience is that, as a rule, the spireme thread and chromosomes stain comparatively lightly as compared with the nucleolus and chromidia. I have never seen the chromosomes so sharply defined as Jörgensen figures them, and I have seen nothing of the so-called tetrad formation which he describes during the oögonial mitoses and in the maturing oöcyte. The chromosomes have always appeared to me much more like those figured by Prof. Minchin and Miss Robertson for the dividing collared cells—small subspherical or irregular bodies, so crowded together and ill-defined that it is impossible to count them accurately. The number characteristic of the oögonia appears to be about eight, as will be seen by reference to figs. 19–23, and this is possibly the somatic number (compare fig. 9).

After these preliminary observations the actual division of the primary oögonia may be described very briefly. The prophases of the mitosis occur while the oögonium is still lying in the mesoglœa outside the chamber which it is about to enter, and while it is in a more or less amœboid condition (figs. 17, 18). Whether or not there is any casting out of chromidia into the cytoplasm I am not certain, but as a few small, densely staining bodies resembling chromidia sometimes appear in the cytoplasm in later stages of this mitosis, it

seems not unlikely that such may occasionally be the case. Apart from the nucleolus, however, there is very little chromatin in the nucleus to be cast out.

The large vesicular nucleus approaches the surface of the oögonium until it is bounded on the outside by only a very thin layer of cytoplasm. In the meantime a spireme thread makes its appearance, and the nucleolus also approaches the surface (fig. 17). The nuclear membrane disappears, and the nuclear sap merges into the cytoplasm. A very curious phenomenon now takes place, the nucleolus being expelled, not only from the nucleus, but from the oögonium. Fig. 18 shows it in the process of extrusion, surrounded by a drop of nuclear sap. The pear-shaped form of the nucleolus at the moment of extrusion suggests that it must be a very soft, perhaps a semi-fluid, body in life. I have only seen this phenomenon exhibited very rarely in what can be considered as at all a conclusive manner. I have frequently seen the nucleolus apparently cast out of the oöcyte, but minute inspection shows that this is (? always) an artificial result brought about in the act of cutting the sections. During the process of fixation, etc., the nucleolus appears to become very hard, and the knife then tears it bodily out of the oöcyte. In the case represented in fig. 18, however, I think there can be no question of the normality of the process of extrusion; indeed, that the nucleolus must be extruded at this stage seems to be indicated by its complete absence in later stages of the mitosis.

It is extremely difficult to determine whether a particular spireme stage under observation belongs to an oögonial or to an oöcyte mitosis. I am inclined to think, however, that in the former case there are few or no chromidia, while in the latter the chromidia are fairly strongly developed (cf. figs. 44, 45). I must admit again, however, that the sorting out of these stages is to a large extent arbitrary.

The spireme thread now breaks up into chromosomes, a typical spindle is formed with a minute centrosome at each pole, and the chromosomes arrange themselves in the usual

"equatorial plate" (figs. 19, 20). The chromosomes now presumably divide, though I can hardly claim to have seen the actual division (cf., however, fig. 21), and the two groups of daughter-chromosomes migrate towards the two centrosomes (figs. 22, 23). The spindle disappears, and we are left with two closely aggregated groups of chromosomes as the foundations of the two daughter-nuclei (fig. 24). Constriction of the cytoplasm between these two daughter-nuclei now follows (fig. 25), and finally the entire oögonium becomes divided into two daughter-cells (fig. 26).

Apparently not until the prophases have been passed through does the oögonium migrate through the layer of collared cells into the adjacent flagellate chamber, as shown in fig. 21. Here it rounds itself off, usually into an oval form (figs. 19, 20), before completing the mitosis. At this stage the cytoplasm exhibits a fairly uniformly and densely granular character, shown especially well in fig. 22. A few small densely staining granules, resembling chromidia, are sometimes visible in it (fig. 19), and may even persist in the daughter-oögonia after completion of the division (fig. 26).

It seems almost certain that a second oögonial division takes place in the flagellate chambers very shortly after the first one. Fig 27 represents a stage which I interpret as an oögonium of the second generation with reconstituted nucleus, and fig. 28 represents what I take to be such a secondary oögonium in mitosis. Without the intervention of such a stage it would be difficult to explain the origin of the next series of stages (figs. 29–39), which occur very abundantly in the chambers, and which I interpret as young oöcytes. With regard to this period of the oögenesis my conclusions differ widely from those of Jörgensen, who makes his oögonia of the second order larger than those of the first order, and his young oöcytes larger still, which would be very difficult to understand in view of the repeated division, and the apparent absence, so far as his account goes, of any process of nutrition.

(d) Growth and Feeding of the Young Oöcytes in the Flagellate Chambers.

The growth of the oöcytes may be divided into two very distinct periods, during the first of which they are found in the flagellate chambers, while during the second they lie in the mesoglœa between the chambers.

As already indicated, it is by no means an easy matter to sort out all the very numerous amœboid cells that occur in the flagellate chambers, some representing stages in oögenesis and others stages in spermatogenesis, into their proper categories. Amongst them, however, may be distinguished a type which occurs very abundantly and exhibits certain peculiarities by which it is more or less readily recognised. The cells in question are small and distinctly amœboid, of irregular form, and usually attached to the wall of the chamber by pseudopodia. They have a rather small nucleus, with a relatively large nucleolus surrounded by a narrow clear space, and then by a broad ring of minute granules extending to the nuclear membrane. The cytoplasm usually exhibits more or less numerous inclusions which may be surrounded by vacuoles. Some of these inclusions, which I interpret as chromidia or yolk-nuclei, stain nearly black, others may stain much more lightly, and look like food-particles undergoing digestion. A typical series of these curious cells is shown in figs. 29–39. I interpret them as young oöcytes engaged in feeding operations.

Figs. 29 and 30 show what appears to be the youngest stage of this series, a stage which may be derived from the mitotic division of a secondary oögonium such as is represented in fig. 27 or 28. The cytoplasm at this stage is seen to be uniformly and rather coarsely granular and contains no chromidia or other inclusions of any kind. The structure of the nucleus even at this early period, with its large nucleolus and broad zone of chromatin granules distinctly separated from it, appears to me to be essentially that of an immature female gamete. Presently the characteristic inclusions make

their appearance in the cytoplasm, which otherwise may come to exhibit a more homogeneous appearance (figs. 31–39). Some of these inclusions are comparatively lightly staining bodies enclosed in vacuoles (figs. 31, 36, 37), and in one case (fig. 39) a nucleated cell was distinctly recognised amongst other bodies. I therefore believe that the lighter coloured inclusions are food-particles undergoing digestion, captured by the young oöcytes from the stream of water that flows through the chambers.

The nature of the intensely black stained bodies that appear in the cytoplasm is easily interpreted. These resemble the nucleolus in appearance, but may be much larger (figs. 33–35). They may or may not be surrounded by distinct vacuoles. On the other hand, they may take the form of small granules or groups of granules (figs. 35, 36). Fig. 38 gives the clue to the manner in which they are formed, for here the nucleolus is seen actually discharging part of its own substance into the cytoplasm through the nuclear membrane. I think there can be no doubt that in these cells very active metabolism, accompanied by the formation of chromidia, or "yolk-nuclei," is going on, and that the bodies in question are of this nature, and are concerned in the elaboration of yolk-granules in the cytoplasm.

Apparently feeding now ceases and the remains of food-particles disappear from the cytoplasm. The oöcyte next migrates through the chamber wall into the mesoglœa (fig. 40).

(e) Growth and Feeding of the Oöcytes in the Mesoglœa.

The formation of chromidia, or "yolk-nuclei," by extrusion of matter from the nucleolus, which was already commenced within the chambers (fig. 38), may now be continued very freely. Specimen 23 contains an immense number of oöcytes, lying in the mesoglœa between the chambers, in which this chromidium-formation is going on (figs. 41, 42, 43), and often giving rise to very fantastic appearances. The nucleolus is

evidently in a liquid or semi-liquid state and appears to be squeezed out into the cytoplasm in threads or drops, just as an artist's colours may be squeezed out of their tubes. During this process the nucleus itself may become distinctly pear-shaped (fig. 43). The drops squeezed out into the cytoplasm are at first enclosed in distinct vacuoles, apparently derived from the nuclear sap. In these vacuoles they disintegrate into granules (fig. 43), which probably became scattered through the cytoplasm. The nuclear membrane may become very indistinct during the process, and there are indications that the nucleus is passing into the spireme stage to be described next. It is doubtful whether the nucleolus is ever completely eliminated from the nucleus at this stage. It seems to me more probable that some of it always remains behind as the foundation of the huge nucleolus which forms such a conspicuous feature in later stages of the oöcyte. The irregularity in shape of the oöcyte during this process of chromidium formation indicates that it is still amœboid, and it seems possible that the extrusion of the chromidia may be due to strong contraction of the cytoplasm, though it must be admitted that the mechanism of the process is very obscure.

The whole of the series of stages representing the feeding and growth of the young oöcytes in the chambers and the remarkable process of chromidium-formation just described appears to have been unobserved by Jörgensen. It is true that that observer worked upon Sycon, but it is unlikely that two such closely related types as Sycon and Grantia should differ in this respect, especially when they agree so closely as regards other features of the oögenesis. Jörgensen figures the young oöcyte, supposed to be directly derived from the last oögonial division, as being very similar to the stage represented in my fig. 47a (compare his fig. 32). This stage may very easily be derived, however, through the intermediate stages represented in figs. 41–43, from the last of the feeding stages observed in the chambers (fig. 39).

It also seems very probable that shortly after leaving the flagellate chambers and undergoing the process of chromidium-

formation just described, the young oöcyte exhibits the prophases of a mitosis which really belongs to the first maturation division. Jörgensen puts this incomplete mitosis, represented by a well-marked spireme stage, immediately after the stage which, in Sycon, probably corresponds to my fig. 47a. I prefer to place it immediately before this stage, where it seems to me to fit in better. Figs. 44 and 45 show two of the prophases in question. The former is evidently a leptotene phase and the latter a pachytene. The latter always shows the characteristic contraction of the spireme described by Jörgensen, which may possibly represent a synapsis or be due simply to the action of reagents. Both these figures show well-developed chromidia in the cytoplasm, which, as I have already said, inclines me to include them in the oöcyte series rather than in the oögonial series, though I do not consider that the evidence is by any means conclusive. It is obvious from fig. 45 that the oöcyte may still be highly amœboid during this phase, exhibiting a very irregular outline.

Jörgensen considers that some of the oöcytes at this stage undergo degeneration and may have something to do with forming the nutrient cells for the older oöcytes, but I have obtained no good evidence of such degeneration and my observations on the feeding of the older oöcytes do not support this view. The spireme thread now disappears (fig. 46) and the oöcyte rounds itself off, takes up a definite position in the mesoglœa behind the layer of collared cells (fig. 47), and enters upon its main period of growth.

Fig. 47a represents a condition of the oöcyte which is very commonly met with. Jörgensen figures a similar condition in Sycon (fig. 32), and speaks of it as a resting condition, but, as I have already pointed out, he regards it as the direct product of the division of an oögonium of the second generation. I am not quite sure, however, that Jörgensen's fig. 32 really represents the same stage as my fig. 47a, for he does not show, or if so only very faintly, the chromidia in the cytoplasm, which appear to me to be very characteristic of this stage. It is by the abundance of these chromidia,

indeed, that this stage is chiefly distinguishable from the primary oögonium just before mitosis, as represented in fig. 16, taken in conjunction with the fact that the latter occurs especially in association with the oögonial mitoses going on in the chambers, while the former occurs especially associated with the later stages of oöcyte growth.

In Grantia the young oöcyte, passing out of this "resting condition," simply flattens out somewhat on one side (fig. 47b), and puts out long branching pseudopodia by means of which it attaches itself to the mesoglœal surface of the layer of collared cells, as shown in fig. 48. Whether these pseudopodia are merely "anchoring" pseudopodia, or whether they also serve to extract nutriment for the growing oöcyte from the collared cells with which they are in contact, must for the present remain an open question. The appearances represented in fig. 48, however, suggest to my mind the latter. We are reminded of the manner in which the superficial cells of the embryo of Stelospongus attach themselves to, and evidently draw nutriment from, the large capsule-cells by which they are surrounded, as described by me many years ago (Dendy, 1888). It will be seen that the collared cells contain abundant "reserve-granules," and similar granules appear in the oöcytes, while the chromidia have entirely disappeared from the cytoplasm. The nucleus is distinctly reticulate, with numerous darkly stained chromatin granules and a large nucleolus, the latter surrounded by a clear space (possibly due to shrinkage ?).

The oöcyte continues to increase in size and the nucleus grows more rapidly than the cytoplasm. Presently we reach a stage which is very characteristic and very frequently met with, and which I propose to call the "contraction stage of the oöcyte." This is represented in fig. 49. It will be seen that the entire cell has rounded itself off again and the pseudopodia have contracted into blunt knobs. The nucleus is very large and pretty uniformly granular, all or nearly all the darkly staining chromatin having evidently been expelled into the cytoplasm in the form of chromidia (yolk-nucleus),

which at this period are apparently not derived, at any rate directly, from the nucleolus. The latter is very large, spherical, and fairly darkly staining. This stage, again, does not appear to have been observed by Jörgensen in the case of Sycon.

It is at about this period that the feeding of the oöcyte by means of nurse-cells begins, a process which continues right on until the oöcyte has reached its maximum size or nearly so, and which will be dealt with separately in a later section (see, in the meantime, figs. 50, 51, 52).

During this process the oöcyte increases enormously in size, and long, root-like pseudopodia are put out again (fig. 54) in a plane parallel to the layer of collared cells against which the oöcyte lies. The nucleus assumes the form of an enormous, thin-walled vesicle, containing a reticulum of lightly staining, flocculent material, which looks very much like a precipitate or coagulation, and includes small, darkly staining chromatin granules scattered through it. Most of the darkly staining chromatin, however, appears to be expelled into the cytoplasm in the form of chromidia, which may be extremely numerous (fig. 53). The nucleolus is very large, and frequently exhibits a differentiation into more and less darkly staining spheres, which may or may not be concentric (figs. 52, 53, 54). Occasionally the nucleolus appears to be cast out into the cytoplasm, but, as already stated, I have come to the conclusion that this is an artificial result, due to the action of the knife in cutting. Sometimes the cytoplasm around the nucleus exhibits a faint radial arrangement of its granules, as shown in figs. 52 and 54. This is a feature upon which Jörgensen has laid some stress in the case of Sycon.

(f) Maturation of the Oöcytes.

The oöcyte now withdraws all its pseudopodia and rounds itself off into a compact ellipsoid body about 0·045 mm. in maximum diameter, preparatory to maturation. The huge vesicular nucleus disappears and its contents are apparently

diffused throughout the cytoplasm, which is dense, and, with the exception of certain inclusions to be mentioned immediately, uniformly and rather coarsely granular. At no stage have I seen any vitelline membrane. The cytoplasm contains a large number of very small chromidia, mostly aggregated in irregular groups or clouds (fig. 55), which are probably, in part at any rate, derived directly from the small chromatin granules that remained in the nucleus at the close of its career. The immense nucleolus in two cases (out of the very few met with) was clearly discernible in the cytoplasm, where it appeared to be undergoing absorption (fig. 55, *no.*). In one case a group of small bodies that might be chromosomes (fig. 55, *chr.* ?) was observed in the cytoplasm at some distance from the nucleolus; but the nature of these bodies is really uncertain, as also is the nature of a protrusion of the surface of the oöcyte opposite to the supposed chromosome-group. It is possible that we have here the beginning of the formation of the first polar body, but I think that that is extremely doubtful.

At a slightly later stage, however, the chromosomes appear unmistakably in connection with the first maturation spindle, which is represented in fig. 56. It is obvious that this agrees closely with the first maturation spindle as described by Jörgensen (cf. his fig. 59), but unfortunately I have only been able to find one really good maturation spindle in my preparations, and I am therefore unable to give any details with regard to the process. I am not even sure of the number of chromosomes, but there appear to be eight or ten in each daughter-group represented in fig. 56. Jörgensen makes the number eight in each group, but represents each chromosome as a sort of tetrad. From my own observations I can only say that the chromosomes are small irregular bodies, and it appears to me that, whenever chromosomes are concerned, Jörgensen's figures must be somewhat diagrammatic.

The formation of the first polar body in Grantia is represented in fig. 57, and it is evident that it takes place very much as described by Jörgensen for Sycon. The polar body

itself is seen to be of large size, and the remains of the spindle are seen as a very distinct cord, slightly thickened in the middle, connecting the group of chromosomes in the polar body with the group that remains behind in the oöcyte.

I have searched in vain for the second maturation spindle and second polar body, but, considering the rarity with which the first occurs in my specimens, their apparent absence has no significance. Even Jörgensen, however, was not able fully to observe the formation of the second polar body, though he tells us that apparently it also contains eight chromosomes, while eight (dyads ?) remain in the mature ovum.

(g) The Nurse-Cells and their Origin; Phagocytosis.

A process of feeding on the part of the growing oöcyte at the expense of certain nutrient cells has been described in the case of Sycon by both Görich (1903) and Jörgensen (1910). Görich says that the oöcytes ("Eizelle") ingest entire cells, which are probably themselves egg-cells of smaller size. The process is represented as taking place very much as in the case of an Amœba ingesting food-particles, except that no vacuole is formed around the ingested nutrient cell, whose protoplasm appears to mingle directly with that of the oöcyte. Each nutrient cell appears to be a small nucleated amœbocyte, with a relatively large, darkly staining particle (? chromidium) in the cytoplasm. Numerous very similar bodies, evidently chromidia, are figured in the cytoplasm of the feeding oöcyte, which appears to be at about the stage figured in my figs. 50 and 51 (compare Görich's figs. 1–6).

Jörgensen tells us that the ingestion of nutrient cells by the oöcyte takes place towards the end of the growth period, and goes on right up to the formation of the maturation spindle. The ingested cells are believed to be mostly oögonia and the ingestion is supposed to be due to chemotaxis. The ingested cells are said to be taken into a preformed gullet in

the oöcyte, and not, as described by Görich, by means of pseudopodia. Jörgensen has, however, observed ingestion by means of pseudopodia in the case of Sycon setosum. He also finds the presence of a compact chromidium in the cytoplasm to be characteristic of the nutrient cell. This chromidium may be taken into the gullet of the feeding oöcyte without the nutrient cell, but usually the entire nutrient cell is taken in. The chromidia received by the oöcyte in this way are distinguished by their size from those cast out from the nucleus, but both undergo like degeneration in the cytoplasm.

I have never seen anything resembling the formation of a gullet ("Schlund") in Grantia, and I think that Görich's account of the taking in of the nutrient cells in Sycon seems the more probable of the two.

In Grantia the process is complicated by the intervention of what I propose to term "nurse-cells," which capture the nutrient cells and bring them to the oöcyte. Possibly the supposed taking in by the oöcyte of the chromidium only from the nutrient cell really indicates something of the same kind for Sycon.

I have observed the feeding of the oöcyte by nurse-cells in specimens 11 and 23 and I have seen many instances of it, and I do not think there can be any doubt either as to the observations themselves or as to their interpretation. The peculiar arrangement shown in figs. 50 and 51 is frequently met with in my sections. I interpret it as indicating that a nurse-cell (*n. c.*) has captured a smaller cell which I regard as a nutrient cell or food-cell (*f. c.*), and is passing it into the cytoplasm of the oöcyte. I shall discuss the origin of the nurse-cell directly; in the meantime I may point out that it exhibits certain fairly well-marked characters of its own. Its cytoplasm is thin-looking and stains very lightly, and the nucleus is of moderate size, with a well-developed nuclear membrane and reticulum and a rather small nucleolus. The cytoplasm usually contains conspicuous inclusions which are evidently the remains of ingested and partially disintegrated cells, but nothing that can be identified with the "chromidium"

of the nutrient cells described by Görich and Jörgensen in Sycon; indeed, the nurse-cell does not appear to be itself a nutrient cell in the sense of these authors. The real nutrient cell (*f. c.*) is, however, a very conspicuous object, lying in the middle between the nurse-cell and the oöcyte. It appears to be always an oval or spherical cell with a reticulate nucleus and rather dense, finely granular cytoplasm, and it appears to be passed on to the oöcyte by the nurse-cell before it has undergone any disintegration, for its outlines are perfectly definite. Judging from its small size and the absence of chromidia in the cytoplasm I am inclined to think that the nutrient cell is probably a rounded-off collared cell, but it is impossible to be certain on this point, though I shall bring forward evidence presently to show that the nurse-cells do capture collared cells.

The process of feeding by nurse-cells does not appear to begin until the oöcyte has attained a considerable size, and it may be fed in this way both in the contracted and in the expanded condition, i. e. when the pseudopodia are reduced to short knobs and when they are fully extended. Figs. 50 and 51 represent the process of feeding by the nurse-cells as usually observed. A somewhat different condition is represented in fig. 52. The nurse-cell is here crushed in between the oöcyte, which is now much larger, and the layer of collared cells against which it lies. The nutrient cell has passed completely into the cytoplasm of the oöcyte, where it is surrounded by a small vacuole in which it appears to be undergoing disintegration, for instead of a distinct nucleus it exhibits two masses of chromatin—one at the middle and one at the side. Both nurse-cell and nutrient cell appear to be much smaller than in the cases previously described. The whole arrangement somewhat resembles the taking in of a chromidium from a nutrient cell as described by Jörgensen, but it is probably simply a more advanced stage of the feeding process shown in the preceding figures.

We come now to the question of the origin of the nurse-cells. I think there can be little doubt that they are derived

from small amœbocytes which occur scattered in the mesoglœa, such as is represented in fig. 58. It will be seen that we have here a cell of about the same size as the nurse-cell shown in fig. 51, and, except for the absence of cytoplasmic inclusions, closely resembling it. The cytoplasm is faintly staining and thin-looking, the nucleus is reticulate, with a small nucleolus and a well-developed nuclear membrane, and even the somewhat angular outline of the entire cell appears to be more or less characteristic, and is frequently met with again in the nurse-cells while in the act of feeding the oöcytes (compare figs. 50, 51). Whence these amœbocytes come it is impossible to say. They do not look like metamorphosed collared cells or epithelial cells, and they are very likely derived direct from embryonic amœbocytes. They probably do not all become nurse-cells, for some, which appear to be of the same nature, grow to a large size and may put out long filiform pseudopodia (figs. 59–61) In this extended condition I have found them both inside the flagellate chambers (fig. 60) and in the mesoglœa between them (fig. 61), while in a more or less rounded-off condition they are sometimes to be seen passing through the layer of collared cells, especially in the neighbourhood of the exhalant apertures of the chambers (fig. 59).

Such amœboid cells sometimes develop into very active phagocytes, apparently entirely on their own account. I have observed this in specimens 11, 22 and 24. Specimen 24 in particular is crowded with large and small phagocytes, which have evidently been feeding voraciously, and apparently chiefly on young germ-cells.

Fig. 62 represents a phagocyte from the mesoglœa, which has ingested a single relatively large cell, too large, I think, to be a spermatogonium, and so large that the cytoplasm of the phagocyte is only able to stretch itself around its prey in the form of a thin envelope. In fig. 63 an actively amœboid phagocyte, with filiform pseudopodia, is apparently in the act of ingesting a collared cell. Fig. 64 represents a phagocyte rounded off in the mesoglœa, with two partially disintegrated cells in its cytoplasm. This one looks as if it might have

become a nurse-cell, though rather large. Fig. 65 represents a very actively amœboid phagocyte, while fig. 66 shows one squeezing itself through the layer of collared cells. Fig. 67 shows one with root-like pseudopodia, half in and half out of a chamber; this one has collected and ingested no less than six cells, which I judge from their size and appearance to be spermatogonia. Fig. 68 represents yet another hanging on to the inner surface of the layer of collared cells.

It appears, then, that while some of these amœbocytes exercise their phagocytic propensities in favour of the oöcytes, and become nurse-cells, others feed voraciously on their own account, even entering the flagellate chambers and apparently collecting the numerous young germ-cells that are found therein. It seems not improbable that this excessive phagocytosis may be regarded as a perverted instinct, for the phagocytes appear to take in food far beyond their own possible requirements, and yet I have never seen a large phagocyte feeding an oöcyte. It is perhaps worth noting that specimen 24, in which most of the cases of phagocytosis by large amœbocytes were observed, had been kept in the circulation for a week before being killed and preserved. The abnormal conditions may have stimulated the amœbocytes to an abnormal activity in phagocytosis.

The possibility also occurs to one that some of the large phagocytes are parasitic Amœbæ which do not belong to the sponge at all. Mr. Orton (1913) has recently described Amœbæ from the gastral cavity of Sycon which certainly seem to be quite independent organisms, though I at first thought otherwise (Dendy, 1913), and it seems by no means improbable that such Amœbæ may enter the chambers and feed upon the young germ-cells, or even force their way into the mesoglœa. The chief argument against this view appears to be the impossibility of distinguishing between the larger and smaller phagocytes, and the apparent identity of the latter with the nurse-cells, which must certainly be regarded as belonging to the sponge itself.

(h) Summary and General Remarks on Oögenesis.

It will be seen from the foregoing account of my observations on the oögenesis of Grantia, that in their main features they agree with what has already been recorded, especially by Jörgensen, for the closely related genus Sycon. In some important respects, however, and especially as regards the derivation of the oögonia from collared cells, my observations differ from those of Jörgensen, and, while I have not been able to obtain anything like such precise results as he claims with regard to the mitotic phenomena, I have been able to describe a great deal that has either escaped his notice or does not occur in Sycon, concerning, for example, the feeding of the young oöcytes in the chambers and the subsequent formation of chromidia by extrusion of nucleolar matter into the cytoplasm, the very remarkable feeding of the oöcytes by means of nurse-cells, and the process of phagocytosis in general.

The process of oögenesis in Grantia may be briefly summarised as follows :

The primary oögonia are directly derived from collared cells, which accumulate reserve material, enlarge, withdraw their collars and flagella, become amœboid and wander into the mesoglœa, re-entering the chambers before dividing mitotically into the oögonia of the second generation. Prior to this division the nucleolus appears to be bodily cast out of the oögonium.

The oögonia of the second generation divide again while in the chambers, and probably almost immediately, into small oöcytes.

The small oöcytes become amœboid, and, while still within the chambers, attached by pseudopodia to the layer of collared cells, take in food-particles and form conspicuous chromidia in their cytoplasm. Having increased considerably in size they leave the chambers and enter the mesoglœa.

Here they continue to undergo a process of extensive chromidium-formation by extrusion of chromatin from the nucleolus into the cytoplasm.

They also probably undergo about this time the prophases of the first maturation division.

They now send out anchoring pseudopodia by which they became attached to the layer of collared cells and probably draw nutriment from them.

Presently they undergo a remarkable contraction, the pseudopodia being almost completely withdrawn, and about this time they begin to be fed by special nurse-cells, which collect smaller cells and pass them into the growing oöcyte.

The oöcyte increases greatly in size, and long root-like pseudopodia are again put out in a plane parallel to the layer of collared cells against which it lies.

The nucleus becomes very large and vesicular, with a huge spherical nucleolus, and chromidia are abundantly formed in the cytoplasm, though apparently no longer directly derived from the nucleolus, but formed probably by extrusion of granules of chromatin from the nucleus. The chromidia (yolk-nucleus), whatever their source, are probably concerned in the elaboration of the deutoplasm, with which the cytoplasm becomes uniformly and densely charged, though definite individual yolk-granules can hardly be recognised.

When the oöcyte has reached its full size it withdraws its pseudopodia and rounds itself off, the nuclear membrane disappears and the contents of the nucleus disperse themselves through the cytoplasm. The nucleolus remains recognisable for some time after this event, but gradually becomes absorbed.

Chromosomes have not been recognisable since the oögonial mitoses, when they appeared in the equatorial plate as a group of eight or ten minute, irregularly rounded bodies, each of which presumably divided into two. They now appear again on the first maturation spindle, but only one really good spindle was found, and that already in the anaphase.

The first polar body is formed in apparently a typical manner, exactly as described by Jörgensen for Sycon, and is of large size.

The second maturation spindle and second polar body,

described by Jörgensen for Sycon, were not met with, though they probably occur.

No evidence was obtained of a reducing division, and, indeed, the number of chromosomes could never be accurately counted.

In spite of the fact that we have no reliable information with regard to the phenomena of meiosis in sponges, we cannot fail to be struck with the close general agreement of the process of oögenesis with the same process as observed in higher animals. The multiplication of oögonia; the formation of chromidia or yolk-nucleus by the oöcyte; the early inception of the first maturation division (if this be confirmed), interrupted by the long period of growth; the co-operation of other cells in the process of nutrition; the character of the nucleus and the formation of the polar bodies; are all features which the sponges share with higher groups, and one can hardly avoid asking the question, Does this close similarity in oögenesis point to a nearer relationship of the sponges with the Enterozoa than is usually admitted in this country? The question is well worthy of consideration, but we can hardly hope for a final solution of it in the present state of our knowledge. We can hardly abandon the choano-flagellate ancestry of the sponges without much stronger evidence than we possess, and we certainly are not justified in attributing a choano-flagellate ancestry to other groups of Metazoa. May we, then, suppose that all the essential processes of oögenesis already existed in pre-choano-flagellate Protozoon ancestors common to sponges and Enterozoa? Such a supposition would certainly be in harmony with the view now generally held that the germ-cells of the higher animals are really equivalent to so many Protozoa, for if this be so then the phenomena of oögenesis must be such as we might reasonably expect to find in Protozoa. Recent advances in protozoology, I think, show that such an expectation is likely to be fulfilled, for we already know that the gametes themselves may attain as high a degree of differentiation (into ovum and spermatozoon) in Protozoa as in Metazoa. We also know that the female gamete may accumulate yolk (e. g. in Coccidium), and that

something that may reasonably be interpreted as a formation of polar bodies may take place (e. g. Paramœcium). I venture to predict that a good deal of light will, in the near future, be thrown upon the complex phenomena exhibited in the life-history of many Protozoa, by comparison of the events that take place in the gametogenesis of higher animals. Some more satisfactory and uniform system of terminology will, however, have to be evolved before much progress can be made in this direction. We shall have to know, for example, exactly what we mean by "chromidia." Prof. Minchin (1912) tells us that "in a great many Sarcodina, especially in those belonging to the orders Amœbæa and Foraminifera, chromidia may be present in the gamete-forming individuals as a permanent constituent of the body-structure. In such cases the chromidia represent, wholly or in part, the generative chromatin, and give rise by formation of secondary nuclei to the nuclei of the gametes." In the present paper I have, following Jörgensen, used the term "chromidia" for all the chromatin which occurs in the cytoplasm. This, I think, is probably all extruded from the nucleus (and nucleolus), and is almost certainly concerned in yolk-formation, and therefore "trophochromatin" and not "idiochromatin." The difficulty of distinguishing between these two kinds of "chromatin" forms perhaps the chief obstacle in the way of further progress in the direction indicated.

(G) Spermatogenesis.

(a) Historical.

I have already referred to Haeckel's discovery of the sperm-morulæ of calcareous sponges in the gastral epithelium of the flagellate chambers, and to his opinion that they arise by division of collared cells. These observations never met with general acceptance, and are usually regarded as having been superseded by Poléjaeff's well-known work, 'Über das Sperma und die Spermatogenese bei Sycandra raphanus Haeckel' (1882).

Poléjaeff lays stress upon the discrepancies between the account of the spermatozoa given by Haeckel and that given by Eimer. According to Eimer, the spermatozoa, if not isolated, occur scattered through the tissues, united in millions in oval balls; according to Haeckel, they lie between the collared cells with their tails projecting freely into the cavity of the flagellate chamber, and it is never possible to find them in considerable quantities. According to Eimer, again, they are to be distinguished from the collared cells by the character of the movements of the flagella; while, according to Haeckel, these movements show no essential differences in the two cases. I think it almost certain myself that, although Haeckel saw the sperm-morulæ in the situation he describes, he did not see the tails of the spermatozoa, for the sperm-morulæ represent a comparatively early stage of spermatogenesis at which no tails have yet appeared. Haeckel probably mistook for spermatozoon tails some of the flagella of the collared cells amongst which the sperm-morulæ lie. The discrepancy as to numbers and position is not a very serious matter. Poléjaeff himself has shown how enormously the number of spermatozoa produced differs in different individuals, and I find myself that in Grantia, although the sperm-morulæ are generally to be observed in the walls of the flagellate chambers, or lying free in the chamber-cavities, some of the early stages of spermatogenesis occur in the mesoglœa, while later stages are found in the inhalant canals (possibly of different individuals), and these might well appear in sections to be lying in the tissues.

Poléjaeff (1882) found that in Sycon raphanus, although the sponge is hermaphrodite, the vast majority of individuals are predominantly female, and only very occasionally a predominantly male specimen is forthcoming. In the latter, however, the sperm-balls ("Spermaklumpen") were so numerous that their whole development could be traced in a single section. He derives these sperm-balls from ordinary amœbocytes ("Wanderzellen") in the mesoglœa (mesoderm), similar to those which, in his opinion, give rise to the ova. These

cells have a diameter of 0·008–0·02 mm., and their bright, vesicular nuclei are distinguished by their relatively large size and their highly refractive nucleolus. Such a cell is represented in Poléjaeff's fig. 3*a* as a spherical body with an excentrically placed nucleus. The nucleus now divides into two somewhat unequal parts, which take up their positions at opposite poles of the cell, which becomes differentiated into two corresponding parts, a cover-cell and a sperm mother-cell ("Ursamenzelle"). The cover-cell does not divide again, but the nucleus of the sperm mother-cell divides repeatedly, and finally gives rise to a large number of very minute, granule-like spermatozoon-heads, enclosed within a capsule formed by the cover-cell. Each of these heads presently becomes provided with a cytoplasmic tail. During this process there is no increase in volume of the sperm-ball, and Poléjaeff remarks upon the extraordinarily small size of the spermatozoa. He also points out that the spermatogenesis of Sycon as described by him differs in several respects from that described for non-calcareous sponges by Schulze, Keller and others. Thus the division of the nucleus of the "Ursamenzelle" is not immediately followed by division of the cytoplasm, so that there arises a multinucleate mother-cell and not a true sperm-morula, but he remarks that this is not a matter of any very great importance. More significant, perhaps, is the absence of the endothelial capsule formed around the sperm-ball by the mesoglœa cells in some of the non-calcarea. This, I think, is a matter of some importance in connection with the problem of how the spermatozoa are transferred from one sponge to another. This problem Poléjaeff does not attempt to solve, and, indeed, we are left in doubt, after studying his paper, as to whether or not he considers that self-fertilisation takes place in Sycon. His fig. 1 shows immense quantities of what appear to be spermatozoon heads in the inhalant canals, the chambers and the exhalant canals, as well as sperm-balls in the mesoglœa, but no attempt is made to decide the question whether or not all this mass of sperm has been derived from the same sponge.

I think the investigation of this question would probably have gone a long way towards reconciling the discrepancies between the observations of Haeckel and those of Eimer, and have shown that the cover-cells retain their wandering propensities for some time, and carry the spermatgonia from the mesoglœa into the collared-cell layer, whence they are discharged into the water-stream and carried out of the sponge altogether, possibly to find their way back again, either into the same or into another sponge, through the inhalant canal-system. My own observations clearly indicate that this is the course of events, though there appear to be noteworthy differences in details of behaviour between the two genera Sycon and Grantia.

During the thirty-two years that have passed since the publication of Poléjaeff's memoir, the only contribution that has been made to the very difficult problem of the spermatogenesis in calcareous sponges is that contained in the paper by Wilhelm Görich—"Zur Kenntnis der Spermatogenese bei den Poriferen und Cölenteraten nebst Bemerkungen über die Oogenese der erstern" (1903). This author again deals with the process as exhibited in Sycon raphanus, and although he describes only a few of the earlier stages in this sponge, his results are in one respect strikingly at variance with those of Poléjaeff. He agrees with the latter in deriving the spermatogonia from mesoglœal amœbocytes, which round themselves off at an early stage of their growth as compared with the oögonia. He also describes the formation of a cover-cell, but in a totally different manner from that described by Poléjaeff. The spermatogonium and the cover-cell, though both derived from amœbocytes of the mesoglœa, differ from one another in certain particulars and do not arise by division of a common mother-cell. They only come into relation with one another secondarily, the cover-cell spreading itself around the spermatogonium, and finally ingesting it, in a way which is evidently very similar to the process of phagocytosis already described by me for Grantia. The result, however, is a spermatogonium enclosed in a mother-cell very much as

described by Poléjaeff. The spermatogonium is represented as dividing mitotically into two and then incompletely into four parts, beyond which its history was not followed.

This is all that is known of the spermatogenesis in Calcarea. Amongst other sponges the most frequently and most fully investigated form is Spongilla, and it is perhaps worth while to say a few words about what is known in this case, which seems to be typical of the non-Calcarea, before proceeding to describe my own observations on Grantia.

As far back as 1888, Fiedler published his memoir, "Über Ei- und Spermabildung bei Spongilla fluviatilis." He describes the formation of cover-cell and sperm mother-cell ("Spermatocyte") by division of a common mother-cell ("Spermatogonium") exactly as described by Poléjaeff for Sycon. The cover-cell, however (of which more than one may be found), not infrequently disappears before the contained "spermatocytes" have completed their development, and the mass of sperm, which may have been derived from several "spermatogonia," becomes enclosed in a secondary follicle formed from ordinary mesoglœal cells ("Parenchymzellen"). The original "spermatocyte" divides repeatedly by mitosis into daughter-cells which are completely separated from one another. The smallness of the objects, however, makes the examination of the process very difficult and the details of mitosis are not very satisfactorily given. The last generation of spermatocytes, the spermatids, develop directly into the spermatozoa. A compact chromatin-ball is formed by contraction of the nucleus, as previously described by Schulze for Halisarca (= Oscarella) (1877) and Aplysilla (1878), and the enveloping cytoplasm is drawn out into a slender tail. In the fully formed spermatozoon the chromatin-ball forms a minute spherical head.

Görich (1903) added some interesting particulars as to Spongilla, especially with regard to the structure of the fully formed spermatozoon, in the paper which I have already quoted. He finds that the number of cover-cells taking part in the formation of the capsule or spermatocyst varies from one

to about six, and maintains that these cover-cells are derived from mesoglœal cells distinct from the spermatogonium as in the case of Sycon. He brings forward very little evidence, however, in support of this view. He describes the often repeated mitotic division of the sperm-cells within the spermatocyst very much as it was described by Fiedler. In the fully developed spermatozoa, however, he finds a far more complex structure than had been observed by any of his predecessors, for in addition to the spherical head and long slender tail he describes and figures middle piece, apical body and centrosomes, thus bringing the structure closely into line with that of the spermatozoon in Enterozoa, as exemplified by Aurelia, which he describes and figures in the same paper.

With regard to the explanation of the close resemblance thus established between the spermatogenesis of sponges and that of the Enterozoa, and its bearing upon the relationship of the two groups, I may refer to what I have already said in my summary on the oögenesis.

(b) Origin and Growth of the Primary Spermatogonia.

In returning to Haeckel's view that the spermatozoa are formed by division of collared cells, I must admit that it is extremely difficult to bring forward convincing evidence that this is really the case. Haeckel, of course, was of opinion that the collared cells become divided up into spermatozoa in sitû, and he says nothing of the existence of the spermatocyst or cover-cell described by Poléjaeff and Görich, while both the latter hold that the spermatozoa develop from amœbocytes of the mesoglœa. I believe that the view of each of these authors expresses part of the truth, and I hope that my own observations may serve to account for, and to a large extent to reconcile, the discrepancies between them. The manner in which I have interpreted these observations and arranged the different stages cannot even yet, however, be regarded as more than tentative.

The first stage in spermatogenesis, as in that of oögenesis, appears to be the enlargement of individual collared cells in the lining epithelium of the chambers (figs. 69, 70). At the same time the cytoplasm acquires a peculiar curdled appearance (if I may use this expression for want of a better), which looks as if it might be due to the running together of the reserve granules. Irregular inclusions of large size may thus be formed, around which vacuoles frequently make their appearance. By these appearances it is possible to distinguish between what I believe to be the primary spermatogonia and the primary oögonia respectively, for in the latter (figs. 11, 12) it will be remembered that the reserve granules remain separate and do not run together in irregular masses.

The nucleus now becomes very distinctly reticulate as compared with that of neighbouring collared cells (figs. 71, 72), collar and flagellum are withdrawn, and the cell puts out pseudopodia and becomes amœboid (figs. 72–74). In this condition the primary spermatogonia are to be found hanging into the chamber from the layer of collared cells by means of their pseudopodia. Definite inclusions disappear from the cytoplasm.

The cell now rounds itself off and assumes a very characteristic appearance (figs. 75, 76). It is readily distinguished from the oögonia by its smaller size and by the character of the nucleus. It is also quite different in character from the young oöcytes, which are of about the same size (figs. 29, 30), especially as regards the nucleus, which is coarsely reticulate and without a really well-defined nucleolus, while that of the young oöcyte has a very conspicuous nucleolus surrounded by a clear zone and then by a zone of fine granules.

I have found the primary spermatogonia in their rounded-off condition in the mesoglœa as well as in the flagellate chambers, so that it seems probable that while still in the amœboid state they may migrate through the chamber-walls as the oögonia and oöcytes so frequently do.

It is interesting to observe that the primary spermatogonia exhibit a good deal of variation in size, as is shown in fig.

75. In one case also (fig. 75 a) I have observed mitosis in the free spermatogonium, but I have no evidence that the spermatogonium ever actually divides until it has been provided with a cover-cell.

(c) Formation of the Spermatocysts or Cover-cells.

It will be remembered that according to Poléjaeff the original amœbocyte in Sycon divides into two parts, one of which forms the cover-cell and the other the primary spermatogonium, while Görich claims that the cover-cell is formed by an independent amœbocyte which approaches and envelopes the spermatogonium in the mesoglœa. My own observations strongly support the latter view, and I regard the process of envelopment of the spermatogonium by the cover-cell as a special case of phagocytosis. Indeed, as already pointed out, spermatogonia are frequently ingested by the phagocytes, and it is difficult, if not impossible, to distinguish an ordinary case of phagocytosis in which only a single spermatogonium has been ingested, from a case of cover-cell formation (cf. fig. 62, in which the ingested cell, however, seems too large to be a spermatogonium). Figs. 77, 80 and 81 represent spermatocysts, with enclosed primary spermatogonia, lying in the mesoglœa. In these cases the cover-cell appears to resemble closely a small phagocyte such as gives rise to the nurse-cells (cf. fig. 58).

The majority of the spermatocysts, however, are found lying in the walls of the flagellate chambers between the collared cells, with the enclosed spermatogonium either still undivided, as shown in figs. 78 and 79, or in process of division, as shown in fig. 82, or, much more frequently, divided up into a sperm-morula (figs. 84, 85). When in this position the spermatocyst certainly looks very much as if it were derived in sitû from a collared cell. Sometimes, it is true, the nucleus is distinguishable from that of adjacent collared cells by its reticulate character (figs. 82, 85), but in other cases (figs. 78, 84) no such distinction can be made out.

I have already pointed out, however, that the nuclei of the collared cells themselves vary very greatly in appearance, and that reticulate and uniformly dark-stained nuclei may occur in adjacent cells. Sometimes the cytoplasm of the cover-cell may even contain reserve granules like those found in the collared cells (fig. 85).

The history of the spermatogonia themselves, however, and the phagocytosis observed in the mesoglœa, seem to me to indicate very clearly that the spermatocysts so often seen lying between the collared cells have reached their position by migration, and there is no sufficient reason for concluding that the cover-cells are ever derived from collared cells.

As the spermatocyst lies in the collared cell layer its nucleus is situate, usually, at any rate, towards the lumen of the chamber, just as are the nuclei of the collared cells themselves, and the spermatogonium or sperm-morula is enclosed in its basal portion (figs. 78, 82, 84).

As the sperm-morula develops the cyst formed by the cover-cell becomes extremely thin (fig. 87) and finally ruptures, discharging the sperm-morula into the cavity of the chamber (fig. 83). Thus the liberation of the sperm-morula from the cover-cell takes place much earlier than in the case of Sycon, where, according to Poléjaeff, spermatozoa are formed while still within the cysts.

(d) Development of the Sperm-morulæ from the Primary Spermatogonia.

The first division of the primary spermatogonium at least appears to take place mitotically, as has already been described by Görich for Sycon. At any rate one sometimes finds spermatogonia in which the nucleus has disappeared, and what appear to be small, scattered chromosomes, eight or ten in number, are scattered through the cytoplasm as shown in figs. 81 and 82, while fig. 80 represents what may be a spireme stage.

I have only once observed what appears to be the two-

celled stage of the sperm-morula (fig. 83), and I regard this as a somewhat doubtful case; it shows, however, two distinct spherical bodies, which I take to be secondary spermatogonia, enclosed in what is presumably a cover-cell (*cov.*) lying in the layer of collared cells.

The four-celled stage I have never been able to find, in spite of prolonged searching. The eight-celled stage, however, I have seen several times (figs. 84, 85, 86), and it appears that the sperm-morula may be liberated from the spermatocyst as early as this (fig. 86).

A stage in which the morula consists of sixteen cells, or thereabouts, is the most frequent in my preparations. This stage sometimes occurs still enclosed in the cover-cell (fig. 87), but more frequently lying free in the cavity of the flagellate chamber (figs. 88–92). A remarkable feature of the eight-celled and sixteen-celled stages is the extraordinary distinctness with which the daughter-spermatogonia are defined, but the exact appearance evidently depends somewhat upon the method of preparation. Very often they appear as little heaps of highly refractive spherical balls, like small shot, stained black or nearly so (figs. 83, 88). At other times they are much more lightly stained, and one or more minute, more darkly stained granules appear within them (figs. 86, 87, 89, 90). How the division takes place I cannot say, but I have seen no sign of mitosis after the first division of the primary spermatogonium. Whether or not the spherical bodies represent entire cells, or nuclei only, I am also unable to say positively, but I conclude from their general appearance that the former is the case. It will be noticed that there is considerable difference in size between the spermatogonia in different sperm-morulæ of apparently the same stage of development (cf. figs. 87, 88), but this is only what might be expected from the differences in size of the primary spermatogonia already mentioned. Although usually spherical, the daughter-spermatogonia sometimes appear to be polygonal from mutual pressure (figs. 89, 91).

A noteworthy feature is the appearance between them in

the sperm-morula of a substance that looks like residual protoplasm (figs. 86–92). As development proceeds (at the sixteen-celled stage), this material seems to swell up so as to separate the spermatogonia more or less from one another.

(e) The Formation of Spermatozoa; Comparison with Sycon, etc.

As to how the spermatozoa are developed from the sperm-morulæ in Grantia I have no definite information to offer. My material does not suffice to settle this question, but I have some reason to believe that the spermatogonia of the sixteen-celled stage undergo further repeated subdivision. I believe that this usually takes place after the sperm-morulæ have been transferred by the water currents to the inhalant canals of another individual. I have occasionally found, both in the inhalant canals and flagellate chambers, small masses of darkly stained granules (fig. 93) which appear to be identical with the masses of granules which Poléjaeff showed to be spermatozoon heads in Sycon. If they be of this nature, as I think highly probable, their minute size certainly serves to indicate a further breaking up of the spermatogonia of the sixteen-celled stage. I have also a small amount of direct evidence of such breaking up of the sperm-morulæ in the inhalant canals, but it is not conclusive enough to bring forward definitely.

Poléjaeff tells us that in Sycon the spermatozoa are formed by repeated subdivision of the spermatogonia within the cover-cell, during which process the spermatocyst does not increase in size. The final products of these divisions are represented as being extremely minute, and each becomes provided with a slender flagellum.

I happen to have in my possession one of Poléjaeff's own preparations of Sycon, sent by him to Mr. Carter in 1883. This preparation shows "sperm-balls" (sperm-morulæ) in the inhalant canals, and I suppose them to have come from another individual. The sperm-balls vary considerably in

size (fig. 94) and each is still enclosed in the cover-cell, whose nucleus is very distinctly visible. In the interior a number of ill-defined, faintly stained bodies are present, which may be daughter-spermatogonia, or, as Poléjaeff supposes, merely the nuclei of these. They are nothing like so definite as the spermatogonia which I find in the sperm-morulæ of Grantia, but this may be partly because they belong to a later stage of development, and partly because of differences in the mode of preparation. According to the information given in his memoir Poléjaeff's preparations were made with osmic acid (0·01–0·05 per cent.) material stained with alum carmine, and this probably applies to the preparation in my possession.

I give in fig. 94 drawings of two sperm-balls from this preparation. It will be seen that they are of just about the same size as the spermatocysts with enclosed primary spermatogonia in Grantia (cf. figs. 77, 78, 81), and, allowing for the longer retention of the cover-cell and the further subdivision of the spermatogonia after the sixteen-celled stage, I think there is no serious discrepancy. I am unable to make a direct comparison between the earlier stages in the two genera as I have found none of these in Poléjaeff's preparations.

That the sperm-morulæ found by me in Grantia are identical with the structures already referred to as described by Haeckel in the lining epithelium of the flagellate chambers of various calcareous sponges, appears to me to admit of very little doubt, although of course I cannot agree with him in the details of his account.

It has occurred to me as just possible that someone may suggest that these bodies are not sperm-morulæ at all, but of quite a different nature; that they may be Sporozoa living for a period as intracellular parasites in the collared cells and possibly also in the amœbocytes of the sponge. Apart from the fact, however, that sporozoan parasites have never yet been observed in sponges, I think the developmental history of the bodies in question is sufficient to negative this view. No infection of the sponge-cells by small forms that might be young Sporozoa has ever been observed, and the cell which

is surrounded by the cover-cell, and which I believe to be the primary spermatogonium, has nothing about it to suggest a sporozoon.

I can only regret that I am unable to give a more satisfactory account of the spermatogenesis, but I hope that what I have said will arouse more interest in this extremely difficult problem, and enable future workers at any rate easily to find the structures in question, and perhaps fill up the many gaps which I have left.

(h) Fertilisation of the Ovum.

I have not been fortunate enough to observe the actual entrance of the spermatozoon into the mature ovum. According to Jörgensen (in Sycon), the head swells up and gives rise to the male pronucleus, in which a nucleolus very soon makes its appearance. The female pronucleus has in the meantime developed from the group of chromosomes that remained behind in the ovum after the formation of the second polar body. These unite together into a compact chromatin ball, around which a vacuole, enclosed in a nuclear membrane, makes its appearance. A nucleolus is very early differentiated in the midst of this mass, the remainder of which is broken up into chromatin granules, which become scattered over the nuclear reticulum.

Sometimes a portion of one of the pronuclei (either male or female) is represented by a small separate "karyomere," so that there appear to be three nuclei in the fertilised egg. The number of nucleoli present in the pronuclei varies.

So far as they go my own observations on Grantia are entirely in harmony with these results. I have been able to study four ova with well-developed male and female pronuclei. In only one of these cases were the two pronuclei both single, and in this case the number of nucleoli was either 4 + 1 or 3 + 2, one of them having been displaced in the cutting. In the other three cases a karyomere was present in addition to the principal pronuclei, and the numbers and distribution of

the nucleoli were 1 + 1 + 1, 1 + 1 + 2, and 1 + 1 + 2 respectively.

Fig. 95 shows one of these cases. The position of the degenerating polar body (*p. b.*) shows that the large pronucleus with the single large nucleolus is evidently the principal female pronucleus, and the small karyomere, with single nucleolus, doubtless belongs to it. The male pronucleus, on the right and below, has two nucleoli.

The formation of karyomeres is a very curious and striking phenomenon, for further details as to which I may refer the reader to Jörgensen's paper. That author points out that karyomere formation also takes place in Sycon in the process of segmentation of the fertilised ovum, and I find the same to be true in the case of Grantia.

It is perhaps worth while pointing out that the nucleoli in the male and female pronuclei do not stain nearly so deeply as the nucleoli of the young oöcytes. The same is also true of the nucleoli in the older oöcytes. The difference may perhaps be correlated with the fact that in the young oöcytes the nucleolus is actively engaged in the formation of chromidia or "yolk-nuclei," while probably it is not directly engaged in this process in the older oöcytes, and certainly not in the fertilised ovum, where yolk-formation has ceased.

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(L) EXPLANATION OF PLATES 23 TO 26,

Illustrating Prof. Arthur Dendy's paper, "Observations on the Gametogenesis of Grantia compressa.

[All figures are magnified about 1650 diameters, and, with the exception of fig. 94, refer to Grantia compressa.]

PLATE 23.

Fig. 1.—Collared cell putting out pseudopodia, from teased preparation of living sponge. *col.* Collar. (Unstained.)

Fig. 2.—Collared cell (*a*), with flagellum still present, putting out pseudopodia and retreating into the mesoglœa from the gastral epithelium. *b.*, *b.* Collared cells still in position in the gastral epithelium. (Specimen 24. Borax carmine.)

Fig. 3.—Amœbocyte (*a*) lying behind the gastral epithelium and probably derived from a collared cell. *b.*, *b.* Collared cells. (Specimen 24. Borax carmine.)

Fig. 4.—Portion of section at right angles to the gastral surface, showing granular epithelial cell (*a*) in the "flask-shaped" condition at the surface, and amœbocyte (*b*) in the mesoglœa. *sp.* Spicules. (Specimen 24. Iron brazilin.)

Fig. 5.—Amœbocyte in the mesoglœa just beneath the gastral cortex. (Specimen 24. Iron brazilin.)

Fig. 6.—Portion of section at right angles to the gastral cortex, showing parts of two granular epithelial cells (*a*) in position on the surface, and a group of amœbocytes (*b*) lying in the mesoglœa and evidently derived by immigration from the epithelial layer. *p. g.* A mass of pigment-granules apparently discharged from an amœbocyte. (Specimen 24. Iron brazilin.)

Fig. 7.—Portion of section through inhalant canal (*i. c.*) and adjacent mesoglœa, showing transition from epithelial to amœboid cells. (Specimen 24. Iron brazilin.)

Fig. 8.—Corresponding portions of two consecutive sections taken tangentially through the layer of collared cells, (*a*) through the nuclei, (*b*) through the cytoplasm below the nuclei; showing variations in the intensity of staining of the reserve granules. α, β, γ, $\hat{c}$, are identical cells in the two sections. (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 9.—A collared cell (?) in mitosis, showing the two groups of daughter-chromosomes, seen end on. (Specimen 24. Borax carmine.)

Fig. 10.—Two adjacent collared cells, one in the normal condition and the other beginning to enlarge to form a primary germ-cell. (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 11.—Part of section through the exhalant opening (*e. o.*) of a flagellate chamber, showing two normal collared cells and the conversion of another into a primary oögonium (*p. o.*). *g. c.* gastral surface. (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 12.—Part of a similar section showing another primary oögonium (*p. o.*) with remains of collar and flagellum and a pseudopodial process wedged in between the adjacent collared cells. (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 13.—Primary oögonium (*p. o.*) after complete loss of collar and flagellum, migrating into the mesoglœa from the layer of collared cells (*c. c.*). (Specimen 24. Borax carmine.)

Fig. 14.—Actively amœboid primary oögonium in the mesoglœa. (Specimen 24. Borax carmine.)

Fig. 15.—Primary oögonium in which small granules of chromatin are beginning to appear in the nucleus around the nucleolus. (Specimen 24. Borax carmine.)

Fig. 16.—Primary oögonium rounding itself off and entering upon resting stage before entering flagellate chamber. (Specimen 22. Iron brazilin.)

Figs. 17, 18.—Two primary oögonia in prophase of mitosis (spireme); the one on the right (fig. 18) showing the expulsion of the nucleolus. *c. c.* Collared cells. (Specimen 22. Iron brazilin.)

Fig. 19.—Primary oögonium in mitosis in flagellate chamber (late prophase), showing equatorial plate, spindle and centrosomes, with chromidia in cytoplasm. (Specimen 11. Iron brazilin.)

Fig. 20.—Oögonium in mitosis in chamber. Same stage as last, but of smaller size. (Specimen 22. Iron brazilin.)

Fig. 21.—Primary oögonium in mitosis (metaphase) forcing its way between the collared cells into a flagellate chamber. (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 22.—Primary oögonium in mitosis in chamber (anaphase), showing separation of the groups of daughter-chromosomes on the spindle. (Specimen 11. Iron brazilin.)

Fig. 23.—Primary oögonium in mitosis in chamber (late anaphase). (Specimen 21. Paracarmine.)

Fig. 24.—Oögonium in mitosis in chamber (late anaphase). (Specimen 22. Iron-brazilin.)

Fig. 25.—Primary oögonium in mitosis in chamber (telophase), showing cell-division; chromidia still visible in cytoplasm. (Specimen 22. Iron hæmatoxylin.)

Fig. 26.—Two daughter-cells (secondary oögonia) resulting from division of primary oögonium, in chamber; nuclei not yet completely reconstituted; chromidia still visible in cytoplasm. (Specimen 22. Iron hæmatoxylin.)

Fig. 27.—Secondary oögonium lying in flagellate chamber, with reconstituted nucleus. *c. c.* Collared cells. (Specimen 11. Iron-hæmatoxylin and picro-indigo carmine.)

Fig. 28.—Secondary oögonium in chamber, in mitosis. (Specimen 22. Iron brazilin.)

Fig. 29.—Young oöcyte, attached to wall of flagellate chamber by pseudopodium. *c. c.* Collared cells. (Specimen 22. Iron brazilin.)

Fig. 30.—Young oöcyte in chamber. (Specimen 22. Iron brazilin.)

Fig. 31.—Young oöcyte in chamber, with one inclusion in the cytoplasm. (Specimen 22. Iron brazilin.)

Fig. 32.—Young oöcyte in chamber, with cytoplasmic inclusions. (Specimen 24. Iron brazilin.)

Figs. 33–35.—Young oöcytes in chambers, with very darkly stained chromidia or "yolk-nuclei." (Specimen 22. Iron brazilin.)

Fig. 36.—Young oöcyte in chamber, with food-particles in vacuoles, and chromidia. (Specimen 22. Iron hæmatoxylin.)

Fig. 37.—Young oöcyte in chamber, with food-particle in vacuole. (Specimen 22. Iron hæmatoxylin.)

Fig. 38.—Young oöcyte in chamber, showing formation of chromidia (yolk-nuclei) by expulsion of chromatin from nucleolus. (Specimen 23. Iron brazilin.)

Fig. 39.—Young oöcyte in chamber, attached to wall at *a*, *a*, containing chromidia or "yolk-nuclei" and remains of ingested cells; *nu.*, nucleus of oöcyte; *n. i. c.*, nucleus of ingested cell. (Specimen 23. Iron brazilin.)

PLATE 24.

Fig. 40.—Young oöcyte leaving a flagellate chamber after feeding, and entering the mesoglœa. *c. c.* Collared cells. (Specimen 11. Iron-hæmatoxylin and picro-indigo carmine.)

Figs. 41–43.—Formation of chromidia or "yolk-nuclei" of the young oöcyte (in the mesoglœa) by extrusion of chromatin from the nucleolus nto the cytoplasm. (Specimen 23. Iron brazilin.)

Fig. 44.—Young oöcyte in prophase of mitosis. Leptotene stage. (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 45.—Young oöcyte in prophase of mitosis. Pachytene stage, showing contraction of the skein. (Specimen 22. Iron hæmatoxylin.)

Fig. 46.—Young oöcyte immediately after the prophase of mitosis, the spireme broken up again. (Specimen 22. Iron hæmatoxylin.)

Fig. 47.—*a.* Oöcyte in "resting condition" behind wall of chamber. *b.* A similar oöcyte flattening itself out against the layer of collared cells (*c.c.*). (Specimen 11. Iron hæmatoxylin and picro-indigo carmine.)

Fig. 48.—Two oöcytes in the mesoglœa, attached by "anchoring pseudopodia" to the layer of collared cells (*c.c.*). (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 49.—Oöcyte in contraction stage, with pseudopodia reduced to blunt knobs. (Specimen 11. Iron hæmatoxylin.)

Figs. 50, 51.—Oöcytes being fed by nurse-cells (*n. c.*), which have captured food-cells (*f. c.*) and are passing them into the oöcyte. (Specimen 11. Iron brazilin.)

Fig. 52.—Oöcyte of nearly full size, lying behind the layer of collared cells (*c.c.*). *ps.* Base of pseudopodium which has been cut off. *n. c.* Nurse-cell, with food-cell lying in the cytoplasm of the oöcyte beneath it. (Specimen 11. Iron brazilin.)

Fig. 53.—Oöcyte of nearly full size, showing very strong development of chromidia ("yolk-nuclei") in cytoplasm. *ps.* Base of pseudopodium that has been cut off; the other pseudopodia are altogether missed by the section. (Specimen 11. Iron brazilin.)

Fig. 54.—Oöcyte of approximately full size, just before withdrawal of the pseudopodia and rounding off. (Specimen 11. Iron brazilin.)

PLATE 25.

Fig. 55.—Oöcyte after withdrawal of pseudopodia and disruption of the nucleus. *no.* Nucleolus undergoing absorption in the cytoplasm (more distinct in next section). *chr.* (?) Group of chromosomes (?). (Specimen 23. Iron brazilin.)

Fig. 56.—Tangential section of maturing oöcyte with first maturation spindle in anaphase. (Specimen 11. Iron brazilin.)

Fig. 57.—Tangential section of maturing oöcyte, showing formation of first polar body (*p. b.*). (Specimen 11. Iron brazilin.)

Fig. 58.—Small amœbocyte of the phagocyte (nurse-cell) type from the mesoglœa between the chambers. (Specimen 11. Iron brazilin.)

Fig. 59.—Large amœbocyte of the phagocyte type; forcing its way through the layer of collared cells (*col.*) near the exhalant opening of a chamber. (Specimen 21. Paracarmine.)

Fig. 60.—Large amœbocyte of the phagocyte type, found in flagellate chamber. (Specimen 24. Borax carmine.)

Fig. 61.—Large amœbocyte of the phagocyte type, from the mesoglœa between the chambers, stretched out against the layer of collared cells (*c. c.*), some only of which are shown. (Specimen 22. Iron hæmatoxylin.)

Fig. 62.—Small phagocyte, with single large ingested cell; from mesoglœa between chambers. (Specimen 24 Borax carmine.)

Fig. 63.—Phagocyte (*ph.*) engaged in ingesting the end collared cell of a row, *i. e.* the one next to the exhalant opening of a chamber. *c. c.* Collared cells. *ps.* Pseudopodium cut off from another amœbocyte. (Specimen 24. Iron brazilin.)

Fig. 64.—Resting phagocyte (nurse-cell?) in mesoglœa just outside layer of collared cells (*c. c.*), with two ingested cells in process of digestion. *n. p.* Nucleus of phagocyte. (Specimen 11. Iron hæmatoxylin.)

Fig. 65.—Active phagocyte in mesoglœa outside chamber, attached by pseudopodia to the outer surfaces of the collared cells (*c. c.*), *i. c.* ingested cells. *n. p.* Nucleus of phagocyte. (Specimen 24. Iron brazilin.)

Fig. 66.—Phagocyte forcing its way through layer of collared cells. Lettering as before. (Specimen 24. Borax carmine.)

Fig. 67.—Large phagocyte with six ingested cells, forcing its way through the layer of collared cells. Lettering as before. (Specimen 24. Borax carmine.)

Fig. 68.—Large phagocyte with four ingested cells, lying in flagellate chamber and attached by pseudopodia to the layer of collared cells. Lettering as before. (Specimen 24. Borax carmine and picro-indigo carmine.)

PLATE 26.

Fig. 69.—Section through parts of two flagellate chambers, adjacent to the exhalant openings, showing one of the collared cells enlarging to form a primary spermatogonium (*p. s.*). (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 70.—Another example of a collared cell enlarging to form a primary spermatogonium. (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 71.—Section through lining epithelium of flagellate chamber showing one of the collared cells enlarging to form a primary spermatogonium (*p. s.*). (Specimen 21. Paracarmine and picro-indigo carmine.)

Fig. 72.—Metamorphosis of enlarged collared cell into a primary spermatogonium (*p. s.*). *c. c.* Ordinary collared cell. (Specimen 24. Borax carmine.)

Figs. 73, 74.—Primary spermatogonia (*p. s.*) in the amœboid condition, attached by pseudopodia to the layer of collared cells (*c. c*). (Specimen 24. Borax carmine.) (Fig. 74 rather doubtful; it has a distinct but not very darkly staining nucleolus with a clear ring around it, and is rather large for a spermatogonium.)

Fig. 75.—Group of primary spermatogonia rounded off; from flagellate chamber. Fig. 75 a.—Another example from an adjacent chamber, in mitosis. (Specimen 24. Borax carmine.)

Fig. 76.—Primary spermatogonium rounded off; from mesoglœa outside chamber. (Specimen 24. Borax carmine.)

Fig. 77.—Primary spermatogonium (*p. s.*) enclosed by cover-cell (*cov.*), behind layer of collared cells (*c. c*). (Specimen 24. Borax carmine.)

Fig. 78.—Cover-cell (*cov.*) and primary spermatogonium (*p. s.*) lying in layer of collared cells (*c. c.*). (Specimen 24. Borax carmine.)

Fig. 79.—Cover-cell (*cov.*) and primary spermatogonium (*p. s.*) lying in layer of collared cells (*c. c.*). (Specimen 24. Iron brazilin.)

Figs. 80, 81.—Primary spermatogonia undergoing mitosis within cover-cells; from mesoglœa. (Specimen 24. Iron brazilin.)

Fig. 82.—Cover-cell (*cov.*) containing primary spermatogonium (*p. s.*) in mitosis, lying in layer of collared cells (*c. c.*). (Specimen 24. Borax carmine.)

Fig. 83.—Section through wall of chamber, showing cover-cell (*cov.*) containing nucleus and two secondary spermatogonia; also 16-celled sperm-morula (*sp. m.*) that has escaped from its cover-cell, the remnant of which (?) is shown at x. *c. c.* Collared cells. *ep.* Epithelial cell of inhalant canal. (Specimen 24. Iron brazilin.)

Fig. 84.—Section through wall of flagellate chamber and inhalant canal, showing cover-cell (*cov.*) with sperm-morula of 8 cells (*sp. m.*), lying in layer of collared cells (*c. c.*). *phag.* Phagocyte with included cells lying in mesoglœa. *ep.* Epithelial cells of inhalant canal. (Specimen 24. Borax carmine.)

Fig. 85.—Sperm-morula of probably 8 cells (*sp. m.*) enclosed in cover-cell (*cov.*), with adjacent collared cell (*c. c.*), in wall of flagellate chamber; seen *en face*. (Specimen 24. Borax carmine.)

Fig. 86.—Eight-celled sperm-morula lying free in cavity of chamber, without cover-cell. (Specimen 24. Iron brazilin.)

Fig. 87.—Sperm-morula of probably 16 cells still enclosed in cover-cell, in lining epithelium of chamber. *n. cov.* Nucleus of cover-cell. (Specimen 24. Borax carmine.)

Fig. 88.—Sperm-morula of probably 16 cells, lying free in chamber and partially broken up. (Specimen 24. Iron brazilin.)

Fig. 89.—Sperm-morula of probably 16 cells, after escape from cover-cell; seen in optical section. (Specimen 24. Iron brazilin.)

Fig. 90.—Sperm-morula of probably 16 cells, lying free in chamber after escape from cover-cell. (Specimen 24. Iron brazilin.)

Fig. 91.—Sperm-morula of probably 16 cells, lying free in chamber after escape fron cover-cell, showing large amount of residual protoplasm. (Specimen 24. Borax carmine.)

Fig. 92.—Sperm-morula of probably 16 cells, lying free in chamber after escape from cover-cell, with swollen residual protoplasm. (Specimen 24. Borax carmine.)

Fig. 93.—Group of spermatozoon heads (?) from flagellate chamber. (Specimen 21. Paracarmine.)

Fig. 94.—Two "sperm-balls" from inhalant canal of Sycon sp. *n. cov.* Nucleus of cover-cell. (From one of Dr. Poléjaeff's preparations.)

Fig. 95.—Ovum during fertilisation, with male pronucleus containing two nucleoli, and female pronucleus represented by two karyomeres with one nucleolus each. *p. b.* Remains of polar body. x . . . x Line of contact of ovum with layer of collared cells. (Specimen 23. Iron brazilin.)

The Chromosome Complex of Culex Pipiens.

By
Monica Taylor, S.N.D., B.Sc.

With Plates 27 and 28, and 3 Text-figures.

Contents.

Introduction.

Miss Stevens, in a paper entitled "The Chromosomes in the Germ-Cells of Culex," gave as one of her conclusions the following:

"Parasynapsis (parasyndesis[1]) occurs in each cell generation of the germ-cells, the homologous maternal and paternal chromosomes being paired in telophase and remaining so until the metaphase of the next mitosis."

Because of the importance of this discovery, especially in the support it lends to the recent theory of parasyndesis, and because of its bearing on the theory of the individuality of the chromosomes, Dr. Agar, in the summer of 1912, at Tay-

[1] I have adopted the word "syndesis" for the conjugation of the chromosomes, and "synizesis" for their clumping together.

vallich, Loch Sween, collected and preserved material in order to investigate the germ-cells of Culex pipiens. This he very kindly gave to me, and in the spring and summer of 1913 a supply of egg-rafts, larvæ, and pupæ from Milngavie and Skelmorlie has been used in conjunction with the original stock.

The results obtained by a study of the Tayvallich material showed clearly that a much more extensive investigation than was originally intended would be necessary in order completely to elucidate the problems that incidentally presented themselves. Hence many of the egg-rafts and larvæ were placed in artificial ponds in order that greater control might be exercised over the material to be fixed, and single specimens were isolated so that their exact age could be determined, and their periods of ecdysis watched.

The life-history of Culex pipiens is to be found set forth in innumerable text-books of Natural History, and much has been written about mosquitoes in connection with malaria, but in no case have I been able to find any adequate account of the behaviour and fate of the imagines which hatch out and live in captivity, nor of the time that elapses between the emergence of the imago and the deposition of eggs. Large numbers of pupæ, developed, some under natural, others under artificial, conditions, from eggs obtained in May or August of 1913, were placed in small ponds which were covered over with large cages made of mosquito-netting so that the resulting imagines could be observed. From many hundreds of these captive-reared creatures I have not succeeded in obtaining any egg-rafts, although some of the imagines have lived for four months. Nor could they be induced to suck blood, which, according to the account of some naturalists, is necessary, even in non-tropical forms of gnats, for the development of the eggs.

A comparison of the spermathecæ of adults which have always been captive with those of adults taken in the open shows that the former never contain spermatozoa, although the latter do. Hence it would appear that captivity is not

favourable to fertilisation. The completion of this study by the detailed investigation of the maturation and fertilisation of the egg-cell will have to be postponed for an indefinite time until a developmental series of imagines caught in the open has been secured, or until the technique of artificial rearing has been mastered.

Another interesting feature in connection with the larvæ is the want of uniformity in the periods of metamorphosis. Although the main body of any collection of larvæ will complete their development in the usual time, there are always some laggards who double or even treble the usual periods. Temperature and food-supply do not wholly account for this retarded development.

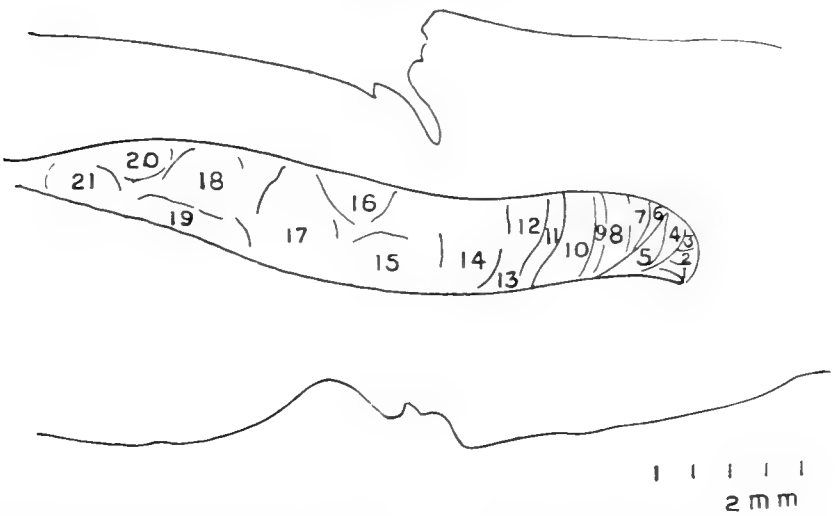
The fixatives employed have been Benda's fluid, acetic bichromate, Gilson's mercuro-nitric, Flemming, and Gilson-Petrunkewitch, the two latter being most successful for the cytology proper; the two former were useful for interpreting cytoplasmic details.

Thionin, iron-hæmatoxylin (prolonged staining), as well as Mayer's cochineal, Ehrlich's hæmatoxylin and safranin were the stains employed. Many slides were first studied in thionin, and then the cover-slip was removed, the thionin washed out, the sections re-stained in iron-hæmatoxylin, and comparisons made between the results of the two stains. For certain stages after prolonged treatment with iron-hæmatoxylin much extraction was necessary, for others little, so that the same slide had often to be studied under various degrees of extraction.

Although aceto-carmine preparations of the whole gonad are very useful for mapping out quickly the main facts of spermatogenesis, and although this stain has the advantage, as Miss Stevens has pointed out, and as my experience has confirmed, of increasing the size of the cellular elements and of thus rendering them easier of examination, they are not permanent, not so good for finer details, and not useful for somatic mitosis. Hence the figures given in this paper have been taken from sections (thickness ranging from 4 μ to 12 μ) and not from aceto-carmine preparations.

Miss Stevens worked on Culex pungens,[1] a form which is very nearly allied to C. pipiens. It is probable that this close relationship between C. pipiens and C. pungens accounts for the great similarity which exists between her figures of primary and secondary spermatocyte anaphases and telophases and those that occur in C. pipiens.

Text-fig. 1.



Outline of testis of pupa of Culex pipiens to show level at which the various stages in spermatogenesis commonly occur. 1–4. Synizesis stage. 5–9. Preparation for spermatocyte 1. 10. Spermatocyte 1. 11, 12. Spermatocyte 2. 13. Spermatids. 14, etc. Spermatozoa in different stages of differentiation.

The Reproductive Organs.

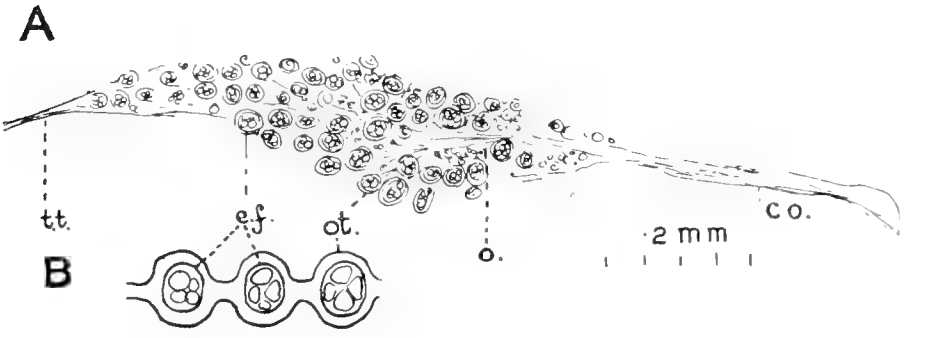
The post-embryonic development of Culex has been worked out by Hurst (**1**). The testes are paired, cylindrical in shape, and possess no receptaculæ seminales, the ripe spermatozoa being stored in the spermiducts.

The ovaries (Text-figs. 2 and 3) are paired and cylindrical, each consisting of large numbers of ovarian tubes which all open into a single duct. The two ducts, one from each side, join to form a common oviduct into which three spermathecæ open.

[1] In a note appended to her paper, however, she expresses a fear that two species were used for her research.

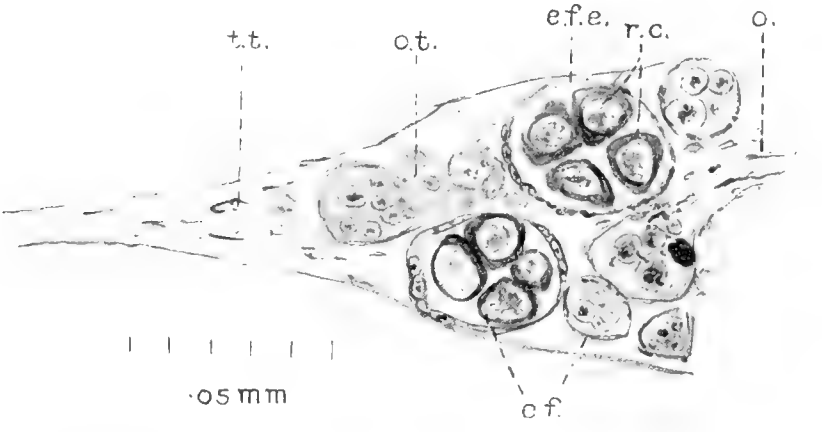
The gonad proper is contained in the third segment from the hind end of the larva or pupa, which also is true for C. pungens (Stevens (2)).

TEXT-FIG. 2.



A. Section through ovary of Culex pipiens. *c. o.* Common oviduct. *e. f.* Egg-follicle. *o.* Oviduct of right ovary. *o. t.* Ovarian tube. *t. t.* Tracheal tube. B. Diagram of ovarian tube with egg-follicles.

TEXT-FIG. 3.



Section through ovary of Culex pipiens. *e. f.* Egg-follicle. *e. f. e.* Egg-follicle epithelium. *o.* Oviduct. *o. t.* Ovarian tube. *r. c.* Reproductive cells. *t. t.* Tracheal tube.

SPERMATOGENESIS.

A figure of a pupal testis drawn from a reconstruction is given in Text-fig. 1. It will be seen that the testis is divided up into a number of small compartments by walls roughly at right angles to the long axis of the organ. These cysts contain cells in different stages of development, the contents

of one cyst being presumably of the same age. In this particular testis the posterior cysts are full of ripe spermatozoa; higher up, the cysts contain spermatids; next to these are cysts with immature spermatids, while higher up the compartments are full of interkinetic nuclei. Higher up still are cells, which, by their more anterior position in the gonad, and by their larger size, are presumably spermatocyte I, while at the head of the gonad are nuclei in synizesis. Thus the topographical relations of the cysts afford a means of identifying the different stages in the spermatogenesis.

After consulting many sections of larvæ and pupæ, and carefully studying the topographical relations of the cysts, it has been possible to distinguish the usual spermatogenesis stages, and this having been done, the recognition of the different stages that occur in the development of each cell generation was not a difficult matter. Although the contents of one cyst are presumably of the same age, it is often possible to find in it extremes of any stage. Thus in a cyst characterised by telophases of the first spermatocyte division there may be a few anaphases, and some metaphases, and possibly a few prophases. Working on this principle a cyst full of early prophases will sometimes contain cells undergoing preparation for prophases, and thus by linking up the information gleaned by a study of these individual variations a full series of stages can be obtained. Stress has been laid on the topographical relations of the cysts for reasons that will be apparent later.

A comparison of Text-fig. 1 with fig. 5 in Stevens' paper (**2**) will show that in the case of Culex pungens any one gonad contains a greater range of spermatogenesis stages than is the case with C. pipiens. Only rarely in the latter case are spermatogonial divisions found in the pupæ and old larvæ, these divisions taking place in younger larvæ. The usual distribution of stages in old larvæ and pupæ of C. pipiens is from synizesis to spermatozoa.

Resting nuclei in the testes of young larvæ resemble the nuclei of the connective tissue (Pl. 27, figs. 1, 2 and 3). In

the densely staining nuclear sap the chromatin is more or less peripherally arranged except for a central mass (Pl. 27, fig. 1). Resting nuclei are not found in old larvæ and pupæ—the synizesis stage apparently replacing the resting stage—nor are they very common even in the young, as there is so much active growth. In Pl. 27, fig. 3, a cluster of resting nuclei is shown from a section of a larva stained in thionin. The highly staining capacity of the cytoplasm at this stage renders the nuclei less conspicuous. Better differentiation is obtained in iron-hæmatoxylin (Pl. 27, fig. 2). In the more posterior parts of the testis of young larvæ the cytoplasm of one cell is more or less clearly marked off from that of its neighbours, but at the head of the gonad the cytoplasm, densely provided with metabolic products, forms a kind of syncytium in which large numbers of small nuclei are indiscriminately scattered. It is important to notice that there are no large dark-staining bodies in the cytoplasm, which at this stage is uniform, these dark bodies being confined to older larvæ and pupæ.

Synizesis.—The telophases of the last spermatogonial divisions are particularly interesting, because they show how the synizesis nucleus has been formed, and, as the synizesis nucleus marks the commencement of preparation for the first meiotic divisions, the whole history of the nuclear changes from the last spermatogonial division to the formation of the spermatid can be traced.

A figure of one such telophase is given on Pl. 27, fig. 4. The two daughter chromatin masses are still connected by the remains of the spindle, and round about these masses is a clear nuclear sap. The nuclear membrane is still present, this persistence of the membrane being characteristic of Culex mitosis as it is of that of many other insects. Each potential daughter-nucleus is possessed of a fairly thin rim of cytoplasm in which are embedded dark-staining bodies. The final separation of these two constituents would result in the formation of two nuclei, each like that drawn in Pl. 27, fig. 5—i. e. the synizesis nucleus. In this nucleus a voluminous and unstainable nuclear sap surrounds a "coagulum"

consisting of chromatin and of a substance which is apparently derived from the spindle apparatus.

This "coagulum" is frequently eccentric (Pl. 27, fig. 6). The cytoplasm of the synizesis nucleus, as was foreshadowed in the telophase of the mother-nucleus, is confined to a narrow rim. Certain dark-staining bodies differentiated by prolonged staining in iron-hæmatoxylin are to be found closely apposed to the nuclear membrane, the rest of the cytoplasm being more sparse. These dark-staining bodies are most readily discovered in material fixed in Benda, aceto-bichromate, and Flemming, and are only characteristic of the cytoplasm of the later stages of spermatogenesis, that of earlier stages being much more uniform, as has already been explained. Pl. 27, fig. 7, shows a cell somewhat older than that given in fig. 5, in which the dark mass of the synizesis nucleus has increased in size, the chromatin being now arranged more or less peripherally around a plasmosome, which stains very palely in the thionin sections, the chromatin being difficultly stainable except after prolonged treatment with iron-hæmatoxylin. In this latter stain the chromatin is seen to form a fairly dense crown of closely matted fine threads around a central space in which lies the plasmosome.

The densely matted masses of chromatin around the plasmosome now become disentangled to a great extent and occupy more space (Pl. 27, figs. 8 and 9). The underlying nuclear sap does not stain so deeply, so that the chromatin is more conspicuous. The plasmosome stains more deeply in thionin than it did in the stage represented by fig. 7. Fig. 8 represents a section through, and fig. 9 an uncut nucleus of this stage. In the latter figure the chromatin threads are shorter and thicker than in fig. 8, in which the nucleus is slightly younger. The cytoplasm of the cell is increasing and is filled with small granules. It has a high staining capacity.

Synizesis has now broken up; the chromatin threads, which have become thicker, lie against the nuclear membrane (Pl. 27, figs. 10 and 11). In only a few cases is it possible to count them, as they are so long and convoluted. Frequently

the apices of the loops thicken and stain deeply. The plasmosome is a highly staining and conspicuous structure.

Very often (Pl. 27, fig. 12) one chromosome thickens up before the others, which are still long and zig-zag, and not easily counted, or one part of the chromosome becomes locally thickened. This stage occurs in cysts along with nuclei containing fully formed chromosomes in late prophase. The threads frequently show a double character.

Late Prophase (Pl. 27, figs. 13 and 14).

The chromosomes have now condensed sufficiently to make the investigation of their number easy. In every case it is possible to count three chromosomes, which are sometimes double, as is often the case in meiotic prophases. The round nucleus is surrounded by a much greater quantity of cytoplasm than formerly. The chromosomes present great variety of shape. They are rod-shaped, club-shaped, dumb-bell-shaped, while crosses and rings are of frequent occurrence, these latter being formed by the partial separation of the chromosomes preparatory to metaphase. This preparation for metaphase is very often evident in the twisted character of the chromosomes, three pairs of twisted chromosomes frequently appearing (compare Stevens' fig. 14).

Metaphase (Pl. 27, figs. 15–18).

The cell itself becomes spindle-shaped. This characteristic change of shape affords help in distinguishing meiotic from spermatogonial divisions, the metaphases and anaphases of the latter taking place in round-shaped cells, the shape of the cell being in no wise affected by the formation of the spindle. The spindle shape is well shown in figs. 15–18.

A typical anaphase is illustrated by Pl. 27, fig. 19. Two of the chromosomes have each almost separated into daughter halves, the two daughter halves being merely united end to end. The third chromosome is not completely shown in the figure, part of it having been cut away.

Telophase (Pl. 27, fig. 20).

The cell now becomes greatly elongated and the chromosomes massed together. Spaces become apparent in the daughter chromatin masses, as shown in the left-hand mass in fig. 20. In the next stage these clear spaces have increased to such an extent that the central mass of the nucleus is free from chromatin, the latter occupying a peripheral position against the nuclear membrane (Pl. 27, fig. 21). It is interesting to compare what takes place here with what happened in the formation of the synizesis nucleus. In the latter case (Pl. 27, fig. 4) the vacuoles appeared round the daughter-mass of chromatin, converting this into the synizesis nucleus. In this case, however, the vacuoles appearing in the daughter mass, the chromatin is squeezed against the nuclear membrane (Pl. 27, fig. 21).

The cytoplasm in the cells, shown in figs. 12–20, is very conspicuous by its bulk, and in specimens fixed in Gilson-Petrunkewitch and Flemming (under certain conditions) it is homogeneous and deeply stainable in thionin. In some cells, notably those fixed in Benda, the cytoplasm is more like that of those cells represented in figs. 49–52, where it appears to be sharply differentiated into a more or less fluid substance and a few darkly stainable bodies.

Second Meiotic Division.

As has already been explained, no reticular resting stage follows the telophase of the first meiotic division. Vacuoles appearing in the daughter chromatin masses push the chromatin against the nuclear membrane rendering it almost invisible (Pl. 27, fig. 21). Next a plasmosome, most readily demonstrated in sections stained in Ehrlich, appears, and the chromatin thickens somewhat (fig. 22). Later (fig. 23) a "clock-face" stage similar to that which occurs in the development of the primary spermatocyte results. The chromosomes then thicken (fig. 24), though they are still in contact with

the nuclear membrane. Finally (fig. 25) three chromosomes appear in the prophase. Crosses and rings are again present, formed by the precocious longitudinal split, together with a divergence of the daughter halves. Often the chromosomes are so thick and close together that it is not easy to count them (figs. 26 and 27).

In metaphase the spermatocyte II cells are again spindle-shaped (figs. 28 and 29), but they are roughly only two thirds the linear dimension of the primary spermatocytes, and, as already explained, their position in the gonad, irrespective of their size, would render their identification an easy matter. Fig. 30 shows an anaphase of this division.

Formation of Spermatids (figs. 31 and 32).

In the round daughter-nuclei which result from the second meiotic divisions the chromatin is again to be found against the nuclear membrane (fig. 31). The nucleus now becomes elongated, the chromatin, being still peripheral, gradually diminishes in size, and finally assumes a rod shape.

Before going on to discuss the spermatogonial divisions, it may be well, in view of the difficulty of recognising the spermatogonial cells, to give some account of the nuclei in the undifferentiated gonads of young larvæ.

In the following account whenever the sex is stated it must be remembered that it is only tentatively given. The presence of cyst walls in the gonad has been the criterion for tentatively assigning the sex in the case of the male larvæ.

The ovary is very richly provided with tracheal tubes, and at a very early stage it is possible to identify these tubes. The somatic cells forming the walls of the ovarian tubes can also be distinguished from the germ cells at a very early stage. By means of these criteria it is often possible to identify as an ovary the gonad of a very young larva. However, in many cases it is not possible to say on which side lies the balance of probability.

The general facts of mitosis in very young larvæ are illus-

trated by Pl. 27, figs. 33–40. From a study of these figures it will be seen that the number of chromosomes in undifferentiated gonads, as well as in those of young male and female larvæ, is 3.

Synizesis nuclei occur in very early stages of both male and female (figs. 33 and 40). The two telophases illustrated in figs. 37 and 38 do not suggest that the daughter-nuclei will pass immediately into the synizesis nuclei (cf. fig. 4). They, therefore, belong to early generations, spermatogonial.

The effects of aceto-bichromate fixation are illustrated in figs. 39 and 40, where various metabolic constituents can be discovered in the cytoplasm (cf. figs. 53, 56, and 58).

Spermatogonial Mitosis in Young Larvæ.

Mitotic divisions in the fully differentiated, though immature, gonad of fairly young larvæ will now be discussed. In such larvæ the hinder ends of the gonad contain the more advanced stages of spermatogenesis, the degree of differentiation of spermatozoa, of course, depending on the age of the larvæ—but the anterior parts of the testis present a great difference in appearance from that of old larvæ and pupæ. Conspicuous in such gonads is the absence of synizesis stages. The cellular elements are much smaller—the cytoplasm forming a syncytium, in which the small nuclei are embedded. This, the "multiplication" stage of spermatogenesis, seems to be confined to young larvæ.

A series of division stages taken from this "multiplication" zone is given, the number of chromosomes being three (Pl. 27, figs. 41–51, and figs. 2 and 3).

The main point which emerges from a study of these nuclei is that the diploid number of chromosomes cannot be demonstrated in Culex pipiens. One cannot, therefore, use the number of chromosomes to distinguish spermatogonial from spermatocyte divisions. Still, a careful study of all the facts seems to show that the occurrence of a synizesis stage marks off the earlier divisions from those of spermatocyte I. The

divisions that occur in young larvæ are spermatogonial divisions, although they possess the haploid number. With regard to those synizesis stages in probable male larvæ, it must be remembered that some cells in quite young specimens differentiate very quickly. Hence the fact that synizesis stages do occur in such young creatures does not argue against the statement that the occurrence of a synizesis stage marks off spermatogonial nuclei from spermatocyte I.

Oogenesis.

As already stated in the introduction, a full history of the facts of oogenesis can only be given when the necessary material has been collected. However, for present purposes it is only necessary to figure a few stages in order to show that the number of chromosomes in the germ-cells is three.

Three chromosomes in prophase are shown in Pl. 27, fig. 52, taken from a young larva before the "rosettes" are well differentiated. The cell in which they occur clearly belongs to an early generation.

A prophase drawn from a section of an imago, ten days old, is shown in Pl. 27, fig. 53. The cell is from a young egg-follicle, and as it is stained in thionin after fixation in Gilson-Petrunkewitch, the cytoplasm is very densely stained.

A metaphase showing six chromosomes is given in Pl. 27, fig. 54.

The drawing in Pl. 27, fig. 55 is taken from an old larva. It shows a prophase where the number of chromosomes is three. The cytoplasm is abundant.

Pl. 27, fig. 56, taken from an egg-follicle of a captive-reared imago, illustrates the oldest stage obtained. The nucleus, with its deep-staining plasmosome, and its fine, well-distributed reticulum of chromatin, forms a striking contrast to the deep-staining, voluminous cytoplasm that surrounds it.

Somatic Mitosis.

Although there is much indirect evidence to show that the divisions described as spermatogonial are really divisions of

the earlier generations, in spite of the haploid and not diploid number of chromosomes, yet, in view of the possibility that they might be precocious spermatocyte I divisions, it has been thought advisable to work out the somatic mitosis of C. pipiens very thoroughly.

Mitosis in the Somatic Tissues of the Ovary (Text-figs. 2 and 3).

The somatic cells of the ovarian tubes of the ovary can be distinguished from the reproductive cells in very young larvæ by their minute size. The developing ovarian tubes look like rosettes in sections cut from older larvæ, the large central cells of the rosette being reproductive cells, the peripheral cells, much smaller in size, being somatic. Later on groups of from four to eight of these reproductive cells become enclosed in an epithelium—the egg-follicle epithelium —also somatic in character. The number of these egg-follicles increases with the age of the creature, the ovary having assumed its definitive arrangement in late pupal life. The exceedingly thin walls of the ovarian tubes, the nuclei of which are very small, are greatly distended by the large egg-follicles, which are made up of one egg-cell surrounded by a varying number of nurse-cells.

The ultimate fate of egg- and nurse-cells has still to be worked out. The somatic tissue of the ovary—whether the epithelium of the tubes, or the egg-follicle epithelium—is a prolific source of mitotic figures. In all cases the number of chromosomes is three.

A cell belonging to an egg-follicle in telophase is shown in Pl. 27, fig. 57, and near it is shown a reproductive cell (fig. 58), which also brings out the difference in size between the somatic and reproductive cell.

Pl. 27, figs. 59 and 60, also show three chromosomes in typically somatic anaphase.

Innumerable figures of prophases could be drawn from the preparations, it being characteristic of Culex pipiens that

metaphases and anaphases are comparatively difficult to find, prophases being much more abundant.

In the case of all the cells of the ovary the number of the chromosomes is three.

Somatic Mitosis (General).

Somatic mitoses, apart from those in the ovarian tissue, are by no means easily found. They do not seem to be confined to any particular period of larval or pupal life, or to take place at any fixed hour of the day or night.

The process, as observed in nerve-cells, is illustrated by Pl. 27, figs. 61–65, and Pl. 28, figs. 66–68.

It will be seen from these figures that there is a great resemblance between the reproductive cells, at certain stages of development, and the somatic cells in the nerve ganglia.

Similar likenesses between the body-wall cells and those of the gonad could also be demonstrated. Thus a very well-marked synizesis stage is characteristic, not only of the later stages of spermatogenesis and of the reproductive cells of the ovary, but also of the cells of the nervous system, and the gradual breaking up of the synizesis resembles, in the main, that which takes place in the gonad. Hence it would appear that synizesis has not the significance in the spermatogenesis of Culex that it is commonly believed to have in other creatures, since this phenomenon is not confined to reproductive cells.

An investigation of the tracheal tube-cells shows that the number of chromosomes is three. Very frequently the daughter halves of the split chromosome can be seen in late prophase (Pl. 28, figs. 69–71).

Evidence as to the number of chromosomes derived from a study of the body-wall cells (illustrated by Pl. 28, figs. 72–79) confirms the results already obtained elsewhere, while in the undifferentiated somatic cells in larvæ just hatched (Pl. 28, figs. 80 and 81) the number is again three.

The alimentary canal wall splits into two layers during

pupal life, the inner layer undergoing disintegration (Hurst (1)). Some of the mitoses that occur in connection with this process are illustrated in Pl. 28, figs. 82–89. The number of chromosomes is three, and in some cases the precocious tendency of the chromosomes to divide in prophase for metaphase is again evident.

Mitosis in Malpighian tubule cells is figured in Pl. 28, figs. 90–95, while fig. 96 shows an equatorial plate from a muscle-forming cell. In all cases the somatic number of chromosomes is three.

Discussion.

In C. pipiens, as has been shown, there appear to be two maturation divisions, though no reduction of chromosomes can be demonstrated. This is contrary to general experience, for, as is well known, when, in any organism, the ripe germ-cell has the same number of chromosomes as the somatic tissues, one of the meiotic divisions is commonly omitted. It must be remembered, however, that while two divisions undoubtedly follow the synizesis stage in C. pipiens, the fact that they follow synizesis is the only one which has led to their separation off from the apparently similar earlier divisions, and to their being described as meiotic rather than spermatogonial. However, as nuclei closely resembling synizetic nuclei can be found in the somatic tissues of this creature, it is, therefore, possible that the synizesis nuclei in the testis have no real value in diagnosing the beginning of the meiotic phase, and that they merely represent a stage of inactivity. The rapid divisions in the "multiplication" zone result in the formation of large numbers of nuclei which, while they are awaiting differentiation into spermatozoa, remain as synizesis nuclei. When the time comes for this differentiation the synizesis nucleus begins to be active, and the stages in this awakening are analogous to the formation of spermatocyte I cells.

From the foregoing account, it will be seen that the parasyndesis for each cell generation which Miss Stevens described for C. pungens cannot be demonstrated for C. pipiens.

No figures indicating the presence of six chromosomes are to be found which cannot readily be interpreted as three chromosomes precociously split for metaphase. For example, conditions like those Miss Stevens gives in her figs. 1 and 2 (oogonia showing three pairs of chromosomes on equatorial plate) and in figs. 8, 9, and 10 (spermatogonial cells showing all three pairs in late prophase), in the light of other evidence, must, when they occur in C. pipiens, be described as three chromosomes already divided for metaphase. The conditions of C. pungens, however, offer a suggestion as to how the haploid number of chromosomes in C. pipiens may have been derived. The permanent fusion of the paternal and maternal members of the pair, i. e. the conversion of parasyndesis into actual fusion, would result in the formation of three out of six chromosomes.

Miss Stevens states that in C. pungens the intimate relationship of the two conjugants persists from one cell generation to the next, the pairing taking place in telophase, and persisting until the metaphase of the next mitosis. From this it would seem that the conjugating chromosomes are only "unfused" in metaphase. In the case of C. pipiens the pairs are fused throughout the whole mitosis, hence the haploid number.

On the other hand, it is quite possible to give a different interpretation of Miss Stevens' figures of parasyndesis from the one she offers. It is significant to note that she gives no figures in support of her statement that each of the six chromosomes (i. e. each member of the three pairs of conjugating chromosomes) found in the oogonial and spermatogonial generations divides longitudinally. She merely states the fact that they do so. Unless this division can be demonstrated, it would seem as though the so-called conjugating chromosomes were merely the daughter-halves of a precociously split chromosome, as is the case in C. pipiens.

An alternative suggestion, therefore, as to the chromosome complex of Culex pipiens and pungens is, that the somatic number is the same as that of the mature gamete, being three

in each case. This alternative would seem to involve the non-participation of one of the gametic nuclei in development.

Whether this is the case, or whether the homologous chromosomes are temporally united in each cell-generation of C. pungens, and permanently so in C. pipiens, can only be settled by an examination of the process of fertilisation, which I hope to undertake in the near future.

I am greatly indebted to Dr. Agar for much valuable criticism; to many friends who have assisted me in collecting material; to Mr. P. Jamieson for cutting the more important sections; and to Professor Graham Kerr for his sympathetic encouragement.

Summary.

(1) The somatic number of chromosomes is three, both in the male and female of Culex pipiens.

(2) The number of chromosomes in the spermatogonia as well as in the primary and secondary spermatocytes and spermatids is three.

(3) The spermatogonial cells are not characterised by a synizesis stage, which latter stage marks off the spermatogonial from the spermatocyte I stage.

(4) The nuclear membrane persists throughout mitosis.

(5) The synizesis stage represents an inactive phase of the nucleus in spermatogenesis.

(6) A synizesis stage occurs in somatic nuclei.

Literature.

1. 1890. Hurst, C. Herbert.—"Post-Embryonic Development of Gnat," 'Trans. Liverpool Biol. Soc.,' vol. iv.

2. 1910. Stevens, N. M.—"The Chromosomes in the Germ-cells of Culex," 'Journ. Exper. Zool.,' viii.

EXPLANATION OF PLATES 27 AND 28,

Illustrating Miss Monica Taylor's paper on "The Chromosome Complex of Culex pipiens."

[All figures (except 3 and 6) were drawn with the Abbé camera under Leitz $\frac{1}{12}$ oil-immersion objective and Zeiss compensating ocular 12, giving a magnification of 3300 diameters. This has been reduced $\frac{2}{3}$, i. e. to a magnification of 2200. Figs. 3 and 6 are drawn to a magnification of 1900, giving a final magnification of about 1270.]

PLATE 27.

Fig. 1.—Resting nucleus in connective-tissue.

Fig. 2.—Resting nucleus from gonad of young ♂ larva.

Fig. 3.—Group of nuclei drawn to same scale as fig. 6 from young ♂ larva; thionin stain.

Fig. 4.—Telophase of spermatogonial cell from head of testis of pupa.

Figs. 5–32 are taken from pupal testes.

Fig. 5.—A synizesis nucleus from head of testis of pupa.

Fig. 6.—A group of synizesis nuclei from a pupa fixed in Benda, and deeply stained.

Fig. 7.—A nucleus showing early stage in the breaking up of synizesis.

Fig. 8.—From a section 4 μ thick, showing later stage in the breaking up of synizesis.

Fig. 9.—From a section 8 μ thick, showing nucleus slightly older than in fig. 8.

Fig. 10.—View of nucleus from one pole, showing the chromatin close to the nuclear membrane.

Fig. 11.—Optical section through a nucleus which at this stage is very characteristically like a "clock-face."

Fig. 12.—Nucleus showing that the thickening up of the chromosomes is not always uniform.

Fig. 13.—Advanced prophase.

Fig. 14.—Advanced prophase.

Fig. 15.—Very early metaphase, showing the spindle-shape of the cell before the mitotic spindle has been organised.

Fig. 16.—A more elongated spindle-shaped cell. Early metaphase.

Fig. 17.—Metaphase, with indistinguishable chromosomes.

Fig. 18.—Metaphase in which one of the chromosomes has divided.

Fig. 19.—Later anaphase. Only one of the three dividing chromosomes is completely shown in the figure.

Fig. 20.—Telophase.

Fig. 21.—Interkinetic nucleus.

Fig. 22.—Interkinetic nucleus stained in Ehrlich, showing a plasmosome.

Fig. 23.—"Clock-face" stage for spermatocyte II nucleus.

Fig. 24.—A nucleus of spermatocyte II, in which chromosomes are becoming visible.

Fig. 25.—A nucleus of spermatocyte II, showing three chromosomes.

Fig. 26.—Advanced prophase.

Fig. 27.—Later prophase; the shape of the cell is just beginning to change.

Fig. 28.—Early metaphase.

Fig. 29.—Metaphase; three chromosomes on equatorial plate.

Fig. 30.—Anaphase.

Fig. 31.—Newly formed spermatid.

Fig. 32.—Immature spermatid.

Figs. 33–40 are drawn from gonads of very young larvæ.

Fig. 33.—A synizesis in a ♂ (?) larva.

Fig. 34.—Prophase from a young ♂ (?) larva.

Fig. 35.—Metaphase from a young ♂ (?) larva.

Fig. 36.—Metaphase from gonad of larva.

Fig. 37.—Anaphase from gonad of larva.

Fig. 38.—Anaphase from ♂ (?) larva.

Fig. 39.—Acetic bichromate and thionin preparation. ♀ (?) larva.

Fig. 40.—Acetic bichromate and iron-hæmatoxylin preparation. ♀ (?) larva.

Figs. 41, 42.—Prophases, spermatogonial; the number of chromosomes is three.

Fig. 43.—Equatorial plate, showing three chromosomes; a young ♂ larva, spermatogonial.

Fig. 44.—Metaphase, spermatogonial, three chromosomes.

Figs. 45, 46.—Late spermatogonial anaphases from young ♂ larva.

Figs. 47—51 are from head of testis of pupa.

Fig. 47.—Prophase, spermatogonial, from head of gonad of pupa (cf. Stevens [fig. 10 (2)]). There are two long chromosomes which have split longitudinally, and one smaller one not yet divided into daughter halves.

Fig. 48 shows the threads of the spindle just beginning to form inside the nuclear membrane, the chromosomes being too massed together to be counted.

Fig. 49.—Metaphase; an equatorial plate where the three chromosomes are just separating into their daughter-constituents.

Fig. 50.—Later metaphase, six chromosomes being easily counted.

Fig. 51.—Early anaphase. Note the round-shaped nucleus, and the somatic-like character of the V-shaped daughter-chromosomes.

Figs. 52–56 are taken from ovaries.

Fig. 52.—Prophase from young ♀ larva.

Fig. 53.—Prophase from ♀ imago.

Fig. 54.—Metaphase from ♀ larva.

Fig. 55.—Prophase from old ♀ larva.

Fig. 56.—From ♀ imago, two months old.

Figs. 57, 58.—Egg-follicle. Epithelium cell with nucleus in late anaphase (57), and nurse or egg cell drawn to same scale (58). (♀ imago section.)

Figs. 59, 60.—Egg-follicle epithelium cells in late anaphase, showing three chromosomes. (From a section of a ♀ imago.)

Figs. 61–65 from nerve-ganglia.

Fig. 61.—Synizesis nuclei deeply stained in Ehrlich from old ♀ larva (cf. figs. 5, 6, and 40).

Fig. 62.—Nerve-cell showing six blocks of chromatin in the "coagulum." Old ♀ larva.

Figs. 63 and 64.—Nerve-cells showing the breaking up of synizesis. Old ♀ larva.

Fig. 65.—Early prophase from ♂ pupa (cf. fig. 10).

PLATE 28.

Fig. 66.—Early prophase of a nerve-cell from a larva just hatched. Compare Pl. 27, fig. 11.

Fig. 67.—Prophase of a nucleus of a nerve-cell from a larva just hatched. Three chromosomes showing precocious splitting.

Fig. 68.—Prophase of a nucleus of a nerve-cell from a young ♂ larva.

Fig. 69.—Three chromosomes in the nucleus of a tracheal tube-cell from a young ♂ larva. Compare this with figs. 1, 9, and 10 of 'The Germ Cells of Culex,' Stevens (2).

Fig. 70.—Tracheal tube-cell from young ♀ (?) larva.

Fig. 71.—Ditto.

Figs. 72–79 are drawn from body-wall cells.

Fig. 72.—Early prophase of a nucleus from a ♀ imago.

Fig. 73.—Three chromosomes in prophase from a ♀ larva.

Fig. 74.—One short and two long chromosomes. Prophase of nucleus from a young ♂ larva. Note indication of two daughter-halves in one of the chromosomes.

Fig. 75.—Three chromosomes in advanced prophase from ♀ larva.

Fig. 76.—Equatorial plate. Side view showing three chromosomes; one of them is cross-shaped.

Fig. 77.—Anaphase from a ♀ larva.

Figs. 78 and 79.—Telophases from ♀ larvæ.

Figs. 80 and 81.—Undifferentiated cells of a larva just hatched, showing three chromosomes in prophase and late metaphase.

Figs. 82–89 are drawn from cells in alimentary canal wall.

Figs. 82, 83, 84.—Prophases from cells in alimentary canal wall of ♀ larvæ.

Figs. 85, 86.—Prophases from cells in alimentary canal wall of a ♀ imago, ten days old. In fig. 85 one of the chromosomes has divided longitudinally.

Fig. 87.—Prophase from alimentary canal wall of ♀ larva.

Fig. 88.—Metaphase from cell of same larva.

Fig. 89.—An equatorial plate showing three chromosomes from wall of intestine.

Figs. 90–95.—Malpighian tube-cells.

Fig. 90.—From young ♂ larva.

Fig. 91.—From ♀ larva.

Fig. 92.—From young ♂ larva.

Fig. 93.—From young ♂ larva.

Fig. 94.—From ♀ larva.

Fig. 95.—From ♀ larva. Note cross-shaped chromosome.

Fig. 96.—Equatorial plate showing three chromosomes; two of them ringed, from muscle-cell of young larva.

Studies on Avian Hæmoprotozoa: No. III.—Observations on the Development of Trypanosoma noctuæ (of the Little Owl) in Culex pipiens; with Remarks on the Other Parasites occurring.[1]

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With Plates 29—31 and 1 Text-figure.

Contents.

Preface.

I propose in the present paper to conclude the account of the work on the parasites of the Little Owl (Athene noctua) which I carried out at Rovigno during the spring and early

[1] For No. II of these Studies, "On the Trypanosome of the Little Owl," etc., vide 'Quart. Journ. Micr. Sci.,' vol. 57, 1911, p. 141.

summer of 1909. I have delayed publishing up to the present my observations on the developmental phases of the Trypanosome in the mosquito, as I hoped to be able, before now, to obtain the corresponding phases, and, indeed, the transmission back again to the bird, of Trypanosoma fringillinarum, here in England, to complete the work. This latter research, however, progresses, unfortunately, very slowly, so that I think it useful to publish my earlier observations without waiting longer, more especially as the only other worker who has written anything of late on this subject, namely, Mayer (**2**), in his account of the parasites of another owl (Syrnium aluco), has upheld the view that the Flagellates occurring in mosquitoes which have fed on an owl are developed from Halteridia present in the blood of the bird. In my "Notes on Sporozoa, No. IV" (**14**), dealing with the nuclear structure of Halteridium and Leucocytozoon, I think I have shown clearly that, considered from the standpoint of these parasites, everything is against such a connection of either with a Trypanosome. And the evidence which I have obtained from the Trypanosome side of the question is equally negative, and does not bear out Mayer's contention in the slightest degree.

General Account of the Experimental Work.

The time at my disposal for experimental work with Culex was only very short—about the last three weeks of June. Earlier than this the bred-out females would not take blood, and at the beginning of July I was unfortunately obliged to leave Rovigno. Five owls were used, to which it will be convenient to refer by their numbers, viz., 15, 16, 19, 22, and 23. The first two were quite free from any infection; No. 22 had only Leucocytozoon, while Nos. 19 and 23 both had Halteridium noctuæ, Leucocytozoon ziemanni, and Trypanosoma noctuæ. As regards Owl 19, the Trypanosome was for some reason or other excessively scanty, and was not, I believe, present at all in the general circulation

(cf. Study No. II). The Halteridial infection of Owl 19 was quite typical, the parasites, which, of course, were in the usual form of male and female gametocytes, being fairly numerous, and many of them full-grown and ripe for liberation from the host-cells. On the other hand, the Halteridial infection of No. 23 was not typical. In this owl nearly every red blood-cell was infected, usually with several (four or more) small forms, which in many cases, as they had increased in size, had united together into a kind of common plasmodium. This condition has been already described by me elsewhere (**13**). I observed hardly any full-grown, normal gametocytes in the blood of this bird. Owls 22 and 23 only arrived on June 12th.

Early in June, finding that my bred-out females would not yet bite, I left Owl 19 one evening in a cage into which I had put half-a-dozen or so "wild" females, caught in the same little outhouse, used as a "dark-room," where Schaudinn himself had caught many in time past. Before midnight two of these had fed. I may note here that the favourite place for feeding of the mosquitoes was the fleshy pad just above the bird's nostrils. One of the two females was examined after about thirty-six hours had elapsed, this being the period when, according to Schaudinn, the ookinetes become transformed into Trypanosomes. Its stomach contained a number of fully-developed ookinetes, i. e. which had lost all the pigment. The majority had the characteristic curved form, but did not appear at all active. A few, however, showed a certain amount of activity, which consisted in tending to straighten out, and again recurve, the body, either slowly or now and again spasmodically. I never observed any marked forward progression of the ookinetes.

Besides the ookinetes certain other bodies were found to occur, very scantily, in the preparation. These elements were more elongated and spindle-shaped, somewhat resembling an Indian club in form, one extremity tapering finely, the other being rounded off. Further, some of them were very slightly curved or crescentic. Apart from the difference in

shape, the general appearance of these bodies, observed living, was not at all unlike that of the ookinetes. They were quite non-motile. While I was in the act of observing one, and wondering whether it was a later stage in the development of an ookinete, it gave one or two very slight, jerky movements, and before I had fully assured myself that these really represented active, voluntary motion on the part of the parasite, to my great surprise it had developed a flagellum. I thought I had just an indication of the tapering end of the body beginning to lengthen, but more than this I did not see. One moment the parasite had no flagellum, an instant later it had a fully-developed free flagellum, about three-quarters as long as its own body. The process must have been exactly comparable, in short, to that which I have since found to occur in Leptomonas ("Crithidia") fasciculata (vide **15**). I hurriedly brought a colleague, Dr. Reichenow, then also working at Rovigno, to look, and he, in turn, saw the process repeated in the case of another individual. These were the only two instances in which we saw the development of the flagellum, and we only found one other flagellated form in the preparation. We both carefully examined several of the other (non-motile) elements, and satisfied ourselves that they had no sign whatever of a flagellum. If I may be permitted the personal reminiscence, I well remember how, in the excitement of the moment, we were both of us firmly persuaded that we had seen the most important stage in the transformation from an ookinete into a Trypanosome, as it had been described by Schaudinn. This confidence did not endure, however, for many days. I continued to watch certain ookinetes during the afternoon, and felt very disappointed that I could not see any indication whatever of a typical ookinete passing into one of the fusiform bodies. After keeping the preparation under observation for about three hours I removed the coverslip and made smears, which were fixed and stained.

Most unfortunately, Owl 19 was taken ill and died on the following day, and for some days I had no owl infected with

Trypanosomes. During this interval I fed several caught mosquitoes on an uninfected bird (either 15 or 16), and these were examined at periods of from thirty-six to fifty-eight hours after being fed. In two cases, elongated, fusiform elements, perfectly similar to those above-mentioned, were found; but none of them was seen to become active or develop a flagellum. In another female, examined about fifty-four hours after being fed, numerous active Flagellates were observed in the stomach; these differed considerably in appearance from the "resting Flagellates," but, on the other hand, agreed closely with the characteristic developmental forms described below, and I have not the least doubt that they also belonged to the life-cycle of some Avian Trypanosome. It is important to note that no ookinetes were seen in any of these mosquitoes. These observations showed not only that the fusiform resting Flagellates might probably have another origin than from the ookinetes of Halteridium, but also that it was essential to use only bred-out females, in order to follow the course of development taken by the blood-parasites in the Culex; hence, from this time onwards only such individuals were used.

On June 12th Owl 23 arrived, and during the night of the 15th–16th I found Trypanosomes in the peripheral circulation; the parasites were of the characteristic fusiform, rather stout type (Pl. 31, fig. A), described by Minchin and myself (l. c.). The Trypanosomes were not at all infrequent—for Avian Trypanosomes, it must be remembered—in the peripheral blood at this time,[1] and were found also on other occasions. In both of the two first mosquitoes which were examined after being fed on this bird, after intervals of about thirty-four and forty hours respectively, numerous active Flagellates were found in the stomach. Digestion was proceeding normally and was about half accomplished, or rather more. (I may state here that females which had taken blood were always kept at a tem-

[1] In any living preparation, consisting of a small drop of blood spread out into a thin layer under a coverslip seven-eighths of an inch square, there would be one or two Trypanosomes.

perature of from 25°–27° C., at which temperature digestion took three to four days to be completed.) The development of the Trypanosomes appeared to be at about the same stage in both the females, and in both similar phases were observed.

Ookinetes of Halteridium were also found, but in both mosquitoes they were few in number—quite scarce, in fact, when compared with the number present in the first female examined, which had fed on Owl 19 (cf. above). This was readily to be understood, bearing in mind the different condition of the Halteridial infection in the two birds; in spite of the very strong infection of Owl 23, there were nothing like so many full-grown, ripe gametocytes as in Owl 19. A living preparation made from one of these two stomachs was kept under observation for some time. Three ookinetes in different fields, all of which had lost their pigment grains, were noted at intervals during two hours, but none of them showed any change in form or the slightest indication of any development into one of the fusiform bodies or into a flagellate condition. The preparation was again looked at two hours later, with the same result in the case of two of the ookinetes; the third could not be found. By this time most of the Flagellates seemed to be dead—at all events, only two or three could be observed, and these were very sluggish.

Altogether, twenty-six female Culex pipiens which had fed on Owl 23 were examined, after intervals varying from about twelve to eighty hours. Flagellates were found in twelve of these, i.e. in about 46 per cent.; sometimes they were numerous, in other cases only few were seen. On the other hand, out of thirty-two females fed on one of the other owls (Nos. 15, 16, 22), none of which was infected with Trypanosomes, the stomachs of which were carefully examined after different intervals (thirty-six to fifty-four hours), in not a single case were the parasites found! Two of these birds were quite free from any Hæmoprotozoan infection; Owl 22, however, had a fairly strong infection of Leucocytozoon ziemanni. Seven of the thirty-two mosquitoes examined

had fed on this latter bird,[1] and in four of them a few of the large ookinetes of Leucocytozoon were found. In form and appearance these were very similar to the ookinetes of Halteridium, but they were considerably larger. Those I observed were quite motionless, and did not change at all in shape.

The fifty-eight mosquitoes examined were barely half the total number (about 120) which fed on blood. The mortality amongst these newly bred-out imagines was high, and it appeared to make little difference whether they took blood or the sugar-water, banana and prune juice with which they were supplied. More than a quarter of those which fed on blood died during the course of digestion. During the short time at my disposal I had, therefore, only very limited material for transmission-experiments. During the last fortnight between fifteen and twenty females which had fed on Owl 23 and successfully completed digestion, were given the opportunity of feeding on an uninfected bird (either 15 or 16). To my very great disappointment, however, not one of these could be induced to feed again. Some of them drank a little water, or partook of the food-supply which was placed in the same cage for a few hours during the day-time for the males to feed upon; nevertheless, many of them gradually died off during this period. I was loath to sacrifice any more for examination, hoping to the very morning of my departure from Rovigno that one or more would bite again and give me the chance of seeing whether one of the uninfected birds would become infected. Unfortunately, the endeavour did not succeed. Owls 15 and 16 accompanied me back to England and lived for many months—much longer than a single bird did at Rovigno—but neither of them ever showed any parasites at all.

While restricting myself to bred-out females for the experimental work, incidentally I examined a few more caught "wild" mosquitoes. None of them was infected with active Flagellates (i. e. Trypanosome developmental forms), but again

[1] This owl died on June 22nd, so that I had it only ten days.

two or three contained the peculiar, spindle-like resting forms, above noted; in no further instance, however, did I see one develop a flagellum. I have since wished that I had been able to devote more time to the study of this parasite, and to ascertain, for example, whether it occurred in male mosquitoes also. But I was intent on proving the origin of the Flagellates which developed in the blood-fed females, and in transmitting them back to the birds, if possible, and this took every minute of my time, as I had no assistance whatever and had everything to do myself.

Description of the Parasites, as they were found in the Mosquitoes.

(i). Trypanosoma noctuae.

I pass on now to describe the developmental phases of the Avian parasites in the female Culex pipiens, and begin with those of Trypanosome noctunae. Considering the Flagellates, first of all, as they were seen in life, in mosquitoes examined about thirty-six hours after being fed on an infected owl, the most striking form, which at once held my attention on first seeing it, was a very long, extremely slender type, which progressed rapidly, by means of its flagellum and the undulating membrane along the anterior part of the body. The membrane appeared to be very narrow along the middle region of the body, and on account of this fact and the active movements of these individuals, I could not determine exactly where it ended, or whether this type was trypanomonad (crithidial), or trypaniform. In fixed and stained preparations, however, it is seen to be distinctly and invariably trypaniform (figs. 13–21). Nevertheless, these attenuated trypanosomes were entirely different in appearance from an ordinary, slender, elongated blood-form of Trypanosome, e. g. a piscine type such as T. granulosum. While the anterior part of the body, where the membrane was conspicuous, was sinuous and flexible, the hinder part, as a rule, nearly half

the entire length or more, was held quite stiffly and did not appear to be actively flexible at all. Frequently it was practically straight, but in some individuals it was quite curved round, like a crook (cf. figs. 19–21); this posterior part would retain this shape, unaltered, even while the parasite was moving rapidly forwards. From a comparison of stained preparations it is evident that this portion of the body represents a prolonged extension of the cytoplasm behind (posterior to) the kinetonucleus. In view of my observations on the living parasites, I regard this cytoplasmic "tail" as differing from the anterior half of the body in lacking anything of the nature of myonemes, and consequently any ability to bend or twist of itself. I consider that it is only, as it were, passively flexible, and that any curving or bending is produced mechanically, as the result of contact with the blood-cells and other elements among which the parasite happens to be working its way. What function this remarkable development serves, I was not able to ascertain.

The above type of individual constitutes a fair proportion of the total number of flagellates present, even at this somewhat early stage of the development. The other types of parasite seen were for the most part relatively short trypanomonad (crithidial) forms and individuals representing every possible transition between such and the extremely attenuated forms. As is shown by the fixed and stained preparations made of stomachs at about this period, the majority of these intermediate forms are really trypaniform, i.e., the kinetonucleus is on the aflagellar side of (posterior to) the trophonucleus. The shorter, more typically trypanomonad individuals resembled the commonly occurring crithidial forms which develop in cultures of an Avian trypanosome, such as I have described in the case of T. fringillinarum. All these forms had what is usually distinguished as the crithidial type of movement. Progression was in a slightly zig-zag manner, the flagellum and the anterior part of the body (corresponding to the position of the undulating membrane)

vibrating actively. The movement of these forms was not nearly as rapid as that of the long, slender individuals. Lastly, other forms noted were short and pyriform, and moved jerkily, not displacing themselves to any extent; these were infrequent. Notwithstanding the great increase in number of the parasites which must have taken place since the blood entered the stomach, I found scarcely any individuals actually dividing. In one or two cases I saw trypanomonad individuals with two flagella, and in one instance a pair of such forms still connected together; in another instance which I noted, division was markedly unequal, a short, pyriform individual becoming separated off from a distinctly larger, broader, club-shaped form.

At a later period of the development the long, attenuated forms predominate more and more, until in females examined fifty-five hours or subsequently after being fed, the stomach contained apparently only such forms; and this is borne out by the study of fixed and stained preparations made of stomachs of this period or later.

All my observations on the parasites relate to the stomach-phases of the life-cycle which occur between thirty-two and about seventy-six hours after the mosquito has fed. I examined four females from twelve to eighteen hours after feeding on Owl 23, but I could not find any Trypanosomes at this early period. It would be necessary to examine many individuals to find the earliest changes in the parasites, because, even if the development is proceeding all right, the Trypanosomes have not yet had time to multiply and give rise to any considerable number of parasites; and it must be remembered that only very few Trypanosomes are taken up by the mosquito from the blood to start with. Neither did I find any phases of the parasites in the intestine; but this did not surprise me, because nearly all the mosquitoes were examined, at any rate, some time before the stomach was quite empty. While digestion is still going on, the stomach is most certainly the principal, if not the only situation in which the Trypanosomes occur. If I had been able to

examine a sufficient number of females after digestion had been completed, or after they had had another meal of blood, the parasites might have been found in the intestine; I shall discuss this point later on. My observations are, I am aware, only incomplete; but they were the fullest I was able to make in the circumstances, and bearing in mind the chief objects on which I concentrated my attention during the short time at my disposal, namely, to determine whether the Trypanosome-phases in the mosquito were derived from the Halteridial parasites or not, and to bring about, if possible, the transmission of the Trypanosomes back again to the owl.

The Trypanosomes in Permanent Preparations; Comparison with the Developmental Stages found in Cultures.—I think, however, by comparing the various forms which I did obtain in the mosquito, with the development which I have found to take place in cultures, in the case of another Avian Trypanosome, T. fringillinarum, that a general idea can be arrived at of the main course of the natural development in the stomach of the insectan host; as already indicated, the chief gap and element of uncertainty relates to the earliest changes undergone by the parasites.

I have no hesitation in making use of the cultural development for this purpose, because practically all the different forms occur in both cases. The reason it is helpful is because, in the culture, the development continues over a much longer period owing to the fact that there is no absorption of the medium, such as occurs in the insect's stomach, and therefore the intermediate forms are met with abundantly and stages in division are frequent, the latter being of much assistance in determining the sequence of the developmental changes. In the Culex, on the other hand, the course of digestion is comparatively rapid and completed in three to three and a-half days, by which time the stomach is empty of blood. It is undoubtedly in relation with this fact that we find the early and intermediate stages in the development of the parasites passed through very quickly, which leads on to the production of the ultimate stages found in the stomach. This develop-

ment proceeds along two lines, the result being the formation of two extreme types. The great difference in the relative frequency of certain forms, on the one hand in the culture, and on the other in the mosquito's stomach, is entirely in accordance with the different conditions prevailing in the medium in the two cases.

The earliest developmental forms obtained are trypanomonad individuals, such as are drawn in figs. 1–3, or *b* and *c* of the scheme. These are medium-sized, fusiform parasites, with a distinct membrane and the two nuclei close together, the kinetonucleus being usually just posterior to the middle of the trophonucleus. Both nuclei are either about the middle of the body or else slightly in the hinder part. This type of individual corresponds exactly with a particular crithidial form which occurs commonly in cultures, such as is drawn in figs. *f′–j′* of the parallel series of stages from cultures, which I have reproduced [1] for ready comparison with the developmental forms in the mosquito. In the mosquito, however, even in my earliest preparations (about thirty-two hours), the number of those trypanomonad individuals is relatively very small. This is in marked contrast to what is the case in the cultures, where the trypanomonad type is by far the predominating one. In the cultures many of these forms are distinctly larger than those with which I compare the mosquito forms just described (cf. figs. *b′–d′*), and have the undulating membrane often better developed. In a small proportion of them, moreover, the kinetonucleus is just in front of the trophonucleus, instead of being alongside, a condition which I have not observed in this phase in the mosquito.

As regards the immediate origin of these trypanomonad forms in the stomach, I think it is most probable that they arise by the division of rather larger, but otherwise similar forms. As is seen very clearly from the full series of the crithidial forms figured in my memoir (l. c., Pls. 27, 29), the

[1] All these figures of cultural forms are taken from my Memoir in the 'Quart. Journ. Micr. Sci.,' vol. 55, 1910, pl. 27, 29, or 30.

division of the larger (earlier) individuals is at first by practically equal binary fission, the only inequality being that the daughter-flagellum may be shorter than the parent one (cf. figs. *e'–g'* of the scheme, Pl. 31). Unfortunately I have not found any typical trypanomonad individuals in the act of the dividing in my preparations, but, as already mentioned, I observed an instance in life in which the parasite was just completing division, and in a condition practically identical with that seen in fig. *g'*.

However, I have very little doubt that the division of these trypanomonad individuals with the nuclei about the middle of the body is almost, if not quite, finished by the time of my earliest preparations. Because there are very few forms still left in this phase of the development; most of the parasites occurring are individuals which have lost the trypanomonad condition already and become trypaniform, and are either at some stage in the development of the elongated, attenuated trypanosome-phase, or have, in fact, attained the latter. I have been able to find, in stomachs of from thirty-two to thirty-six hours, a regular series of transitional forms in this connection (cf. Pl. 29, figs. 4–10). The elongated trypaniform condition is reached by a progressive modification of the form of the above trypanomonad individuals. I think this change is most probably unaccompanied by further division, at any rate, to any extent; otherwise, I ought to have found some indication of the process, as the parasites are fairly numerous, and all degrees in the gradual change of the type of form are to be met with.

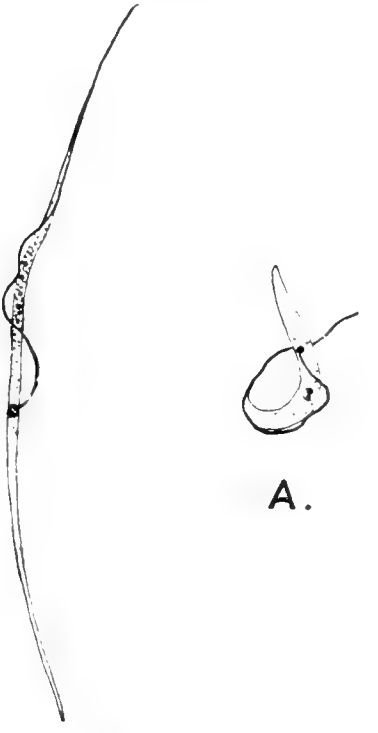
The earliest change is the passage of the kinetonucleus, and, of course, of the blepharoplast and attached flagellum, definitely behind the trophonucleus, the latter retaining its position (figs. 4 and 5). Next, the general cytoplasm in the posterior half begins to increase considerably in length; in this way the parasite becomes longer, but it does not increase much in breadth. On the contrary, as the length increases, the body becomes ultimately not only relatively, but actually narrower and more slender. The change is most probably the

result of two factors combined, namely, growth to some extent, and a thinning-out of the general cytoplasm. The latter factor is evident from the change in the form of the trophonucleus. This body, in the early stage, has the shape of a short and broad oval (figs. 4–6); as the trypaniform condition develops it becomes elongated, and appears as a narrow oval, compressed laterally (figs. 7–10). Meanwhile the kinetonucleus has passed well back behind the trophonucleus, but it never approaches anywhere near the posterior end of the body, the extension of the cytoplasm backwards, as the "tail" develops, being so great (figs. 8–12). The final position of the kinetonucleus is usually about the middle of the body, or slightly in the posterior half. Nevertheless, the attached flagellum and the membrane are relatively long; the membrane is always narrow and rarely at all wavy. Although, as the parasite assumes its final attenuated condition, the anterior part of the body also becomes thinned out and tapering, it does not appear to increase in length to anything like the extent that the posterior half does; so that the trophonucleus lies, as a rule, distinctly in the anterior part of the body, at times comparatively near to the anterior end (cf. figs. 13–20).

As the final stage in the development of this form is nearly attained, a characteristic change usually occurs in the appearance of the trophonucleus. This organella, which has already become narrowed and compressed, becomes broken up into many oblong or rather rod-like blocks, arranged with their length transversely to the long axis of the body of the parasite, thus giving the whole nucleus a remarkable ladder-like appearance (figs. 13–17). Apparently this change represents really the break-up of the original large karyosome, which is not seen, as a rule, in the nucleus when stained by Giemsa, into several small karyosomes arranged in a row. This is shown by the comparison of wet-fixed preparations stained by iron-hæmatoxylin, in which slender elongated trypaniform individuals, approaching the final form, have, instead of one large karyosome (as in Text-fig. 1 *a*), four or

more small karyosomatic bodies (Text-fig. 1*b*). Probably each rod or block in the Giemsa-stained parasites corresponds to a karyosomatic grain of the greatly extended trophonucleus. Whether this change in the nucleus is absolutely necessary before the final form can be said to be completely

TEXT-FIG. 1.



× 1600. Stages of Trypanosoma noctuæ in the mosquito, showing different conditions of the trophonucleus (from wet-fixed film, stained by iron-hæmatoxylin). A. Intermediate trypaniform phase. The karyosome of the trophonucleus is apparently budding off a small daughter karyasome. B. Approximately final (propagative) form. There are five distinct karyosomes forming the elongated trophonucleus.

formed, I do not feel certain ; occasionally individuals, which in other respects seem to have reached the final stage, are found where the nucleus is not ladder-like (cf. figs. 19–21).

In my preparations of stomachs of fifty-five hours or later, scarcely any intermediate changes are present, practically all the individuals having attained the attenuated trypaniform

condition. These are certainly very striking in appearance; their thread-like form can be judged from the fact that their length is generally from 52 to 56 μ, while they are rarely more than 1 μ broad at the widest part, and often scarcely that! Superficially, they almost deserve the name of spirochætiform Trypanosomes, were that not so very misleading. The half of the body in front of the kinetonucleus—the actively motile part—is frequently spirally twisted or coiled to some extent; while the long, passive cytoplasmic "tail" behind the kinetonucleus may be either practically straight (figs. 13, 14, 18 and 20) or bent up on itself in a curve (figs. 15–17, 19 and 21).

The above-described trypaniform type also occurs in the cultural development. I have observed individuals both in the intermediate condition, and in what undoubtedly corresponds to the ultimate form attained in the mosquito's stomach (cf. figs. *k'–m'*). It is important to note that the proportion of such forms to the trypanomonad individuals in the cultures is just the reverse from what is the case in the mosquito after thirty-two hours. This provides a very interesting commentary on the course of the cultural development as compared with that of the natural one. Whereas, in the latter, the enviromental conditions favour and lead on to the production of the attentuated trypaniform type, by the modification of the ordinary trypanomonad individuals, in the cultures there is not the same stimulus (the approaching completion of digestion) to the production of this form, and in consequence it is only developed, as it were, in isolated instances; the multiplicative ("crithidial") form, on the other hand, persists and increases in numbers, far beyond what occurs under the natural conditions. The intermediate forms in the cultures resemble closely the corresponding individuals from the insect's stomach (cf. figs. *e–g*). The few final forms which I have found (fig. *m'*) also agree quite obviously in their chief features; there is the same long, cytoplasmic "tail," and even the ladder-like nucleus may be developed. The principal difference is that the individuals in the cultures are

much broader relatively than the natural "spirochætiform" individuals; their body has apparently more bulk. I think this is simply an indication that these forms have grown more in the cultural medium than they do in the stomach; and division having most probably ceased, the result is the marked increase in "girth."

To consider next the other line of development followed by the parasites in the mosquito, this proceeds also from the original type of trypanomonad individual, by a modification in form and the mode of division. Here, again, I think the course of events can be understood from the cultural development. In certain of the ordinary trypanomonad individuals, the nuclei show a tendency to be in the hinder part of the body. This may be in the first place due to a slight obliqueness in the direction in which the preceding nuclear division has occurred. At any rate, in such individuals the direction of the nuclear division is usually oblique, and we find not only the persistence of the nuclei in the hinder part of the body, but a distinct tendency already for the division of the body to be slightly unequal (cf. fig. *n'*). These individuals with the nuclei definitely in the posterior half of the body pass, or grow into the characteristic club-shaped forms,[1] the general body-protoplasm tending to be concentrated in the region of the nuclei (fig. *o'*). Now, in these forms, the division is always markedly unequal. It is important, I think, to note this particular point, that where the nuclei are situated about the middle of the body, the division is practically equal (as in the ordinary trypanomonad individuals); where, on the other hand, the nuclei are distinctly in the hinder part of a club-shaped individual, the division of the cytoplasm is unequal. This is seen in figs. *p'* and *q'* of my scheme and also in several other figures in my memoir (**12**). I consider this mode of division is primarily due to the drawn-back position of the nuclei, and consequently of the blepharoplasts, and to the fact that the new daughter-flagellum is, at any rate, largely

[1] I formerly distinguished these by the cumbrous term of "accentuated trypanomonad" individuals.

formed by free, independent growth. Individuals which are manifestly the product of a similar division are seen in figs. *r* and *t'*.

Now, what I have found in the mosquito agrees entirely with the above description. One of the earliest stages along this line of development is the characteristic club-shaped trypanomonad form of fig. 22, or *k*. This individual corresponds very closely, it will be seen, to that of fig. *o'* or *p'*; it is almost ready for division. As remarked in my account of the living observations, I saw one instance of such a form in the act of unequal division. The smaller of the daughter-individuals about to result was a short, pear-shaped form, with scarcely any membrane; the larger one was a rather club-shaped individual, long and tapering, and probably with a long, narrow membrane (cf. fig. *q'*). Unfortunately, I have not obtained any examples of individuals just dividing in my permanent preparations; in view, however, both of the living observation and of the fact that numerous individuals belonging to both the distinct forms which are produced by such division occur in my preparations (cf. figs. 24, 26, and again, figs. 23, 25), I cannot doubt that such unequal division takes place as a normal and typical phase of the development in the mosquito.

By further division of the larger daughter-individual (possessing the long membrane), smaller forms are produced (figs. 30–32 or *n* and *o*); these can at first be recognised as corresponding to one or the other of the two types, but in the smaller individuals the distinction between the daughter-forms tends to be less marked. Here and there I have found a stout pyriform individual, one of the early-developed pear-shaped daughter-forms, just about to divide, after further growth (fig. 27); and also a quite small parasite, one of the ultimate stages, I should say, undergoing division (fig. 34). There can be no doubt that this line of development leads ultimately to the production of small, pear-shaped or oval parasites, with the nuclei close together and situated about the middle of the body, or nearer the posterior end, and with

the flagellum drawn back but with practically no membrane (figs. 29, 32 and 33). Here we have, unmistakably, the haptomonad phase of the Trypanosome, as I have proposed (**16**) to term the so-called "gregariniform" phase, which serves for attachment (and coincident multiplication). If these figures, for instance, are compared with certain of the text-figures in my description (**15**) of Leptomonas fasciculata, as I found this parasite in Culex pipiens, it will be perfectly clear that they represent the corresponding phase of the Flagellates in both cases. This agreement, I may incidentally mention, affords an excellent example in favour of the point I have urged, that from the haptomonad phase alone (having regard only to the morphology) it cannot be said with certainty whether a particular Insectan parasite represents a Leptomonad, a Crithidia or a Trypanosome.

From the above account it is clear that the early development of Trypanosome noctuæ in the mosquito culminates in the production of two distinct and extreme types. Whether the above description includes all the modifications of form which occur in the life-cycle in the Insectan host, I am not able to say. I think, however, that it is not difficult to interpret the significance of the end-stages, and if the view I favour is correct, any further stages in the life-history represent in the main a repetition, or re-development of the above sequence of forms, consequent on the persistence of the parasites in the mosquito and their response to fresh meals of blood.

The haptomonad forms most probably—as, indeed, is implied by thus designating them—become attached to some part of the wall of the alimentary canal, lose practically all the flagellum and enter upon the resting phase; "resting," that is to say, as regards locomotion, but not in regard to nutrition or multiplication. I think there is no reason to doubt that the chief situation favoured by these haptomonads for attachment, as the digestion becomes finished, is the anterior end of the stomach and especially the invagi-

nated epithelium of the proventriculus, as was so graphically described by Schaudinn (**10**) and illustrated by him in Text-fig. 14. Just because the parasites were in this situation, I feel sure that in regard to this point Schaudinn's account relates to actual developmental phases of T. noctuæ and not to a purely Insectan form (such as, possibly, Leptomonas fasciculata). In the case of the latter, on the other hand, the haptomonad forms are restricted to the intestine apparently. Whether the haptomonad phase of T. noctuæ also invades the intestine to any extent, I am unable to say; considering that these forms tend to mass themselves at the anterior end of the stomach, it is quite likely that they do not—unless they are also able to form cysts for passage to the exterior.

Returning now to the first line of development, what is the further destiny of the remarkable thread-like individuals? I am decidedly of the opinion that they represent the propagative phase of the Trypanosome in the mosquito, the form, that is, in which the parasite is finally transmitted back again by inoculation to the owl. Unfortunately, I have no proof of this, but certain considerations point strongly to this being the right explanation of the significance of the above very characteristic type. In the first place, a brief comparison with the known course of the life-history in piscine Trypanosomes is most suggestive. It has been recognised for some time that a given species of fish Trypanosome shows—at all events, in many cases—very considerable polymorphism during that part of the cycle undergone in the blood of the Vertebrate host; thus certain individuals of a species may be quite small, slender forms (representing a young stage), others large, massive forms, with all intermediate grades between cf. T. granulosum, T. percæ, Minchin [**3**]). Now I have shown that the same pronounced polymorphism also obtains in Avian Trypanosomes (vide [**12**] and, with Minchin [**5**]). Whereas earlier writers frequently described a small form from a particular host as one species, and a large, massive form from the same host as another species, the true meaning

of these different types is that they are different stages in the life-history of one species. Thus the large, massive, "blue" Trypanosomes of the little owl, originally described under the name T. ziemanni, and connected by Schaudinn with Leucocytozoon ziemanni, are really only the large forms of T. noctuæ and not a separate species at all. And a corresponding great variation in form and size is found in T. fringillinarum, in the chaffinch. Hence there is a strong family resemblance between piscine and avian Trypanosomes, as regards the types met with in the Vertebrate host; and it seems quite clear that both belong to the same group or division of Trypanosomes, and stand somewhat apart from Mammalian forms, for instance. Turning now to the life-cycle of piscine forms in leeches, which has been fully worked out by Miss Robertson in two or three cases (e.g. T. raiæ in Pontobdella [7 and 8], T. danilewskyi in Hemiclepsis [9], it is found that, after a varying period of multiplication by the parasites in the trypanomonad (crithidial) phase, as the digestion approaches completion—the time occupied varying considerably according to the conditions—a trypaniform type is developed, which becomes very elongated and slender. This passes forwards from the crop into the proboscis-sheath, and is the propagative, inoculative form, which transmits the infection to a fresh fish.

This type of form is essentially similar to that above described in Culex, the significance of which I believe to be also the same; in the leech it does not apparently attain the same degree of tenuity and the remarkable thread-like appearance which it does in the mosquito, but it may at times show the same peculiar ladder-like nucleus. It may at first be thought that from thirty-four hours onwards is too soon for the final, propagative form in the mosquito to be already present. But this rapid development appears quite explicable when the habits of the mosquito are considered. Unlike most of the Invertebrate hosts (transmissive agents) of Trypanosomes, mosquitoes do not feed solely on blood. On the contrary, it is generally recognised that, so far as the species of

temperate climates are concerned, meals of blood are not by any means the rule, and in many cases only taken in connection with the development of the eggs. In the case of most females blood appears to be necessary for this purpose; it certainly is so in Culex pipiens, and as I have recently shown (l. c.), two meals of blood will suffice to bring about the growth and oviposition of the fertilised ova. After laying one, or perhaps two, batches of eggs, most of the females either die or go into hibernation. Hence, it is perfectly clear that unless the propagative phase of the Trypanosome is developed in time for inoculation at the second (or at most the third) meal of blood, the chances of the parasite passing back to the bird are very uncertain—an entirely different state of affairs from what is the case among tsetses or leeches. For the above reasons, therefore, I think there is no difficulty in assuming that the thread-like "spirochætiform" individuals which I have described represent the inoculative type, or in understanding their early development in the mosquito.

While I consider it is quite likely that these forms are inoculated again into the blood at the second time of feeding, I do not overlook the possibility that they (or some of them) pass first into some other organ of the mosquito (such as the salivary glands, or œsophageal diverticula), and there await a third meal; but this has still to be ascertained. I do think, however, Schaudinn was mistaken in stating that the Trypanosomes cannot be transmitted back again to the owl until the fourth meal inclusive. I should say, if the parasites had to wait until the mosquito took a fourth meal of blood, they would rarely, if ever, have the opportunity of getting back again at all, unless, perhaps, they were in a female which was going to hibernate. It is obvious from Schaudinn's account that he was following chiefly the multiplication of the parasites in the haptomonad condition (vide his description of their attachment in vast numbers to the walls of the alimentary canal). Schaudinn does not appear to have seen the real propagative phase at all! He nowhere describes the

attenuated forms with the characteristic cytoplasmic "tail" (cf. on the other hand, Mayer's account, referred to below). Schaudinn's slender, "spirochætiform" individuals, which he describes under "Spirochæta" (Trypanosoma ziemanni), agree fairly well with the intermediate trypaniform stages in the development of the final type (such as, e.g. figs. 7 and 8); but I must add, nothing has astonished me more than the lack of close correspondence, in the main, between the different forms of the Trypanosome occurring in the mosquito, as Schaudinn figured them, and as I have found them.

II. The Ookinetes of Halteridium noctuæ and Leucocytozoon ziemanni.

A few words next concerning the ookinetes of Halteridium and Leucocytozoon, as they occur in my permanent preparations. As regards their general appearance, I have little to add to the description previously given by Mayer (l. c.). The ookinetes of both forms are fundamentally similar in type, as was of course to be expected, considering that two essentially similar parasites are concerned. In both cases the body is very frequently more or less coiled up in the form of a C. Practically the only difference between the two is that the ookinetes of Leucocytozoon are much larger, especially in regard to length, than are those of Halteridium (c. f. figs. 35–40 and 41 and 42).

In my preparations of the Culex which fed on Owl 19, made from thirty-three to thirty-six hours after feeding, the ookinetes of Halteridium are very numerous. In preparations made from mosquitoes which fed on Owl 23, ookinetes are also usually to be found (up to thirty-six hours), but they are very scanty; this difference is due to the different conditions of the infection in the two owls respectively (see above, pp. 400–401). Speaking generally, all the ookinetes observed are in the same phase of development, and with one or two exceptions have lost all the pigment. In none of them is anything like

a kinetonucleus, let alone a developing flagellum, recognisable. In a certain number a small chromatinic area, or clump of chromatinic grains, is present, in addition to the nucleus (figs. 35 and 36); but these stain quite similarly to the nucleus, and in no case have the characteristic appearance of a kinetonucleus, as seen when stained by Giemsa (contrast the "resting flagellates" referred to below). Unfortunately I am unable to say whether the nucleus possesses a central karyosome or not, but at any rate there is certainly no excentric or extranuclear karyosome, such as occurs at certain periods both in Halteridium and Leucocytozoon when in the blood,[1] and which often simulates a kinetonucleus in so marked a manner that it was formerly mistaken for one, until I showed clearly (**14**) what its true significance was. Frequently the ookinete shows one or two vacuoles in the cytoplasm; I have not been able to find any ookinetes in my preparations of mosquitoes which were made later than fifty-four hours after feeding.

I have only come across very few ookinetes of Leucocytozoon on my smears; I was generally able to find one or two during the living examination of the stomachs of mosquitoes which had fed on an owl infected with this parasite, but unfortunately, in several cases, owing to their scantiness, I have not succeeded in obtaining any on the permanent slides made. Those I have found are all practically similar in form and size (figs. 41 and 42). The nucleus is always situated fairly near to the more rounded end of the body, as it is also in Halteridium; it varies in size to some

[1] Reichenow, in a note on Leucocytozoon ziemanni in his account of the Hæmogregarines in 'Handb. d. Path. Protozoen' (**6**) states that he was unable to find a karyosome in the male gametocytes. As I showed in my "Notes on Sporozoa," published about the same time, there is certainly such an organella present, excentric or even extranuclear as in the female forms. It is unmistakable in preparations stained by iron hæmatoxylin, but it is rarely shown in Giemsa smears. Reichenow does not say whether the figure he gives is from a Giemsa smear or not, but from its general appearance (e. g. the hypertrophied host-cell nucleus stains quite differently after iron-hæmatoxylin), I should say it was.

extent. Now and again separate chromatinic granules occur close to it (cf. fig. 42). In these ookinetes, also, one or two vacuoles are often present. None of the ookinetes of Leucocytozoon, any more than those of Halteridium, are in any later stage of development. I have not seen the least indication of the remarkable skein-like formation so graphically described by Schaudinn, accompanied by nuclear multiplication and the eventual development of a number of very small Trypanosomes! I must say that I doubt very much now the correctness of all this.

III. The "Resting Flagellates."

As was described in the account of the living observations (see above, pp. 401–402), in a small number of female Culex pipiens caught wild, which were examined at an early stage of the experimental work before I restricted myself to the use of bred-out mosquitoes, certain characteristic and rather peculiar motionless bodies were sometimes present. I have mentioned how on one occasion such resting forms were actually seen to develop into active flagellates, and the temporary illusion fostered by the observation. But not only were they seen in the first "wild female," which fed on Owl 19, well infected with Halteridia, they also occurred now and again in females which had fed on an uninfected bird, or which were examined before being allowed to feed on a bird at all! Hence, whatever their origin, there is no reason for associating them with Halteridium. For some cause or other, with the above-noted exception, these resting forms were never observed to become active, nor were active flagellates corresponding to them ever found.

In my permanent preparations, practically all these parasites occur in the resting condition (figs. 43–45): in one or two instances, however, there is an unmistakable wavy border in the anterior part of the body, accompanied by a drawn-out, tapering anterior end (figs. 46–47). I am strongly of the opinion that the flagellum is actually developed in both

these cases, but it is difficult to be quite certain because for some reason or other, it has not stained red in the usual manner after Giemsa. Especially in the individual drawn in fig. 47, however, a definite line is evident along one edge of the anterior, tapering part, which is continued free for a short distance, which in all probability represents a flagellum. The general appearance of these forms, moreover, closely resembles that of the particular individual referred to above, just the instant before a well-marked, free flagellum became apparent. I think no one can have any doubt that these are really Flagellates, because they are certainly binucleate forms; in all cases, a definite kinetonucleus is present, usually immediately in front of the trophonucleus, which has the characteristic staining reaction to Giemsa. It may be pointed out that there is no question of this element being a karyosome, because, the karyosome of the nucleus can at times be seen, appearing, as is always the case in Giemsa-stained smears, as a clearer area, with a distinct centriole in the middle (cf. figs. 43 and 46). The cytoplasm always stains more darkly and much more bluish-purple in tint than any of the developmental phases of Trypanosoma noctuæ, in which, by the way, aflagellate phases appear to be entirely lacking. In all my experience of crithidial forms, whether as developmental phases of Trypanosomes, or "Crithidial" parasites, I have never observed any in which the cytoplasm stains in this peculiar dark manner, or in which the flagellum is so faint.

This parasite is certainly a crithidial form; this is evident from the contiguity of the two nuclei about the middle of the body, as well as from the distinct undulating membrane, where an individual is in, or about to assume, the active condition. In one respect it appears to be unique, i. e., in being non-flagellate, and moreover, without any trace of a rhizoplast, and quite motionless, when still possessing the elongated, fusiform shape, which is always associated in other Flagellates of this kind, with the active, flagellated condition; in all other cases of which I am aware, when the

parasites are "resting" and non-flagellate they are in the short, more or less oval, haptomonad phase. This form does not agree with any of the "Crithidiæ" hitherto described from mosquitoes; until more is known about it I am inclined to regard it as a distinct parasite. At present I have obtained the impression that this may be a purely Insectan form, a parasite more particularly, perhaps, of the larval Culex. Somehow, it does not look like a form accustomed to a blood-medium; its appearance and behaviour are so different from all the other crithidial forms which I have had occasion to study.

General Conclusion regarding the Question of a Connection between the Trypanosome and the Hæmosporidian Parasites of the Little Owl.

For the last time, I hope, that it will be necessary, I return to this subject, more particularly in order to point out the bearing upon it of the observations recorded above on the developmental phases of these parasites in the mosquito. It must be apparent, indeed, that these observations support and further strengthen the conclusion at which I had already arrived (14), that there is no connection whatever between these different types of parasite; and I have found nothing that in any way corroborates Mayer's account (2), in which he has upheld the opposite view.

In the first place, with regard to the mosquito which fed on Owl 19. As was to be expected from the typical ripe Halteridial infection of this bird, numerous fully-formed ookinetes were found without difficulty in the stomach when dissected. But not one of these ever showed any sign of passing into a flagellate condition; nor were any active Flagellates observed of the different types which I subsequently found frequently in mosquitoes fed on an owl known to have Trypanosomes. This fact is very significant when it is remembered that no Trypanosomes were ever seen in the blood of Owl 19, either in life or in searching smears, and if

they were present, must have been so rare at the time as to be negligible.

As was discussed above, the matter was complicated just at the outset by the use of a few "wild" females in which a "resting Flagellate" occurred, which on one or two occasions developed into the active condition. In my own opinion, however, it is perfectly clear that these resting Flagellates have nothing to do with the ookinetes. Although there is undoubtedly a general resemblance in appearance between these two bodies when observed in life, there are several important reasons for concluding that this is only a coincidence. In stained preparations the two types of element appear fundamentally distinct, for the resting Flagellates show without exception the binucleate condition; the ookinetes, on the other hand, never do. In not a single instance, whether of Halteridium or of Leucocytozoon, have I been able to find an ookinete which possesses the binucleate condition, the first essential for it to be regarded as connected with a Hæmoflagellate. Again, the staining reaction of the general cytoplasm in the two cases is entirely different; and though, knowing what I do of the dangers, no less than the advantages, associated with the use of the Giemsa stain, I should be the last to lay stress upon casual staining differences in different cases; nevertheless, where such a difference is constant and uniform, weight may be laid upon it. Further, these resting Flagellates occurred also in "wild" females fed on owls totally uninfected with any of the parasites under discussion, and in which no ookinetes, of course, were found. Finally, these resting Flagellates have certainly nothing whatever to do with the developmental phases of Trypanosoma noctuæ.

Now, in the bred-out mosquitoes which fed on Owl 23, ookinetes were always scanty; particular individuals were carefully watched on different occasions, but in these also no change or development of any kind was ever seen. Nevertheless, in about 46 per cent. of these mosquitoes examined, active Flagellates were observed in different stages of development; and it is to be re-

membered that Owl 23 was the only bird in which Trypanosomes were found in the peripheral circulation, and actually at this period. If the Trypanosomes had indeed developed from ookinetes, it is difficult to understand why they should occur frequently when the ookinetes were scanty (and sometimes not actually noticed), and yet not at all in the case where the ookinetes were numerous (the female which fed on Owl 19, with the normal Halteridial infection). All my observations point clearly to these active Flagellates being the developmental forms of T. noctuæ in the mosquito, and derived directly from the stumpy, or stout fusiform Trypanosome, present in the blood in the summer (as previously described (**5**). I have never once seen the slightest indication of these developmental Trypanosome-stages in any mosquito fed on a bird which did not contain this stumpy, transmissive phase of the Trypanosome in the peripheral circulation—whether it contained Halteridium, or Leucocytozoon, or neither.

A few remarks, in concluding this discussion, about Mayer's account (l.c.). The most important statement of this worker is that, in hanging drops of blood from an owl infected with Halteridium, in which careful examination failed to reveal any Trypanosomes after four days or so Flagellates were found to be present; the inference is, of course, that these had developed from the Halteridium-ookinetes. I can only say that I feel absolutely convinced that Mayer was mistaken in supposing no Trypanosome-individuals to be present in the drop at the beginning of the experiment. I know well from experience how easily one of these forms can be overlooked, especially if the blood-corpuscles are in a fairly thick layer. Another possible explanation would be that certain very minute or ultramicroscopic phases of the Trypanosome were present, which later gave rise to flagellates. But this idea is quite unnecessary when I have shown clearly that the same particular flagellate developmental stages occur regularly, both in cultures and in mosquitoes, as a result of inoculation with a definite Avian Trypanosome form. For my own part,

I say candidly that I can see no sufficient evidence up to the present for believing in "cryptotrypanosomiasis"[1] or in "infective granules," etc. In all the life-cycles of Trypanosomes which are now known in the invertebrate host, there is no suggestion of such a thing, and such an explanation is not, I consider, required. In birds, and even more so in cattle and sheep, Trypanosome individuals may be so excessively scanty in the circulation that the greatest difficulty is entailed in finding them; but let a single individual succeed in passing into a culture or into its right invertebrate host, and it will multiply so rapidly that before many days have passed the forms to which it gives rise are readily found. I may say also that I am no longer inclined to think that any small form of these Avian Trypanosomes occurs in the red blood-corpuscles, which might perhaps be mistaken for a young stage of Halteridium. Since very considerable doubt has been thrown upon the occurrence of such a stage in Trypanosoma cruzi, it is becoming more and more probable that Trypanosomes do not get into the red cells at all; at all events I am disinclined now to postulate the occurrence of such a phase in the Avian Trypanosomes which I have studied until we have an authentic instance of it in some other case. As in the case of fish-Trypanosomes, with which, as I have shown above, Avian Trypanosomes have much in common, I firmly believe that unless the transmissive trypanosome-form of an Avian Trypanosome passes into culture or the invertebrate host, no development of Flagellates will occur.

Mayer describes and figures further certain "large forms" which developed in mosquitoes, which he regards as the developmental stages of Leucocytozoon, in contradistinction to Halteridium. But these forms are obviously the same as certain of those which I have described and figured as the developmental stages of Trypanosoma noctuæ; his fig. 56, for instance, represents an individual nearly arrived at the final attenuated form. The stages which Mayer associates

[1] By this term I understand some definite phase of the parasite, hitherto unrecognised and possibly ultramicroscopic.

with Halteridium, equally with those which he associates with Leucocytozoon, constitute part of a regular series, and belong to a definite life-cycle, as I have clearly shown. Correspondingly, it will be remembered, Minchin and I showed also that the small forms of the Trypanosome in the blood, associated by Schaudinn with Halteridium noctuæ, form part of a regular series with the large individuals, regarded by him as belonging to Leucocytozoon ziemanni, and altogether represent only one species—Trypanosoma noctuæ; there is no species T. ziemanni. If, therefore, one still held to the idea that the small Trypanosomes are connected with Halteridium and the larger ones with Leucocytozoon, one would be led to the impossible position that Halteridium and Leucocytozoon are different phases of one and the same thing.

I do not suppose that Mayer any longer considers that an ontogenetic connection exists between any of these different parasites, especially as in addition to the above-mentioned paper, I have also published my account of the cytology, in which I have shown that, after all, there is no nuclear dimorphism in Halteridium and Leucocytozoon, and that these are not related to the Binucleata. But as Mayer's account is the only one published of late years on the developmental stages of these parasites of the owl in mosquitoes, I have been obliged to point out where it is erroneous in the light of my own observations on the whole subject.

The conclusion of the whole matter is, that the three parasites of the little owl—Trypanosoma noctuæ, Halteridium noctuæ and Leucocytozoon ziemanni—are entirely distinct and separate types; and the same is undoubtedly true for other species of these parasites in other birds. So far as T. noctuæ is concerned I have been able to outline above the main course of its development in the mosquito (Culex pipiens), though there are, unfortunately, gaps still to be filled up. The development of the Halteridium and the Leucocytozoon in the mosquito remains to be ascertained—supposing, that is to say, that

there is any development beyond the ookinete stage. Other workers (e. g. the Sergents (**11**), Aragao (**1**) and Mayer (loc. cit.) have obtained the development of one or both of the parasites up to the same stage, but never any farther. As regards Halteridium, Aragao, who has succeeded in transmitting H. columbæ from a Hippoboscid fly (Lynchia) back again to the pigeon, is doubtful whether there is really any further development in the Insectan host; it is possible, moreover, that the so-called schizogony in the lung represents the delayed sporogony of the ookinetes, as has been hinted at by Minchin, in his text-book on the Protozoa (**4**). If that be so, then I see no reason why the ookinetes of H. noctuæ should not behave similarly; in which case, Culex pipiens may prove, after all, to be a true host—transmissive agent—of this parasite also. At any rate, for all that one can yet say to the contrary, the ookinetes may be inoculated back again into an owl, at the same time as the final propagative forms of the Trypanosome. It is, perhaps, not without significance that in every owl which was infected with the one parasite, we found the other also to be present (cf. Minchin and Woodcock, loc. cit.). The development and transmission of Leucocytozoon are still more a matter of uncertainty. As far as Schaudinn's observations are concerned, if such a remarkable nuclear multiplication and skein-development does occur, it is very strange that neither Mayer nor I myself have seen any signs of it. One thing is, I think, practically certain; if the ookinete does produce a number of small elements by rapid division, these will not prove to be Trypanosomes or other binucleate Flagellates!

The Lister Institute;
May 12th, 1914.

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EXPLANATION OF PLATES 29–31.

Illustrating Dr. H. M. Woodcock's paper on "Studies on Avian Hæmoprotozoa: No. III.—Observations on the Development of Trypanosoma noctuæ (of the Little Owl) in Culex pipiens; with Remarks on the Other Parasites occurring."

[All the figures on Pls. 29 and 30 are magnified 2000 times linear; those of the scheme, Pl. 31, are × 2000 (nearly). I am indebted to Miss Rhodes for kindly drawing and colouring most of the figures of the Halteridium ookinetes.]

PLATE 29.

All the figures relate to the development of T. noctuæ in the mosquito (Culex pipiens).

Figs. 1–3.—Typical trypanomonad forms.

Figs. 4–6.—Earliest stages in the development of the trypaniform type.

Figs. 7–10.—Intermediate trypaniform stages.

Figs. 11, 12.—Approximation to the final inoculative form. Fig. 11 shows an intermediate stage in the change in the nuclear condition; three fairly large chromatinic masses (karyosomes) are present.

Figs. 13–17.—Typical attenuated thread-like forms, with ladder-like nucleus. These are considered to be the inoculative type.

Figs. 18–21.—Individuals which agree in character with the final forms, except for the fact that the nucleus has not become ladder-like.

Figs. 22, 23.—Club-shaped trypanomonad forms. Early stages in the second line of development. (See text.)

Figs. 24, 26.—Pear-shaped forms, with the kinetonucleus tending to be in front of the trophonucleus and with hardly any membrane. These individuals result from the smaller member of an unequal division of

such a form as that of fig. 22. They represent the beginning of the haptomonad phase.

Fig. 25.—Individual resulting from the larger half of the same or a similar unequal division. It tends to be slightly club-shaped, and has the nuclei well back, with a long, narrow membrane.

Fig. 27.—Pyriform individual commencing division.

Figs. 28, 29.—Other pyriform haptomonad forms.

Figs. 30, 31.—Small individuals which still show to a slight extent the characters of the differing products of an unequal division (probably of such a form as that of fig. 25).

Figs. 32, 33.—More haptomonad forms, or more strictly, perhaps, forms which are going to become attached.

Fig. 34.—One of the smallest forms found undergoing division.

PLATE 30.

Figs. 35–40.—Ookinetes of Halteridium noctuæ. In fig. 38 part of the skin or pellicle of the ruptured erythrocyte is seen still attached to the ookinete. This occurs now and again.

Figs. 41, 42.—Ookinetes of Leucocytozoon ziemanni.

Figs. 43–47.—"Resting Flagellates" found in "wild" mosquitoes. The individuals of figs. 46 and 47 have almost certainly developed a flagellum, marginal, if not free.

PLATE 31.

Scheme comparing the development of T. noctuæ in the mosquito with the developmental stages of T. fringillinarum as found in cultures. (For explanation, see text.)

Studies in the Experimental Analysis of Sex.

Part 11.—On Stylops and Stylopisation.

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With Plates 32–35.

It has long been known that the presence of Strepsipterous parasites on solitary bees and wasps may exert a remarkable influence on their hosts, and the paper by Professor J. Pérez in 1886 (1), gave a full account of the effect of Stylops melittæ on the bees Andrena, which must always be referred to as a classical contribution to the study of parasitic castration. Although many authors have subsequently written on these parasites, the observations of Monsieur Pérez remain unsurpassed for fulness of detail and interest, and we are indebted to the veteran entomologist for additional information which he has kindly put at our disposal in answer to inquiries. The re-examination of the subject, which is undertaken here, seemed desirable, not so much for the purpose of bringing new facts to light, but for confirming reported facts and collating them with the newly acquired results on parasitic castration brought about by Sacculina, since the effect of Stylops on its hymenopterous hosts seemed to be parallel to that of Sacculina on crabs,

and yet to differ from it in many important respects. The Strepsipterous parasites differ from Sacculina in that they have the sexes separate, whereas Sacculina is hermaphrodite, so that we can test whether the sex of the parasite has any influence on the effect excited. Moreover, it is clear that in certain cases the female host is induced to assume certain male characters as the result of parasitic castration by Stylops, an effect which is never observed in the case of Sacculina, and which requires careful examination. We have been able to study a large number of stylopised bees of various species, but the most fruitful material consisted of a large colony of heavily stylopised Andrena nigroænea, established in a grassy bank close to the University Museum, which was kept under observation for several years and afforded us a rich collection. The following observations on the structure and history of the parasite are based on the bees taken from this colony.

1. Notes on the Parasite.

Stylops melittæ, like all the Strepsiptera, has the sexes separate; the adult male being an extremely active-winged insect, showing a possible relationship to the Coleoptera (Pl. 33, fig. 8), while the adult female is a degenerate grub-like creature which remains permanently inside the body of the bee in which it is parasitic. The male, before hatching out as a winged insect, also develops inside the abdomen of the bee, undergoing its larval stages and pupation in this situation. The male pupa, in fact, closely resembles the adult female parasite, and protrudes a little cap between the segments of the bee's abdomen to the exterior, which closely resembles the head of the female parasite which is similarly protruded. When the adult-winged male emerges from its pupa and from the bee, it pushes off the protruded cap of the pupa and leaves the old empty pupal case inside the abdomen of the bee where it can often be recognised as a hollow cavity communicating with the exterior. Since the male Stylops emerge from their pupa soon after the bees

come out of their burrows for the first time in spring, the empty pupal case of the male Stylops is much more frequently met with in the bee than the pupa with its cap on containing the male itself. In the case of the female Stylops, it is quite different, since she remains permanently in the bee, and may be found all through the spring and early summer with her body distended with developing eggs or larvæ.

The appearance of a bee's abdomen with three female Stylops in it is shown on Pl. 32, fig. 1. The heads of the female Stylops are seen protruding between the segments of the abdomen, while the rest of their bodies are hidden inside the abdomen of the bee. If we dissect out the whole of the female parasite from the bee (Pl. 32, fig. 2), we see that its body consists of two chief parts, a hard yellow chitinous portion, the cephalo-thorax, which is protruded to the exterior, and a soft white segmented portion or abdomen, which is buried in the bee's abdomen, occupying a very large proportion of the hæmocoel or body cavity of the bee.

There was for a long time some doubt as to whether the hard protruded chitinous part was really the head or the tail end of the Stylops, but it is now quite certain that it is really the head end, as it is possible to prove by means of a median sagittal section, such as is shown on Pl. 32, fig. 3, that the brain (*gn.* 1) and the subœsophageal ganglion (*gn.* 2) are situated there. We can also establish from the position of the ganglia and the ventral nerve cord (*n*), that the surface of the cephalo-thorax exposed to view when the parasite is *in sitû* on the bee is the ventral surface.

Looking at this ventral surface of the cephalo-thorax we can see (Pl. 32, fig. 2), certain tubercles and slits which are of importance. A median tubercle near the extreme anterior end is placed just behind a minute pore which represents the mouth (*m*). On each side of the mouth are a pair of lateral tubercles which probably represent the atrophied mandibles (*md*). Behind these tubercles is a bow-shaped slit (*o*), the opening of the brood passage, by which the larvæ are

finally liberated. This slit at maturity communicates with a superficially-placed passage running all along the ventral surface of the body. The lateral limits of this passage are indicated by the ridges (*r*) shown in the figure. The relations of this brood passage are shown in the sections on Pl. 32, figs. 3 and 4 (*b*). It will be seen that the passage is simply a space between the chitinous integument and the body wall, the epithelium of which (*ep*) is peculiarly modified by the cells being produced into spiny processes. The function of this curiously modified epithelium of the brood-passage has never been suggested, but possibly its rough surface is convenient for the young larvæ to travel over in their journey to the exit at *o*.

The eggs complete their development in the body of the female, which at maturity consists of a mere sack containing them, all the other organs being reduced to vestiges. An idea of the appearance of a fully ripe female with the body full of active larvæ and embryos is given by the photograph on Pl. 33, fig. 6. The fully-developed larvæ reach the brood passage from the body cavity of the parent by means of five peculiar trumpet-like invaginations which lead into the brood passage and acquire openings into the body of the parent at the moment that the larvæ are ready to escape. The five trumpets are shown attached to the epithelium of the brood passage, the rest of the body having been removed, in the photograph on Pl. 33, fig. 7, and a trumpet with its opening into the brood passage is shown in the sections on Pl. 32, figs. 3 and 4.

We may make some mention of the other organs of the body of the female parasite. The skin, except where the epithelium of the brood passage is specially modified, is exceedingly thin, and the nourishment of the body must take place by absorption through this thin skin. There are no special cells for seizing on or elaborating a special kind of food either in the skin or elsewhere, so that we may suppose that the hæmolymph of the bee affords a ready-made medium which supplies the parasite with all that is requisite. This is in

perhaps significant contrast to Sacculina, where a special and highly-developed system of roots ramifies through the body of the host, and is engaged in seizing on a special constituent of the food, viz. fat.

The alimentary canal (Pl. 32, figs. 3 and 4, *g*), of the Stylops is clearly recognisable, but in a quite degenerate and useless state. There is a minute mouth opening (*m*), and an equally minute anus at the hind extremity, but the lumen of the gut through the body is obliterated. The whole apparatus is obviously functionless. There is a peculiar mass of cells with dark staining nuclei and eosinophilous cytoplasm (Pl. 32, fig. 3, *c*) situated in the ventral part of the cephalo-thorax; the function of these cells is unknown, but possibly they are of the nature of supporting-cells analogous to cartilage, to stiffen the exposed region of the body. Dorsally to the gut the remains of the dorsal blood-vessel or heart (Pl. 32, figs. 3 and 4) can be recognised. The nervous system consists of three ganglionic masses (Pl. 32, fig. 3, *gn* 1, 2, and 3), the dorsally-situated ganglion, (*gn* 1), being connected by means of a thin commissure round the œsophagus with a large ganglion in the thoracic part of the cephalo-thorax (*gn* 2). From this ganglion a thin, nervous strand (*n*), representing the ventral nerve cord, passes to a third ganglion (*gn* 3) in the abdomen, and from this a thin filament passes away, but no other ganglia could be found in the adult female.

Of great importance for the economy of the parasite is the tracheal system. There are two main tracheal trunks which open on conspicuous tubercles (Pl. 32, fig. 2, *tr.*) on each side of the cephalo-thorax. These two tracheæ (see Pl. 32 and 33, figs. 4 and 7, *tr*) pass right through the body, giving off numerous branches, which ramify among the developing eggs and supply them with oxygen from the outside, quite independently of the bee. We may note again a contrast between Stylops and Sacculina here, since the developing Sacculina roots have to obtain their oxygen from the blood of the crab.

The above account of the structure of the female parasite

is sufficient to give an idea of its mode of life and nourishment; for further details the reader may refer to the papers of v. Siebold (**2**), Pierce (**11**) Nassonow (**10**) and Brues (**8**). There are certain points relative to the life-history of the parasite which remain obscure but suggest features of great interest. It is known that the larvæ, which are called Triungulins and have the form shown in ventral view on Pl. 32, fig. 5, leave the body of the parent by means of the opening of the brood passage, and find their way on to flowers visited by the bee. They then clamber on to another bee which visits the flower, and clinging on to its hairs are carried back to the burrow, where they ultimately enter the cells and infect the next generation. At exactly what stage they enter the young bee larvæ is not known, but it is presumably at an early stage of development.

The really obscure part of the life-history concerns the mode of development of the eggs, and the question, how the female parasite is fertilised by the male, if, indeed, fertilisation ever takes place.

In our account of the structure of the adult female parasite it was shown that the portion of the body protruded to the exterior is the head, and not the tail, as certain authors have supposed. Apart from the anomaly of fertilisation taking place through the head end of an insect, we have been unable to find any opening or organ such as a spermatheca for the entrance or reception of the spermatozoa of the male, either on the protruded cephalo-thorax or on the rest of the body inside the bee. It has been suggested by some authors (**16**) that fertilisation may take place through the opening of the brood passage, but this appears to us improbable, as there is no means of entrance from the brood passage into the body of the parasite, the trumpet-like invaginations being completely closed until shortly before the larvæ are ready to emerge. It has also been suggested that fertilisation might take place through the mouth and alimentary canal, but this rather extravagant suggestion is not supported by the actual state of the alimentary canal, which appears in sections as a con-

tinuous narrow tube passing through the body without any outlet into the body cavity where the eggs are contained.

It has already been suggested that the eggs of Strepsiptera may develop parthenogenetically in certain cases. Thus Brues (**8**), describing the oogenesis in Acrochismus wheeleri (Pierce), a Xenid parasite on the wasps Polistes, contends that the eggs develop parthenogenetically, the second polar body re-entering the egg and fusing with the egg nucleus. Since Brues, however, did not follow polar-body formation his evidence is incomplete.

Dr. R. C. L. Perkins (**5**) has expressed the view that the parasites of the bee Halictus must be parthenogenetic, at least in certain cases, as when the eggs begin to develop it is impossible that a male could have fertilised the female. Dr. Perkins also has kindly informed us that out of 500–1000 specimens of this parasite seen by him only one or two were males, so that evidently the males are on the point of disappearance. Taking these facts in conjunction with the practical impossibility, as it seems to us, of the spermatozoa in Stylops ever entering the body of the female at the time the eggs begin to develop, we are led to the conclusion that development is always parthenogenetic in the Stylopidæ. If this is correct, it follows that the active winged males are useless for the propagation of the species, a conclusion which few would accept without misgiving.

Various observers have attempted to observe the act of copulation in the Strepsiptera, but mostly with no or very equivocal success. Pierce (**11**), in his valuable revision of the Strepsiptera, remarks—"That the female must be fertilised can hardly be doubted, and yet the nature of the act and the fact itself has been but slightly proven." Observations on the behaviour of the male by Saunders and Crawford (**11**) show that he is actively attracted by the female Stylops, or, at any rate, by the bee on which a female Stylops is situated, and that he runs about on the body of the bee, evincing the greatest excitement. F. Muir (**17**) has made similar observations, but is uncertain how copulation takes place.

The following account, compiled by one of us (A. H.) from notes on the habits of the male, may be given:

At the end of April and beginning of May, 1912, the male Stylops was not uncommon in the vicinity of the colony of Andrena nigroænea, being seen on the wing at mid-day in sunshiny weather. The singular flight of the male Stylops has often been seen and commented upon by many observers. When once recognised they can never be forgotten, the peculiar flight and milky-white wings at once distinguishing them from all other insects. None were observed actually flying over the burrows of the bee, and nearly all, when first seen, were some 10 or 15 feet from the ground, sufficiently high to prevent some of them being captured. All the specimens caught were boxed alive for further observation or experiment. On three occasions a male Stylops was, immediately after capture, introduced into a large glass-bottomed box containing a freshly caught bee, infected with one or more female Stylops. In each case the behaviour of the male was identically the same. The male Stylops, directly it was introduced into the box, fluttered on to the bee, and quickly ran over its body to where the head of the female Stylops was everted between the bee's abdominal segments. At this time the male is rapidly vibrating its wings and protruding its last two or three apical segments, which are long and tapering like an ovipositor. The insect is thus quite unlike a dried specimen in which these segments are invariably telescoped into the body. Actual pairing did not occur on any of the three occasions. The bee, which had been resting quietly in the box, became extremely restless as soon as the male Stylops flew on to it, and kept repeatedly climbing to the top of the box and then suddenly dropping, as if in the endeavour to rid itself of its unwelcome rider. After about ten or fifteen minutes of ceaseless running to and fro over the bee, the male Stylops voluntarily quitted the Andrena, but still continued to run and vibrate its wings for about two hours longer, after which time it dropped apparently exhausted, and died shortly afterwards. The other males which had

been boxed also continued to flutter and to vibrate their wings ceaselessly, until, in about two hours, all movement came to an end, and they were apparently dead. It will be seen from this account that the male Stylops, besides retaining its structure and activity unimpaired, also possesses the instinct for attaching itself to the bee, but in no case has actual copulation been observed.

The copulatory apparatus of the male Stylops (fig. 8, A and B) consists of a hollow chitinous penis, shaped rather like a pick-axe, which can be everted on a hinge, but when withdrawn is covered by a grooved sheath. Although the penis is a slender organ it has a sharp point, and might be used for hypodermic injection of spermatozoa into the body of the female. It is, however, very difficult to see how the injection of spermatozoa into the body of the female would result in fertilisation, because the eggs never quit the ovary, and are always completely surrounded by follicular epithelium, which would prevent the access of spermatozoa casually injected into the body-cavity. The male does not show any trace of degeneracy in its internal reproductive organs, the vesiculæ seminales being crowded with active spermatozoa.

In several females the eggs have been found in an early stage of development, the features of which strongly confirm our suspicion that development is parthenogenetic. In these cases all the developing eggs are at approximately the same stage of development, exhibiting two, or, in some cases, more segmentation nuclei (Pl. 32, fig. 4 A,*bl.*), while at the periphery of the egg a mitotic spindle is observed (p. 1), which invariably exhibits a single large chromosome and three, or four smaller ones, often in process of division. Each egg is completely invested by the follicular epithelium (*f*).

Now, it is quite clear that the mitotic spindle must represent the first polar body in process of division. There is, however, no trace of a second polar body, which there certainly ought to be if a second polar body was given off and fertilisation effected in the usual way.

It is possible to explain the appearances in these eggs on three suppositions: (1) That no second polar body is formed and that the female pronucleus develops parthenogenetically, (2) that a second polar body is formed but fuses with the female pronucleus, which then develops parthogenetically, or (3) that a second polar body is formed but disappears and leaves no trace, although the first polar body is still only in the metaphase of its mitosis, and that fertilisation is effected by a spermatozoon.

It must be admitted that the third supposition is exceedingly improbable from what we know of the development of any other egg, the entire disappearance of the second polar body before the completion of the mitosis of the first polar body being altogether unknown.

During the present year (1914) the burrows of the colony of A. nigroænea were carefully watched at the beginning of the season, and the first bee carrying a female Stylops was captured, and the parasite preserved and its eggs examined by serial sections. These eggs were found to be already in a fairly late segmentation stage, which is strong presumptive evidence that development had already begun before the bee had left its burrow, and before the Stylops would have had a chance of being fertilised.

It will therefore be seen that the cumulative evidences in favour of the parthenogenetic development of the eggs of Stylops are exceedingly strong, consisting in the following main heads: (1) There is no opening or apparatus in the female adapted for conveying the spermatozoa to the eggs; (2) the eggs remain throughout their development encased in the follicular epithelium of the ovary, so that access to them by spermatozoa which had entered the body cavity is very difficult to imagine; (3) parthenogenesis must occur as a normal rule in the parasites of Halictus; (4) the known stages in the polar-body formation of Stylops are inconsistent with the view that fertilisation by a spermatozoon has been effected; (5) actual copulation by the male has never been adequately observed.

We may finally note that in a large number of colonies of infected Andrena it would appear that the male parasite is very much scarcer than the female, and in certain cases may have almost entirely died out. This rule is, however, not invariable, and in the case of Xenos, Wheeler (**12**) records an actual preponderance of males over females.

The most difficult thing to explain, on the assumption that the males are now useless, is their marked instinct for clambering on the body of the bee when infected by a female Stylops. This surely indicates that at some time pairing took place in this situation. In explanation of this it must be remembered that under present conditions the female Stylops never develops beyond what is really a larval or rather pupal stage, and that at some previous period in the history of the parasite it is certain that the female developed further and probably issued from the bee as a fully-formed and possibly winged imago. It may well have been that when this was the case the males waited for the emergence of the females and paired with them directly they issued from the bee, and that they still retain the instinct of attaching themselves to the infected bees and waiting for the appearance of the female imago, an appearance which now is never realised. For an explanation we are forced to fall back upon some such hypothesis as this, since no transitional forms are known to exist in nature which might show the intermediate steps by which the endo-parasitic habit and arrested development of the female parasite have been acquired.

If we are correct in supposing that the males of the Stylopidæ are useless and that development is invariably parthenogenetic, it may be pointed out that such a condition of affairs is not altogether without parallel according to the results of recent researches. In the Rhizocephala (**13**) degenerate Cirripedes parasitic on various Decapod crustacea, we find that in certain genera, e.g. Sacculina and Peltogaster, the parasites are hermaphrodites which propagate themselves by a continuous round of self-fertilization. Nevertheless, degenerate larval males are found, often in

numbers up to twenty or thirty, fixed round the mantle opening of about 80 per cent. of young Sacculinæ, and these larval males are entirely degenerate and useless in the reproduction of the species. Other genera of the Rhizocephala, e. g. Sylon, are purely female, and reproduce entirely by parthenogenesis, and have thus got rid of the marked disharmony (to borrow Metchnikoff's term) which characterises the sexual economy of Sacculina and Peltogaster. Another instance of sexual disharmony has been described by Maupas in certain free-living Nematodes of the genus Rhabditis, where again the majority of the individuals are self-fertilizing hermaphrodites, but a certain number of males are still produced which are useless for reproduction. In other species these males have been entirely eliminated.

These instances of undoubted disharmony, or imperfection of adaptation in sexual economy, should make us pause before we assume that the males of the Stylopidæ must still be functional in those species in which they occur in considerable numbers.

2. The Effect of the Parasites on their Hosts.

We will consider first the effect of the parasites on the internal reproductive organs of the hosts.

In the case of twenty female Andrena nigroænea, of which four carried male Stylops puparia and sixteen female Stylops, it was found in every case that the ovary was very greatly reduced in size and was incapable of producing mature ova. The appearance of such reduced ovaries, as compared with that of normal ovaries, is shown on Pl. 33, figs. 9 and 10.

No marked difference was observed in cases where male parasites were present from those in which female parasites were concerned.

It was found, therefore, without exception, that stylopisation brought about a reduction in size of the ovary and complete sterility.

This result is in agreement with the observations of Pérez (1).

In the case of fifteen male A. nigroænea, of which four carried male puparia, ten female Stylops, and one had a male and a female parasite, it could not be observed that the presence of the parasites in any case had exerted any effect on the development of the testes or their ducts. The figures given on Pl. 33, figs. 11 and 12, show the male reproductive apparatus in normal and stylopised males, and it will be seen that there is no reduction in size in the stylopised individual. In order to test whether the testes of the stylopised males produce ripe spermatozoa, it was found necessary to examine bees early in the year soon after their emergence from the burrows, since both normal and stylopised individuals later in the year were generally found with the vesiculæ empty of spermatozoa.

If, however, stylopised males are taken early in the year, it is possible to show that their testes and ducts are in the same condition as normal males, and that abundance of ripe spermatozoa are present in the large vesicles which lead from the three testicular tubes on each side into the vas deferens. The section on Pl. 33, fig. 13, through the three testicular tubes and the vesicle of one side of a stylopised bee, shows the presence of abundant spermatozoa in the vesicle. The testicular tubes in this section are more or less empty with a rather ragged degenerate epithelium, but this appearance is due to the fact that spermatogenesis is over, and is equally to be noticed in normal males.

This absence of any effect of the stylopisation on the male internal organs is on the whole in agreement with what other authors have found, though Pérez records some cases of a one-sided damage being inflicted on the testes by the parasite, and Theobald (7) is inclined to believe that the damage may be considerable. Perkins (5), on the other hand, both for males and females, tends to minimise the effect of the parasites on the internal organs, and records the fact that stylopised males have been taken in copula with

stylopised females, showing that the sexual instincts may still persist in stylopised individuals.

There can be no doubt from our own observations, and from the general consensus of opinion, that the female bees suffer far more serious reduction in their ovaries than the male bees experience in the case of their internal reproductive organs.

The reason for this difference in effect appears to us fairly obvious. The testes of the male bee are exceedingly minute structures, about a hundredth part of the ovaries in size, and they, therefore, require a small fraction of the nutriment which is demanded by the ovaries. The presence of the parasite, therefore, while cutting off a large part of the necessary nutriment from the ovaries, does not succeed in depriving the minute testes of the small amount of nourishment which they require, and hence they are able to attain their normal development, though the ovaries are seriously starved.

We may now proceed to the effect on the external characters.

The males and females of A. nigroænea differ, firstly from one another in their external genital armature which consists of a complicated copulatory apparatus in the males and of an ovipositor in the female. After examining a long series of stylopised males and females, we are unable to find any reduction or abnormality in these structures as the result of stylopisation. In this respect we are not in complete agreement with Pérez (1), who reports a marked reduction in the development of the genital armature as the result of stylopisation in several cases. We do not doubt that this is correct, but the effect in any case is a comparatively slight one, and there is never the slightest difficulty in at once recognising the male and female stylopised individuals by the genital armature which is always typically developed, though it may be in certain cases somewhat reduced in size.

Another important secondary sexual character, affecting the hard chitinous structure of the bee is found in the

antennæ, which are 13-jointed in the males and 12-jointed in the females, of all Andrena (see Pl. 33, figs. 14–15).

This character in our experience and in the experience of all other observers is quite unaffected by stylopisation, the infected individuals always having the number of joints typical of their sex. Pérez again is of opinion that in certain cases the relative length of the joints in infected individuals is slightly altered, but here the effect is admittedly very slight indeed, and the figures given to illustrate the effect do not appear to us to bear out the contention.

A marked distinction between the sexes of all Andrena is found in the structure of the femur and tibia of the hind legs, which are thin and not markedly hairy in the male, but in the female are greatly enlarged to form the scopa or pollen-collecting apparatus. The condition of the normal male (A. nigroænea) is shown on Pl. 34, fig. 16, of the infected male, in Pl. 34, fig. 17, of the normal female in figs. 18 and 19, and of two stylopised females in figs. 20 and 21.

These figures bring out the fact, which we have found invariably in A. nigroænea, that as the result of stylopisation the male does not acquire in any degree the scopa of the female, while the scopa of the female is always to some extent reduced in size by the action of stylopisation.

We also find that stylopised females never carry any pollen on their scopæ, in marked distinction from the normal females, the majority of which are found with their scopæ plastered with pollen as shown in fig. 18. The stylopised females have evidently entirely lost the instinct for collecting pollen, though they still continue to visit the burrows. Of the hundred or so stylopised females examined not a single individual had pollen on it, but we are not in a position to say that such a thing cannot ever occur, as there is certainly a great degree of variation in the intensity of the effects of stylopisation in different individuals and species.

We have found that stylopisation affects the punctuation of the chitin of the abdomen to a certain extent, though the effect can only be appreciated by examining a good series of

normal and infected individuals together. If we look at a series of normal males and females together, we shall notice that the males reflect the light more brightly than the females, owing chiefly to the less degree of punctuation and hairiness of the abdomen.

The stylopised males, on the other hand, tend to have the abdomen dull, very much as in the female, and this appears to be due to the deeper and more frequent punctuation on the abdomen, and not to a greater hairiness. The stylopised females do not appear to be affected either in punctuation or hairiness.

We have now dealt with the most important secondary sexual characters which concern the structure of the hard chitinous parts, and it will be recognised that the effect exerted by stylopisation is small and consists in a reduction of certain sexual characters, and never in a real assumption of characters proper to the opposite sex. The most constant and striking effect is the reduction of the scopa in the female and the loss of the instinct for collecting pollen. Comparing these effects with the effect of Sacculina on the secondary sexual characters of Inachus (**14**), it will be admitted that the complete inversion suffered by the males of Inachus has no parallel in the bees modified by stylopisation, so far as structure is concerned.

There remains for consideration, however, a very important character which may undergo a very complete inversion as the result of stylopisation. In certain species of Andrena, e. g. A. chrysosceles and A. labialis, the female has the ordinary black clypeus, but the male has a yellow or white one (see Pl. 35, figs. 22, 23, 25 and 27). Pérez discovered that as the result of stylopisation the female might assume completely or in part the coloured clypeus of the male (see Pl. 35, fig. 26), while the male might undergo considerable retrogression and lose a great part of the yellow colouration (fig. 28). Pérez makes it clear and has personally informed us that this remarkable effect is by no means invariable and that very frequently stylopised males and females of A.

labialis may exhibit their proper clypeus colouration without any modification. It is therefore necessary in this case to be able to examine a long series of infected individuals, and it would appear that other observers have not been fortunate in securing such a series because no confirmation of Monsieur Pérez's discovery has hitherto been published. We are fortunately in a position to produce confirmatory evidence in the case of A. chrysosceles. Pl. 35, fig. 22, depicts the head of a normal female specimen, while fig. 23 shows the head of a normal male with the coloured clypeus. Fig. 24 shows the head of a stylopised female, taken at Sandford near Oxford by one of us (A. H. H.), which has developed the coloured clypeus of the male in a typical manner. This specimen was parasitised by a male Stylops. It may be stated that long series of normal A. labialis and chrysosceles have been examined, and that in no case has any assumption of the coloured clypeus by the female or *vice versâ* been observed apart from the effects of stylopisation.

This acquisition of clypeus colouration by the female is by far the most striking alteration brought about by stylopisation, because it really amounts to a true acquisition of a positive character belonging to the opposite sex, and not to a mere negative suppression of characters that should normally be developed. The alterations in the hard parts and the blackening of the clypeus in the males, can all be interpreted as mere negative suppressions, but the acquisition of the yellow clypeus by the stylopised females is in a different category. In the majority of Andrena the clypeus of both sexes is black, so that the loss of the yellow colour in the stylopised males of A. labialis may be considered a mere repression, but not so the acquisition of the yellow colour by the female.

Before discussing these results there are two points which merit attention. Since the Stylops parasites are of separate sexes, it appeared possible that the sex of the parasite might have an important influence on the effect exerted upon the bee. For instance it might be found that

only females stylopised by male Stylops could develop the white clypeus characteristic of the normal male bee. Of course if such a contention could be proved it would have a most important bearing on the theoretical interpretation of how the effect is brought about. It would suggest, in fact, that the male Stylops exerted a specific male influence on the bee, and the female Stylops a specific female influence.

In answer to our inquiries Monsieur J. Pérez has kindly sent us some of his specimens of A. labialis, which satisfactorily settle this point. Among these specimens there are two female A. labialis parasitised by female Stylops which show a considerable amount of white colour on the clypeus, and there is also a male A. labialis parasitised by a male Stylops, which shows a very marked reduction of the white colour on the face.

Dr. R. C. L. Perkins has also sent us two valuable instances bearing on this question, viz., two females of A. labialis, with their faces coloured as in the male, both of which are parasitised by a single female Stylops. These instances are abundantly sufficient to demolish the view that the sex of the parasite has any determining influence on the effect produced on the secondary sexual characters. It is probably true that the presence of a male Stylops has a more generally damaging effect on the bee, but there is no evidence of the male parasite exciting a specifically male effect and of the female exciting a female effect upon the host.

The theoretical importance of this fact will be given its due weight in a later paragraph.

The second point to which attention may be called is the great amount of variation exhibited by Andrena and other insects in their reaction to strepsipterous parasites. This variation does not only subsist as between different species of hosts, but also as between different individuals of the same species of host. Wheeler (**12**), who has made a most detailed and exhaustive examination of the effect of Xenos on the wasps Polistes came to the conclusion that the parasite had no definite effect on the secondary sexual characters of the

host, and it is clear that his conclusion is perfectly correct. The same may probably be said of the effect of Eleuchus on the Homoptera.

We have already seen that no other observer has apparently described the effect of Stylops on the clypeus colouration of certain Andrena, noticed by Pérez, until we came across the case of A. chrysosceles published here. These apparently contradictory results have led to much confusion, but to anyone familiar with the facts of parasite castration in other branches of the animal kingdom they will occasion no surprise. In cases where the effects of the parasite lead to the most startling and complete inversions, as in Sacculina on Inachus and Peltogaster on Eupagurus (**14**), there is always a certain small percentage of individuals which remain almost completely unaffected by the presence of the parasite, while in other cases, such as Sacculina on Carcinus, the effect of the secondary characters is often nil, and never consists in more than a slight approximation of the male to the female type. Such variations then, whether due to differences in individual or specific susceptibility or to some casual event in the history of the disease, are to be expected, and should warn the observer not to draw conclusions without examining a long series of infected individuals.

(3) Discussion of Results.

If we compare the effects of stylopisation with those of Sacculina on Inachus, we shall recognise that they are much slighter and less radical in the former than in the latter case. Thus in the internal reproductive organs stylopisation only causes a reduction in size of the ovary, and prevents ripe ova from being produced, while the testes of the male are practically unaffected. The presence of Sacculina, on the other hand (**13**), may occasion the complete destruction of all the internal genital organs, with the exception of some remnants of germinal epithelium, while infected

male crabs may be induced by the Sacculina to produce ripe ova in their testes.

Correspondingly the effects of stylopisation on the secondary sexual characters are comparatively slight, and amount to no more than a reduction of certain characters, such as the scopa of the female, while in the case of Sacculina the whole morphological structure of the male crab may be entirely converted to the female state.

In one case, however, that of the colour of the clypeus, the female bee when stylopised may assume the positive male character.

It is clear, therefore, that the reaction of the bee to the Stylops does not go so far as that of the crab to Sacculina, either internally or externally, and whereas in sacculinisation we are forced to the conclusion that the Sacculina exerts an active feminising influence on both sexes of infected crabs, in the case of stylopisation it is sufficient to hold that the action here consists merely in an arrest of development incident on the cutting off of a certain amount of nutriment from the ovaries, and to a less extent from the testes. In sacculinisation we have argued (13) that the Sacculina roots, by demanding a certain type of nutriment, viz. fat, stimulate a certain type of metabolism in the crab, which is characteristic of the adult female when maturing its ovaries, and that this internal change of metabolism brings in its train all the deep-seated changes in the internal and external genital structures. The Stylops, on the other hand, does not initiate such wide-spreading changes; it stops short at abstracting a certain amount of nutriment from the blood, and so causes a merely quantitative alteration in the development of the internal and external genital organs. It has been pointed out that the Stylops parasite does not appear to be taking up any special nutriment from the blood of the host, but rather to receive the nutriment from the blood, ready-made, and thus it would not be expected to stimulate any special line of metabolic changes. Further, the Stylops always receives its oxygen from the outside air, while the

Sacculina roots are living amærobically, and must split off their oxygen from the blood of the host. This implies a more intimate relation between the metabolism of host and parasite in the case of Sacculina.

We thus see that the effects of stylopisation may be interpreted as due to a merely quantitative abstraction of nutriment normally destined for the reproductive glands, and that this abstraction brings in its train a reduction in size of these glands, especially the ovaries, and a corresponding reduction in the development of some of the secondary sexual characters.

It may be urged, however, that this explanation does not apply to the assumption of the yellow clypeus by the female, as this is a positive male character. We have an excellent analogy for this case in sterile female birds (**15**) which, either as the result of operative ovariotomy or else of ovarian disease and atrophy, may assume male plumage to a very marked extent. The assumption of the yellow clypeus by stylopised female bees with reduced ovaries seems to us exactly parallel to the assumption of cock's plumage by female birds with ovaries either atrophied or removed by operation. It seems that in both cases the mere atrophy or suppression of the ovary is sufficient in both cases to induce the development of certain male characters in the colouration.

It is not necessary, therefore, to ascribe a special masculinising influence to the Stylops parasite in the same sense as one must ascribe an active feminising influence to the Sacculina roots. The masculinising influence resides in the female bee itself, just as in the female bird, and is called into activity by the mere suppression of the ovarian function.

In this manner we may look upon the acquisition of the yellow clypeus by the female as due to the same cause as the other alterations brought about by stylopisation, viz. to the mere quantitative cutting off of nutriment from the ovary, and not to any specific or qualitative action of the parasite, as in the case of Sacculina on Inachus.

It appeared possible to us at one time that a qualitative action might account for the assumption by the female of the male clypeus in the following manner: It might be possible that this assumption by the female only followed when she was parasitised by a male Stylops, which might exert a specific male influence on the host. This supposition is not confirmed by the facts, as the presence of a female Stylops can equally bring about the assumption of the male clypeus by the female.

The fact that the sex of the parasite has no influence on the effect exerted on the host is in reality a strong confutation of the idea that the effects of parasitic castration are due to a specific internal secretion produced by the parasite. For if such a secretion were produced by the parasite, we should certainly expect that the female parasite would produce a female internal secretion and the male a male one, whereas we find that the parasites of both sex exert a similar effect. This is perfectly intelligible if we suppose that the parasites of both sexes act on the host merely by cutting off a certain amount of nutriment from the gonad, a process which reacts more profoundly on the female than on the male, owing to the larger size of the ovaries and the larger demand made by them on the nutriment in the body.

The peculiarity of the case of Sacculina consists in the fact that the roots of the parasite happen to demand an excessive supply of the same sort of nutriment which the ovary of a normal female crab requires, and so bring about a series of profound metabolic changes leading to the feminisation of the host.

The result of all the above considerations is to show that a parasite may act on the sexual characters of its host in two ways. Firstly, it may simply take up a certain amount of nutriment from the blood so as to deprive the gonad of its proper supply and lead to its partial atrophy, but without bringing about any deep-seated alteration in the metabolism or stimulating any special set of metabolic changes. The abstraction of this nutriment, by depriving the blood of its

proper supply, may lead to the atrophy of the gonad and to the reduction of the secondary sexual characters, and even to an inversion of certain secondary sexual characters, e. g. the colour of the clypeus in Andrena. Secondly, as in the case of Inachus parasitised by Sacculina and of Eupagurus parasitised by Peltogaster, the parasite in obtaining its food from the blood of the host, may set going a special set of metabolic changes in the host, and these changes may result in diverting the metabolism of the host to the female state, so that the host assumes female characters throughout and the infected male may even be induced to produce ova in its testes. This second type is not a mere passive inhibition like the first, but an active reaction explicable, as has been shown, on the basis of an immunity reaction (**14**).

Another point which emerges from this study is that the bee, Andrena, belongs to the same category as many birds (Pheasants, Fowls, Ducks, Ostriches, etc.), (**15**) in that a mere atrophy of the ovary is followed by an assumption of a positive male secondary sexual character. In all these animals it would appear that the normal ovary exerts an inhibitory action preventing certain male characters from emerging, and that when the ovarian influence is removed or interfered with the stimulus is given for the development of these characters.

Summary.

(1) From a study of the anatomy and life history of Stylops, it appears that despite the existence of active winged males, fertilisation cannot occur and development is always parthenogenetic.

(2) The parasite obtains its oxygen from the outside air by means of tracheal openings on the cephalo-thorax, and it does not possess any special absorptive organs for taking up a special kind of food from the host. Nutrition appears to take place by simple filtration from the host's blood through the very thin skin of the parasite.

(3) The effect of the parasite on the internal genital organs is slight, as compared with the effect of Sacculina on Inachus, and leads to a reduction in the size of the ovaries to about quarter the normal size, while the testes are usually unaffected. The ovaries of stylopised bees never produce ripe ova, but the testes generally produce normal ripe spermatozoa.

(4) The effect on the secondary sexual characters is again slight as compared with that of Sacculina on Inachus. The external gonapophyses are usually unaltered, or they may be slightly reduced in size; the antennæ are unaltered. The scopa of the parasitised female is generally reduced in size, and she never or very rarely collects any pollen. The punctuation on the abdomen of the male may be increased.

(5) The most striking effect occurs in certain species (e. g. A. labialis and chrysosceles) in which the male normally has a yellow clypeus and the female a black one. Stylopisation in those cases may lead to the female assuming a yellow clypeus as in the male, while the male may lose the yellow and acquire a partially black clypeus.

This acquisition of the yellow clypeus by the female is the only change which can undoubtedly be interpreted as a positive acquisition of a secondary sexual character proper to the opposite sex.

(6) This effect may be brought about by male or female Stylops indifferently, the sex of the parasite having nothing to do with the nature of the effect exerted.

(7) The effects of stylopisation may be ascribed to a merely quantitative abstraction of nutriment from the gonad, leading to its partial atrophy, and not to a qualitative alteration of the metabolism such as is brought about by Sacculina. This also applies to the assumption of the yellow clypeus by stylopised females, on the analogy of the assumption of male plumage by many female birds as the result of simple ovariotomy or ovarian atrophy.

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EXPLANATION OF PLATES 32–35,

Illustrating Mr. Geoffrey Smith's and Mr. A. H. Hamm's "Studies in the Experimental Analysis of Sex." Part II.—"On Stylops and Stylopisation."

Lettering.

b. Brood-passage. *b. v.* Dorsal blood-vessel. *bu.* Bulb on vas deferens. *c.* Supporting cells. *ch.* Chitinous investment. *ep.* Modified epithelium of brood-passage. *g.* Gut. *gn.* 1. Brain. *gn.* 2. Sub-œsophageal ganglion. *gn.* 3. Abdominal ganglion. *m.* Mouth. *md.* Mandible. *n.* Ventral nerve. *nu.* Nurse-cells. *o.* Opening of brood-passage. *ov.* Ova. *te.* Testes tubes. *tr.* Tracheal tube. *vd.* Vas deferens. *v. e.* Vasa efferentia. *ves.* Vesicula seminalis.

Fig. 1.—Abdomen of Andrena nigroænea ♀ with heads of three Stylops melittæ ♀, protruding in natural position.

Fig. 2.—Stylops melittæ ♀, from ventral surface, removed from bee.

Fig. 3.—Stylops melittæ ♀, in sagittal section.

Fig. 4.—Ditto, transverse section through middle of body.

Fig. 4A.—Section through developing egg with first polar body (*p.* 1), blastomeres (*bl.*), and follicular epithelium (*f*).

Fig. 5.—Triungulin larvæ, from brood-passage, ventral view.

Fig. 6.—Stylops ♀, adult, with body full of triungulins ready to hatch.

Fig. 7.—Ditto, triungulins removed, showing five trumpets attached to epithelium of brood-passage. Two tracheæ spread out laterally.

Fig. 8.—Stylops melittæ, ♂, dorsal view; abdomen telescoped.

Fig. 8A.—Copulatory apparatus of S. melittæ, lateral view. *B.* Penis.

Fig. 9.—Ovary of normal Andrena nigroænea ♀.

Fig. 10.—Ovary of Stylopised A. nigroænea ♀.

Fig. 11.—Testes and ducts of normal A. nigroænea ♂.

Fig. 12.—Testes and ducts of Stylopised A. nigroænea ♂.

Fig. 13.—Section through testes, ducts, and vesicula of one side of Stylopised A. nigroænea, showing ripe sperm in vesicula.

Fig. 14.—Antenna of normal A. nigroænea ♀.

Fig. 15.—Antenna of normal A. nigroænea ♂.

Fig. 16.—A. nigroænea, normal ♂.

Fig. 17.—Ditto, stylopised ♂, showing unaltered legs and gonapophyses.

Fig. 18.—Ditto, normal ♀, carrying pollen on scopæ.

Fig. 19.—Ditto, normal ♀, showing scopæ without pollen.

Fig. 20.—Ditto, stylopised ♀, showing reduced scopæ, otherwise unaltered.

Fig. 21.—Ditto, stylopised ♀, showing reduced scopæ, otherwise unaltered.

Fig. 22.—Andrena chrysosceles, head of normal ♀.

Fig. 23.—Ditto, head of normal ♂.

Fig. 24.—Ditto, head of stylopised ♀.

Fig. 25.—Andrena labialis, head of normal ♀, after J. Pérez.

Fig. 26.—Ditto, head of stylopised ♀, after J. Pérez.

Fig. 27.—Ditto, head of normal ♂, after J. Pérez.

Fig. 28.—Ditto, head of stylopised ♂, after J. Pérez.

The Rat-Trypanosome, Trypanosoma lewisi, in its Relation to the Rat-Flea, Ceratophyllus fasciatus.

By

E. A. Minchin

and

J. D. Thomson.

With Plates 36—45 and 24 Text-figures.

Contents.

PART I. INTRODUCTORY.

(1) PERSONAL NARRATIVE.

IN this memoir we give a detailed account of investigations which have occupied us intermittently, with many interruptions, during the past five years. When we undertook this task our object in view was to work out as fully as possible the life-history and mode of transmission of a trypanosome, so that in at least one species of these important parasites its relation to the invertebrate host might be as thoroughly known as, for instance, the relation of the malarial parasite to the mosquito, thus furnishing a standard with which the life-histories of other species of

trypanosomes might be compared and contrasted as they become known. How far we have succeeded in our task must be left to our readers to judge.

The species which we selected as the subject of our investigations was Trypanosoma lewisi, the common parasite of rats which is apparently of world-wide distribution. This species offers many advantages for such a study. It is common in London and easily procured when required; its vertebrate host, the rat, is a mammal of small size, the domesticated variety of which lives well and breeds rapidly in confinement, is inoffensive, and is easily handled; its invertebrate host, the rat-flea, is also easy to keep in captivity and is extremely prolific, and it is of a size which, though it increases to some extent the difficulties of manipulation, has a great advantage that the material to be searched and studied microscopically is confined within a small compass[1]; and finally, by no means the least of the advantages of working with T. lewisi is the fact that it is non-pathogenic to its natural host and cannot live at all in human blood.

Since there is no difficulty whatever in obtaining the vertebrate host in abundance, either in the clean (i. e. non-infected) or infected condition, our first care was to obtain a stock of the invertebrate host, the flea. This we succeeded in doing from rats trapped in the open near Mr. Gurney's Laboratory at Sutton Broad, Norfolk. Fifty specimens of Ceratophyllus fasciatus were obtained in this way in the autumn of 1908, and were kindly identified for us by the Hon. N. C. Rothschild, and with these fleas a breeding-cage was stocked and a flea-farm started. The cages used were of the type used by the Plague Commission, as figured in the 'Journal of Hygiene,' vol. vi, pl. iv. A rat was kept in the cage to feed the fleas, and they were left to themselves. Early in 1909 one of us (E. A. M.) went to Rovigno for some

[1] An advantage which those will appreciate who have had practical experience of searching for trypanosomes through many centimeters of the digestive tract of the tsetse-fly, for instance.

three months, during which time the fleas were left to breed under the care of an assistant, whose duties consisted of attending to the rat and replacing it if it fell ill or died. When the cage was examined after Easter it was found to be swarming with fleas, and our work began in May, 1909. We have worked ever since then with the fertile progeny of the original fifty fleas from Norfolk, and have never added further to our stock from without. The fleas breed so fast that it is often necessary to keep their numbers down, otherwise they take too much blood from the rat and affect its health. Fresh breeding-cages have also been started, and during the greater part of the time that we have been at work we have kept two cages constantly going, one in which the fleas are fed always on a clean healthy rat, and another in which an infected rat is always kept. We shall refer to these two cages as the non-infected and the infected breeding-cages respectively. As will be shown below, the stock of fleas with which we have worked all along was fortunately quite free from any natural infection with leptomonad or other flagellate parasites. Thus we have been saved from a fertile source of confusion and error, since we can be quite certain that any flagellates found in our fleas are stages of T. lewisi and nothing else.

Although we cannot claim that in our work we have solved completely every problem presented by the transmission of the trypanosome and its development in the flea—a result which probably no man could achieve in a life-time—we think it now fitting that we should publish such results as we have obtained, after having done as much as we were able to do in the time and under the circumstances. We claim at least that we have not jumped to our conclusions; our note-books contain not only the records of many experiments, but also of the dissection and examination of over 1,600 fleas, and we have over 700 drawings of stages of the development of the trypanosome, from which those given in this memoir are a selection. It would, indeed, have been easier for us to have written a plausible and apparently complete account of

the development of T. lewisi, full of positive statements, after one year of our work than it is after five years, during which we have been forced by the logic of facts to abandon or modify many of our earlier conclusions or beliefs.

It is our pleasant duty at this point to express our thanks to those of our friends and colleagues to whom we are indebted for assistance. To Dr. Woodcock and Miss Robertson we are grateful for much advice, friendly criticism, and valuable suggestions. Our work could not have been carried out, certainly not in the time at all events, without the assistance of Miss Rhodes, who has not only drawn all the illustrations with a skill to which it is quite unnecessary for us to draw the reader's attention (since the figures speak for themselves), but has also relieved us of a large part of the wearisome drudgery of searching through the microscopic preparations. Mr. George Kauffmann has been most helpful in every part of the investigation, not only assisting in making preparations, examining rats, and other similar duties, but more especially in carrying out intelligently and enthusiastically all the details of the experiments, in which his extraordinary skill and resourcefulness in controlling the wayward flea were invaluable. Dr. D. J. Reid has given us the benefit of his skill and experience in microphotography. From our colleagues of the Lister Institute, Dr. C. J. Martin, the Director, and Mr. Bacot, who have been themselves engaged in studying the transmission of plague by fleas, we have had many valuable hints and help in various ways. To each and all of these we desire to express our cordial thanks and gratitude.

(2) Notes on the Flea, Ceratophyllus fasciatus.

(a) Anatomy. Methods of Dissection.

The fleas collected for dissection and examination were thrown, or allowed to hop, on to the surface of a small

quantity of salt-citrate solution[1] placed in a suitable glass capsule. The fleas are quite helpless on the surface of the liquid, and each flea that it is required to dissect can be picked off the surface of the liquid and transferred to a small drop of the same solution on a slide for further operation.

The examinations of the fleas were usually conducted by both of us acting in concert. One of us worked with the dissecting-microscope, extracted the parts of the flea required, placed them on slides, covered them with glass slips, and handed them to the other, who proceeded to search them carefully through under a microscope, using dry lenses of fairly high magnification (Zeiss D or apochromatic 4 mm.). In some cases one of us worked entirely alone, but it is difficult for one person to carry out satisfactorily both the dissection and examination of the flea; the various parts of the digestive tract often require prolonged and careful searching to find the flagellates, and if the operator be working single-handed, one preparation may dry up while he is searching through another.

For the dissection of the flea[2] the following apparatus was used: A pair of fine needles mounted in wooden handles, a fine pair of forceps, and a dissecting-microscope, besides slides and coverslips. The needles used were sharpened on a hone, one to a sharp point, the other to a flat, chisel-like edge with rounded corners. The pointed needle was the more useful for holding, the flat-edged needle

[1] Made up as recommended by Laveran and Mesnil, namely: 1 grm. of sodium citrate and 1 grm. of sodium chloride dissolved in 200 cc. of distilled water. This mixture appears to be most favourable for the examination of living trypanosomes.

[2] If an operation can be properly called dissection which consists in treating the flea as the Thracian women are said to have treated Orpheus: "Discerptum latos juvenem sparsere per agros" (i. e. fields of the microscope, in this case). It need hardly be said that our object was not to study the anatomy of the flea, but to extract from its body those organs which might possibly harbour developmental stages of the trypanosome.

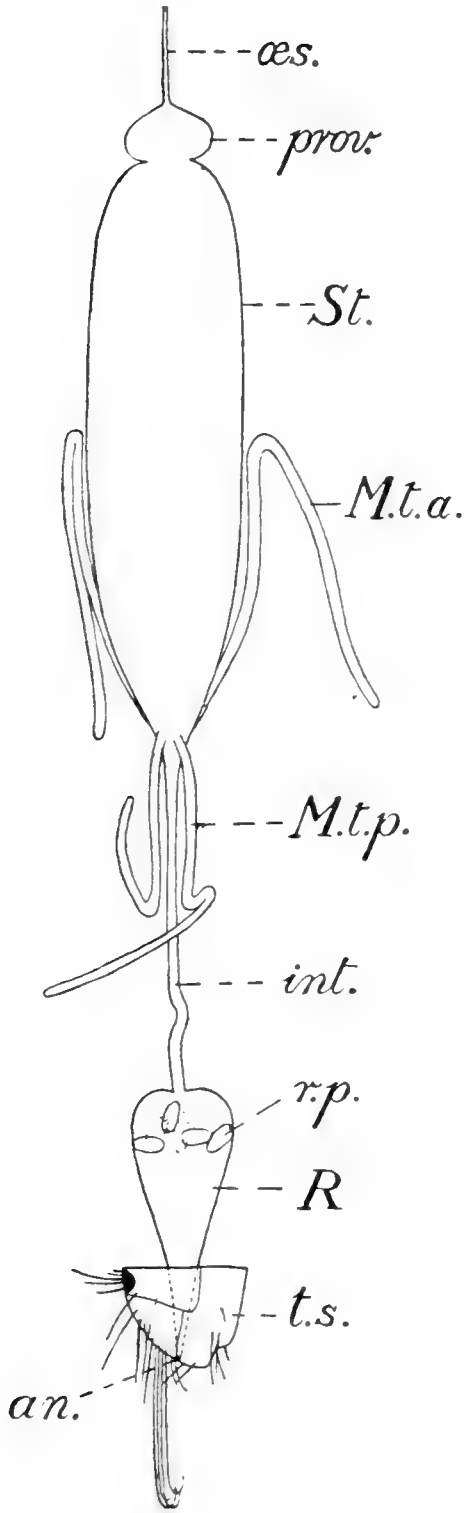
for cutting. The dissecting-microscope used was a Zeiss binocular, with No. 4 eyepieces and the paired objectives F^{55}. It is also necessary for the dissector to have at hand an ordinary microscope armed with a low power, since it is often difficult to distinguish the minute organs of the flea under the dissecting-microscope; the intestine, for instance, if severed from its connections might easily be confounded with a portion of a Malpighian tubule.

In the following paragraphs is described the method of procedure for making what may be considered an exhaustive examination of a flea for trypanosomes; it was not always necessary, however, to attempt so much, nor is it claimed that the entire operation was always successfully carried out, since both our knowledge of the flea's anatomy, and our skill in extracting the organs required, advanced considerably during the progress of our investigations.

The flea, as stated above, is picked up with a fine pair of forceps, holding it by its head, and placed on a slide (slide 1) in a drop of salt-citrate solution. The first operation is to cut off the head, which is not always easy if the flea be a lively one, in which case it is best to asphyxiate or drown the flea partially by holding it under water with the forceps for a short time. To decapitate the flea, hold it still by pressing the pointed needle across the thorax, and with the flat-edged needle cut across the head in the region of the eyes. The severed head may then be removed to another slide (slide 2), covered with a cover-glass, and the contents of the proboscis examined; but as the proboscis was never found to contain trypanosomes we ceased to trouble about it in our later studies.

It is frequently the case that the flea has its rectum filled with fæces or with partially digested blood, and when this is so it happens commonly that the rectum empties itself by a violent contraction at the instant that the head is severed (sometimes also eggs are extruded); or if the evacuation does not take place at this point in the proceedings, it is very difficult to avoid squeezing out the contents of the distended

TEXT-FIG. 1.



Digestive tract of a female flea, dissected out and drawn with the camera lucida at a magnification of 60, reduced in the reproduction to 40. The anterior part of the dissection is seen in ventral view; the rectum and its surroundings in side view. *œs.* Œsophagus. *prov.* Proventriculus. *St.* Stomach. *Mt. a.* Malpighian tubule of the anterior pair; that on the left side of the stomach is shown in its normal position, that on the right has its distal limb pulled out and away from the stomach. *Mt. p.* Malpighian tubule of the posterior pair. *int.* Intestine. *r. p.* The six rectal papillæ. *R.* The rectum. *t. s.* Terminal segments. *an.* The anus.

rectum during the subsequent operation of opening the abdomen. In cases where fæces are thus extruded the body of the flea is removed at once to another slide (slide 3), and the fæces left on slide 1 are covered with a slip and examined.

Through the integument of the flea the stomach can be seen lying ventrally in the anterior $\frac{2}{3}$ of the abdomen, and often the rectum can be seen at the hinder end in the dorsal region. The ventral posterior and dorsal anterior part of the abdomen is seen to be occupied by a whitish mass, most conspicuous in the female, and consisting chiefly of the reproductive organs.

The next stage in the proceedings is to open the body of the flea. This is done near the hinder end, at about the level of the fourth or fifth tergite of the abdomen. The body is held still with the pointed needle, with which the thoracic region is pressed down or speared, and with the flat-edged needle the body-wall is cut through dorsally and ventrally in the region indicated, and the hindermost segments of the abdomen gently detached in such a way as to separate the integumental portions without rupturing or tearing the internal organs. It is especially important, if it be desired to examine the contents of the body-cavity, that the digestive tract should not be in any way torn or punctured. By holding the anterior part of the body and pulling gently on the detached hinder part, the gut can be stretched out and seen in nearly its full length; the stomach, usually containing a greater or less amount of more or less digested blood, is seen projecting from the anterior part of the body, the rectum is contained in the detached hinder part, and stretching between the two is the intestine like a delicate white filament, exposed in its whole length, but more or less obscured by the fat-body, Malpighian tubules, and generative organs, especially by the large ovaries in the female; these organs render the female flea much more difficult to dissect, in spite of its larger size, than the less-encumbered male. The generative organs and as much as possible of the fat-

body are now pulled out on to the slide and cut off from the body, care being taken not to injure the gut. The carcase of the flea, with the hinder part hanging on by the still intact intestine, is now removed to another slide (slide 4), and the extracted contents of the body-cavity on slide 3 can be covered with a slip and passed on for examination; but so far as stages of T. lewisi are concerned, it is superfluous to do so, since they are never found in the body-cavity unless the gut has been punctured or ruptured.

The next step is to divide the digestive tract into two parts, thereby severing completely the hinder part of the body from the fore-part. This is done at the point at which the Malpighian tubules are given off at the junction of the stomach and intestine, the region which represents the transition from the mid-gut, lined by endoderm, to the hind-gut or proctodæum, lined by ectoderm. The Malpighian tubules are four in number in the flea; two of them run forward a short way on the wall of the stomach right and left, attached to it by fine tracheal tubes, and then turn backwards again with a sharp, elbow-like bend towards the dorsal side of the body; the other two tubules run backwards parallel to the intestine and alongside of it towards the hinder end of the body. The posterior pair of the tubules are also bent on themselves towards their distal extremities, but not so regularly as the anterior pair. The gut is cut across with the flat-edged needle at the point of origin of the tubules, and if this be performed accurately one pair of tubules (the anterior pair) remains attached to the stomach, the other pair to the intestine; sometimes, however, all four tubules remain attached to one or other of these organs. The hinder part of the body, with the intestine and rectum, is now removed to another slide (slide 5). The stomach is then pulled backwards out of the anterior part of the body on slide 4, and with it come out also, continuous with its anterior termination, the proventriculus and the œsophagus, these two parts representing the embryonic stomodæum, lined by ectoderm, while the stomach represents the whole of the embryonic mid-

gut. The proventriculus is lined by a thick chitinous cuticle prolonged into stiff, curved, pointed spines, densely planted and forming, apparently, a straining apparatus; it is approximately globular in form and usually contains blood. The œsophagus is a delicate tube, its walls composed of the chitinous cuticle internally and a delicate network of muscles externally; it generally performs active movements, twisting from side to side, when freshly extracted.

The two pairs of salivary glands are situated in the anterior region of the abdomen right and left of the stomach. Each gland has the form of a simple oval pouch, the wall of which is composed of a single layer of large cells with very large nuclei. From each gland comes off a duct, which, after running a short distance, unites with the similar duct of the other gland of the same pair. (In one instance we have seen the two glands of one side of the body fused into one, but with their ducts quite separate; on the other side of the body there was a pair of distinct glands in their normal relations). The common duct of each pair of glands then passes forwards alongside the gut through the thorax into the head, where it meets and joins with the corresponding duct from the other side of the body. The common salivary duct then runs a short distance and opens into the proboscis, doubtless on the hypopharynx as in other insects. The salivary ducts are recognisable at once under the microscope by their trachea-like structure, being lined by a thick cuticle which has ring-like thickenings; the rings are, however, somewhat irregular and easily distinguishable from the very even and regular spiral thickening of the wall of a tracheal tubule. Externally to the cuticular lining the tubule is covered by an investing layer of protoplasm, of uneven thickness in different parts and containing fairly large nuclei at irregular intervals. The ring-like thickenings of the cuticular lining become less marked as the ducts approach their point of junction, and cease altogether before they unite; the cuticular lining being quite smooth in the common duct and for short distances in the paired ducts.

Not infrequently the salivary glands come out with the stomach when it is pulled out; more usually, however, they do not do so, but remain in situ. In such cases the anterior part of the body is removed to another slide (slide 6), and the stomach, left on slide 4, is teased up, covered, and handed on for examination.

Now the dissection of the hinder part of the body, on slide 5, is proceeded with, in order to extract and separate the intestine and rectum. The rectum, situated dorsally to the accessory reproductive apparatus, penis or receptaculum seminis, is a fairly large pear-shaped organ, the stalk of the pear terminating in the anus. The slender intestine joins the rectum at its broad end, and in this region are situated the six conspicuous rectal papillæ, remarkable and very characteristic structures, the presence of a single one of which makes it easy to recognise even a small fragment of the rectum. Behind the papillæ the rectum has a thin wall, to which the crithidial stage of the trypanosome, when present, is usually found attached, sometimes in vast numbers. In its anterior part, the region of the papillæ, the rectum has only circular muscle bands, between which are wide interspaces. In the hinder region, behind the papillæ, there are both circular and longitudinal muscle-bands; the latter can be traced forward to just behind the papillæ, at which point each band becomes rapidly narrowed to a tendon-like fibre, and at the same time the striations of the muscle disappear. The tendinous continuations can be traced forwards, in the living condition, for some distance, but we have not made out the exact points of their insertion.

The intestine is characterised by a continuous coat of ring-like muscle-bands, with interspaces, arranged very regularly external to the epithelium. When the edge of the intestine is focussed under the microscope, the layer of circularly-disposed muscle-fibres is seen in optical transverse section like a string of beads. The intestine is frequently seen to be performing active peristaltic movements, and it may be thicker in some parts than in others, owing to the contraction of the muscles.

The rectum must be dissected carefully out of the hinder part of the body, so that it remains on the slide, free from all the adjacent organs or chitinous plates of the integument. The easiest way to do this is to make an obliquely longitudinal cut with the flat-edged needle so as to sever the ventral-anterior half of the hindmost segments, together with the genitalia, from the dorsal-posterior half containing the rectum and anus. The genitalia can then be removed and the rectum extracted without much difficulty. It requires some care to separate it from the anus without injuring it. When this has been accomplished, all unnecessary débris is cleared away. If it be desired to make separate examinations of the intestine and rectum, the intestine is cut off as close as possible to its junction with the rectum. To effect this it is best to spear the rectum with the pointed needle and make the cut with the flat-edged needle; or the operation of cutting off the intestine may be performed before the rectum has been dissected out from the hinder part of the body. In either case, the intestine is removed to another slide (slide 7), and both rectum and intestine, on their respective slides (5 and 7) are teased up, covered, and passed on for examination. It is not difficult to tear the rectum into several pieces with the needles, but it is not so easy to tease up the intestine; it is too slender to make sure of splitting it lengthways, except by good luck and more or less accidentally, and it is necessary as a rule to content oneself with cutting it transversely into two or three short pieces, the contents of which are generally squeezed out during the process.

Finally there remain the salivary glands, on slide 6, in the portion of the carcase consisting of the thorax and fore-part of the abdomen from which the gut has been extracted. The salivary glands, as has been stated above, are lodged in the fore-part of the abdomen beside the stomach, and it is generally by no means difficult to extract them when the stomach has been removed. To do this it is best first to spear the thorax with the pointed needle, then insert the flat-edged needle into the abdominal cavity from behind, and rake

out gently the contents of the abdomen. The salivary glands sometimes come out fairly clean, but more often they are embedded in fat-body, tracheæ, etc., from which they must be carefully freed as much as possible. In such cases they are sometimes a little difficult to detect under the dissecting microscope, but their position may be traced by their long, thread-like ducts. They are much smaller in the male flea than in the female. Another method which sometimes succeeds better in extracting the glands is to pull on the integument of the thorax with one needle and on that of the abdomen with the other. The body-wall then often tears across at the junction of the thorax and abdomen, and the salivary ducts are seen at once stretched out between the two. By continuing to pull the thorax forwards, the glands may be pulled out of the abdominal cavity and are seen hanging on to the back of the thorax, from which it is not difficult to detach them. By this method the glands may often be obtained very clean and free from encumbering fat and other tissue. When the glands have been extracted, other débris is cleared away and the coverslip is put on. The glands are very soft and are crushed immediately by the weight of a coverslip if there is no other tissue under it; but for examination of their contents this is not a disadvantage.

In the foregoing paragraphs we have given a detailed account of a full examination of the flea, such as we practised in the earlier periods of our investigation. But when it became evident to us that the trypanosome, during its development in the flea, never strays beyond the limits of the digestive tract proper, we were able greatly to curtail the ritual of the examination and to omit entirely the proboscis, body-cavity, and salivary glands. It is also unnecessary, as a rule, to separate the intestine and rectum in the dissection. Consequently, our later examinations were reduced to (1) the excluded fæces, if any, on the slide on which the flea was decapitated; (2) the stomach, on a second slide; and (3) the rectum and intestine, on a third.

It was no part of our task to make a special and detailed study of the

anatomy of the flea, but a few points observed by us incidentally in our dissections may be noted briefly here.

The nervous system, of which some beautiful dissections were made in this laboratory by Major Christophers, I.M.S., consists, as in insects generally, of (1) the brain or supra-œsophageal ganglion-complex, sending off the peri-œsophageal connectives which pass on either side of the œsophagus to connect with the foremost of (2) the three large thoracic ganglia, joined by connectives to form a series which passes on into (3) the abdominal chain of ganglia. It is a very difficult operation to dissect out the brain and the first two thoracic ganglia, but it happens very frequently that in the ordinary dissections of the flea the third thoracic ganglion and the abdominal chain of ganglia are exposed entire and in continuity. It is then seen that the abdominal chain consists of a series of small ganglia terminated posteriorly by a larger ganglion; and further that in the male there are seven smaller ganglia, in the female only six, in the abdominal chain. The larger hindmost ganglion, from which nerves are sent off to the genitalia and rectum, evidently represents a fusion of several ganglia equivalent to the more anterior smaller ganglia. Consequently it is seen that the concentration and fusion of ganglia at the hinder extremity of the ventral chain has proceeded a step further in the female than in the male.

The genitalia consist, in the male, of a conspicuous pair of testes, situated dorsally in the abdomen, and a pair of filamentous glands (prostates?) not unlike Malpighian tubules at first sight, but of slightly smaller calibre, and differing entirely in histological structure. There is no separate seminal vesicle, but each testis is a tightly convoluted tubule, the lower end of which is dilated to contain the ripe spermatozoa. Ducts from the testes and prostates unite to form a median Ductus ejaculatorius, which opens into a large penis of very complicated structure. In the female the two ovaries occupy practically the same position as the testes, but take up much more space and extend forwards to the most anterior limits of the abdomen. Each ovary consists usually of four egg-tubes or ovarioles, but in one specimen that we have mounted as a permanent preparation there are five ovarioles on each side. The ducts of the ovarioles unite to form the paired oviducts, and these unite in their turn to form the common oviduct. Ventral to the common oviduct lies the unpaired receptaculum seminis, consisting of a brown, chitinous capsule of a peculiar shape. The main body of the capsule is spherical, but gives off a curved, horn-shaped diverticulum, ending blindly. The horn-shaped portion has its concave curve turned towards, and connected by striped muscles with, the spherical part of the capsule. A slender duct of great length, and much convoluted near its origin, arises from the spherical part of the capsule, and runs back to open probably into the distal extremity of the oviduct or into the

genital vestibule. The spherical part of the capsule and duct of the receptaculum are surrounded with unicellular glands, thickly clustered round the capsule and the convoluted portion of the duct, but thinning out and becoming smaller towards the distal end of the duct. The receptaculum, dissected out, stained and mounted for the microscope, is a singularly beautiful object. It usually contains a dense mass of spermatozoa.

The heart is frequently seen in dissections at the hinder end of the body as a delicate filament, which by its own contractions twists and lashes itself about. Under the microscope it appears a delicate tube, beset towards the hinder end by the pericardial cells which are attached to it on either side, right and left, and are crowded together towards the hinder end, but occur more sparingly towards the middle region and are wanting in the anterior third of the heart. The ostia appear to be confined to the posterior region of the heart, but we have not made out their exact number or arrangement. For the pericardial cells, see Minchin (1910).

(*b*) Notes on the Parasites of the Fleas.

In a former publication one of us (E. A. M., 1910) has described some parasites found in our stock of fleas. The most important was a form to which the name Malpighiella refringens was given, occurring, as the generic name implies, in the Malpighian tubules of the flea. Since that time this infection seems to have died out entirely in our fleas, and we have not seen any Malpighiella in the fleas dissected by us for the last three years or more. Why this parasite should have died out in our fleas it is impossible to say, but it may be remarked that no conditions could possibly be more favourable for contaminative infection from flea to flea (whether from adult to adult, or larva to larva, or adult to larva, or vice versa) than those in our breeding cages, where vast numbers of fleas in all stages of development are herded together in a confined space. Consequently the disappearance of Malpighiella in our cages rather indicates that the fleas do not acquire infection with this parasite by the contaminative method.

In the publication referred to, numerous yeast-like bodies were described and figured from the digestive tract of the flea. Since then we have found organisms of this kind abundantly in smears of the salivary glands (text-fig. 24, p. 642).

In the larvæ of fleas that we have dissected and examined from our cages we have found the gregarine Agrippina bona (Strickland, 1912).

The cysticercoids of tapeworms are found not infrequently in the fleas. Nicoll and Minchin (1911) described two species of cysticercoids

from our fleas, representing Hymenolepis diminuta and another species of the same genus. We have found the same two species frequently, and also have in our possession specimens of a third species not identified. The cysticercoids appear sporadically, and are sometimes quite common for a period, and then are not found again for a long time. This uncertainty in their occurrence is quite intelligible, since their appearance must be caused by the introduction into the cage of a rat infected with tape-worms, which doubtless infects a large number of the larvæ that later become adult fleas.

The point upon which we wish to lay special stress is the absence in our stock of fleas of any flagellate parasites, and more especially of the leptomonad described by Swingle (1911) under the name Herpetomonas (Leptomonas) pattoni. We have been at great pains to convince ourselves upon this point. In the first place we dissected at various times about eighty[1] fleas from the non-infected breeding-cage without finding any flagellates of any kind in them, while flagellate parasites occur in a very large percentage of those known to have fed upon infected rats, though not in all, since the trypanosome often fails to establish itself in the flea, and even when the insect has been fed on a rat with trypanosomes swarming in the blood, they often disappear completely from the digestive tract of the flea within twenty-four hours of its having fed.

We give here three tables (A, B (1), and B (2)) showing the results of dissections of fleas from our stock which had been put upon infected rats, and so had had the chance of acquiring an infection of T. lewisi. Fleas do not always feed, however, when given the opportunity to do so, especially in cold weather,[2] and if the fleas are dissected and examined within twenty-four hours after having been put on the rat (the fleas in all cases having been kept hungry for

[1] As a matter of fact we dissected far more than this, all with negative results, but we have not kept exact records of more than seventy-nine.

[2] Note especially the twenty-one fleas of November 11th, 1911, in Table A, of which eighteen did not feed. This was due to a sudden cold snap, the first breath, so to speak, of winter.

two or three days previously to being put on), it is quite easy to distinguish those which have been fed from those which have not availed themselves of the opportunity of doing so. In this way useful controls are obtained for determining whether the fleas contained any flagellate infection before being used for putting on the infected rat.

TABLE A.—Fleas Examined within Twenty-four Hours after being put on an Infected Rat, to show the Numbers that had or had not Fed, and the Numbers of those that had Fed but in which no Flagellates were Found.

Date on which the fleas were examined.	Time since fleas put on infected rat.	Number of fleas put on the rat.	Number of fleas apparently not fed and containing no flagellates.	Number of fleas which appeared to have fed and which contained: (a) Stages of T. lewisi.	(b) No flagellates.
5 : vii : '10	6 hours	3	1	2	0
19 : vi : '11	12 ,,	10	2	8	0
13 : ii : '13	15 ,,	14	3	11	0
23 : v : '11	18 ,,	4	1	3	0
26 : v : '11	18 ,,	12	1	7	4
25 : i : '13	18 ,,	9	3	5	1
30 : i : '13	18 ,,	5	1	4	0
6 : ii : '13	18 ,,	8	3	5	0
4 : iii : '13	20 ,,	14	12	2	0
10 : vi : 10	24 ,,	4	2	2	0
6 : vii : '10	24 ,,	3	2	1	0
17 : iii : '11	24 ,,	5	1	3	1
15 : vi : '11	24 ,,	11	6	4	1
26 : ix : '11	24 ,,	17	7	8	2
30 : ix : '11	24 ,,	22	2	18	2
3 : x : '11	24 ,,	13	1	12	0
6 : x : '11	24 ,,	14	2	11	1
7 : xi : '11	24 ,,	17	2	11	4
11 : xi : '11	24 ,,	21	18	2	1
17 : xi : 11	24 ,,	17	5	10	2
24 : xi : '11	24 ,,	20	2	12	6
5 : xii : '11	24 ,,	12	3	7	2
12 : xii : '11	36 ,,	6	1	5	0
2 : vii : '12	24 ,,	14	8	6	0
20 : vi : '11	24 ,,	14	3	8	3
Total . . .		289	92	167	30
Percentage . .		100	31·83	57·79	10·38

From Table A it is seen that of 289 fleas which were put on infected rats, 92 (31·83 per cent.) had not fed and contained no flagellates, 167 (57·79 per cent.) had fed and contained T. lewisi, and 30 (10·38 per cent.) had fed but contained no flagellates.

In addition to these negative data we have had the opportunity of comparing our stock of fleas with another stock which was actually infected with Leptomonas pattoni. When one of us (E.A.M.) was in Paris in January, 1913, he was very kindly presented by Dr. E. Chatton, of the Pasteur Institute, with some living fleas (Ceratophyllus fasciatus) from a stock infected with Leptomonas pattoni. These fleas were brought back to London and a fresh breeding-cage colonized with them. The fleas were left to breed for a year, during which time the rat in the breeding-cage was changed frequently, but none of the rats put in acquired any trypanosome-infection. When the fleas were examined at the beginning of 1914, they had multiplied enormously, and were found to contain Leptomonas-infections. We did not keep any exact records of our dissections of the Leptomonas-fleas, but, roughly speaking, about 50 per cent. of the fleas contained teeming infections. The leptomonads appear to establish themselves in the fleas as readily as does T. lewisi, perhaps more so, since, as will be seen from Table B (1), barely more than 14 per cent. of our stock of fleas contained swarming infections when exposed permanently to infection with T. lewisi in the infected breeding-cage, and Table B (2), if we count only those known to have fed on an infected rat, not less than six, not more than fourteen days previously, gives but a slightly higher percentage (21·19).

We may conclude, therefore, from a comparison of our stock of fleas with those bred from Dr. Chatton's stock infected with the leptomonad, that, had our stock also been infected with leptomonads, we should not have failed to find fleas containing leptomonads in those fed on clean rats in the first place, and secondly, that the percentage of fleas

TABLE B.—Summary of the Condition of Fleas known to have Fed on Infected Rats. (1) Fleas taken at Random from the Infected Breeding Cage.

Date. (Fleas dissected.)	Trypanosomes.				Experimental infectivity of the flea.
	Fleas.	None.	Scanty.	Swarming.	
18 : ii : '10	3	1	1	1 (r)	Infection produced by 5 fleas of which 3 were dissected (Table J).
23 : iii : '10	4	1	2	1 (s)	
30 : iii : '10	6	1	3	2 (s)	
5 : iv : '10	6	0	3	3 (1r, 2s)	
8 : iv : '10	2	0	1 (si)	1 (si)	
10 : iv : '10	2	1	1	0	
22 : iv : '10	4	3	0	1 (s)	
3 : v : '10	4	4	0	0	
25 : vii : '10	5	2	3	0	
27 : vii : '10	3	1	2	0	
5 : viii : '10	5	2	3	0	One positive (Table K).
6 : ix : '10	3	3	0	0	
7 : ix : '10	4	3	1	0	
14 : ix : '10	3	0	2	1 (s)	
15 : ix : '10	3	0	3	0	
22 : ix : '10	3	1	1	1 (s)	One positive (Table K).
23 : ix : '10	1	1	0	0	Negative (Table K).
24 : ix : '10	1	1	0	0	
26 : ix : '10	2	1	0	1 (r)	
27 : ix : '10	3	3	0	0	
28 : ix : '10	3	3	0	0	
29 : ix : '10	3	2	0	1 (ir)	
3 : x : '10	2	2	0	0	
6 : x : '10	2	0	1	1 (r)	
10 : x : '10	4	1	2	1 (r)	
13 : x : '10	3	3	0	0	All negative (Table K).
14 : x : '10	3*	3	0	0	One positive (Table K).
17 : x : '10	3	1	1	1 (r)	All negative (Table K).
18 : x : '10	3	2	1	0	,, (Table K).
24 : x : '10	3	3	0	0	,, (Table K).
25 : x : '10	3*	3	0	0	One positive (Table K).
4 : xi : '10	3	2	1	0	,, (Table K).
8 : xi : '10	3	2	1	0	All negative (Table K).
15 : xi : '10	3	2	0	1 (r)	One positive (Table K).
16 : xi : '10	3	1	1	1 (r)	All negative (Table K).
21 : xi : '10	2	2	0	0	Both negative (Table K).
22 : xi : '10	2	2	0	0	,, (Table K).

Date. (Fleas dissected.)	Trypanosomes.				Experimental infectivity of the flea.
	Fleas.	None.	Scanty.	Swarming.	
29 : xi : '10	1	1	0	0	
30 : xi : '10	2	1	0	1 (r)	
1 : xii : '10	2	0	1	1 (s)	
5 : xii : '10	5	1	4	0	Injection of sal. gl. negative.
6 : xii : '10	2	0	2	0	
7 : xii : '10	2	0	2	0	
9 : xii : '10	2	2	0	0	Both negative (Table K).
13 : xii : '10	3	2	1	0	All negative (Table K).
14 : xii : '10	3	2	1	0	"
15 : xii : '10	6	5	1 (sr)	0	Fleas infected rat 239, stomachs rat 257 (Table I).
12 : i : '11	5*	5	0	0	Fleas positive (Table I).
13 : i : '11	6	4	2 (r)	0	Stomachs injected, positive (Table I).
26 : i : '11	6	5	1 (ir)	0	Negative (Table I).
26 : i : '11	6*	6	0	0	Fleas infected rat 251, stomachs rat 261 (Table I).
27 : i : '11	5*	5	0	0	Fleas positive (Table I).
14 : ii : '11	10	2	6	2	Stomachs injected, 2 positive.
28 : ii : '11	10	7	2	1 (r)	Stomachs positive, recta negative.
7 : iii : '13	8	8	0	0	
13 : iii : '13	4	0	1 (sr)	3 (2s, 1sr)	
14 : iii : '13	3	0	0	3 (1s, 2sr)	
17 : iii : '13	8	8	0	0	
28 : iii : '13	9	5	4 (3s, 1sr)	0	
31 : iii : '13	7	7	0	0	
4 : iv : '13	5	4	0	1 (sr)	
7 : iv : '13	6	0	3 (sr)	3 (s)	
10 : iv : '13	6	1	4 (1s, 3sr)	1 (sr)	
14 : iv : '13	2	0	1 (s)	1 (i)	
Total .	249	144	70	35	
Percentage	100	57·83	28·11	14·06	

The batches marked * were fleas of which the stomachs and other organs were kept for injection into rats, and were therefore examined hastily and imperfectly.

s = stomach; r = rectum; i = intestine.

TABLE B.—Summary of the Condition of Fleas known to have fed on Infected Rats. (2) Fleas fed at Definite Periods.

Age of infection in flea (approximately).	Date.	No. of fleas.	Fleas containing Trypanosomes.		
			None.	Scanty.	Swarming.
6 hours	14 : vi : '10	4	0	1 (s, r)	3 (2s, 1sr)
,, ,,	5 : vii : '10	2	0	1 (s)	1 (s)
12 ,,	28 : iii : '11	5	0	2 (s)	3 (1s, 2sr)
,, ,,	19 : vi : '11	9	1	2 (1s, 1r)	6 (5sr, 1r)
18 ,,	21 : iii : '11	9	0	6 (s)	3 (1s, 2sr)
,, ,,	23 : v : '11	4	1	1 (s)	2 (sr)
,, ,,	26 : v : '11	11	4	3 (2sr, 1r)	4 (1s, 3sr)
,, ,,	25 : i : '13	6	1	2 (1sr, 1r)	3 (2s, 1sr)
,, ,,	30 : i : '13	4	0	2 (1s, 1sr)	2 (sr)
,, ,,	6 : ii : '13	5	0	1 (si)	4 (2s, 2sr)
21 ,,	4 : iii : '13	2	0	0	2 (s)
24 ,,	10 : vi : '10	4	2	2 (1sr, 1r)	0
,, ,,	6 : vii : '10	3	2	1 (r)	0
,, ,,	17 : iii : '11	5	2	1 (s)	2 (sr)
,, ,,	15 : vi : '11	5	1	4 (s)	0
,, ,,	27 : vii : '11	28*	10?	12	6
,, ,,	1 : viii : '11	11*	8?	1	2
,, ,,	3 : viii : '11	29*	12?	13	4
,, ,,	30 : ix : '11	10	2	6 (4s, 2sr)	2 (1s, 1sr)
,, ,,	3 : x : '11	20	2	10 (6s, 4sr)	8 (6s, 1sr, 1r)
,, ,,	7 : xi : '11	13	1	4 (1s, 1sr, 2r)	8 (4s, 4 sr)
,, ,,	11 : xi : '11	12	1	9 (7s, 2sr)	2 (2s)
,, ,,	17 : xi : '11	13	2	8 (5s, 2sr, 1r)	3 (2s, 1r)
,, ,,	24 : xi : '11	3	1	1 (s)	1 (s)
,, ,,	5 : xii : '11	12	2	6 (4s, 2sr)	4 (3s, 1sr)
,, ,,	9 : xii : '11	18	6	12 (s)	0
,, ,,	25 : vi : '12	10	0	4 (2s, 2sr)	6 (1s, 5sr)
,, ,,	2 : vii : '12	9	2	2 (1s, 1sr)	5 (1s, 4sr)
,, ,,	24 : vi : '13	12	0	7 (2s, 3sr, 2r)	5 (sr)
36 ,,	20 : vi : '11	6	1	0	5 (4s, 1sr)
48 ,,	11 : vi : '10	6	5	1 (r)	0
,, ,,	16 : vi : '10	5	5	0	0
,, ,,	7 : vii : '10	5	5	0	0
,, ,,	18 : iii : '11	3	2	0	1 (s)
,, ,,	26 : iv : '11	6	2	4 (1s, 2sr, 1r)	0
,, ,,	26 : vi : '12	10	1	3 (sr)	6 (5sr, 1s)
,, ,,	3 : vii : '12	10	4	3 (r)	3 (1s, 2r)
60 ,,	30 : iii : '11	4	3	1 (sr)	0
,, ,,	11 : vii : '12	16	2	12 (1s, 3sr, 8r)	2 (1s, 1sr)
3 days	7 : ix : '09	4	2	2 (1s, 1r)	0
,, ,,	16 : ix : '09	5	4	1 (s)	0
,, ,,	23 : ix : '09	3	2	0	1 (s)

Age of infection in flea (approximately).	Date.	No. of fleas.	Fleas containing Trypanosomes.		
			None.	Scanty.	Swarming.
3 days	27 : xi : '09	5	1	2 (sr)	2 (1s, 1r)
,, ,,	17 : vi : '10	5	5	0	0
,, ,,	8 : vii : '10	4	4	0	0
,, ,,	27 : iv : '11	10	6	4 (2s, 1sr, 1r)	0
,, ,,	27 : vi : '12	10	1	5 (1s, 2sr, 2r)	4 (1s, 3sr)
,, ,,	4 : vii : '12	12	6	5 (1s, 1sr, 3r)	1 (r)
$3\frac{1}{2}$,,	12 : vii : '12	16	4	8 (1s, 7r)	4 (1s, 3r)
4 ,,	8 : x : '09	6	1	4 (sr)	1 (sr)
,, ,,	15 : x : '09	8	4	2 (sr)	2 (sr)
,, ,,	19 : ix : '10	3	0	1 (sr)	2 (1s, 1sr)
,, ,,	28 : iv : '11	10	5	5 (1s, 4sr)	0
,, ,,	5 : vii : '12	12	9	3 (sr)	0
,, ,,	18 : vii : '12	12	8	3 (r)	1 (r)
5 ,,	9 : x : '09	2	2	0	0
,, ,,	22 : vi : '10	2	0	0	2 (sr)
,, ,,	20 : ix : '10	3	2	1 (i)	0
,, ,,	29 : iv : '11	10	6	1 (s)	3 (r)
6 ,,	20 : vii : '12	12	7	5 (r)	0
7 ,,	1 : v : '11	4	3	1 (s)	0
$7\frac{1}{2}$,,	26 : v : '13	6	2	2 (r)	2 (1sr, 1r)
8 ,,	12 : x : '09	2	2	0	0
,, ,,	19 : x : '09	5	3	1 (r)	1 (r)
,, ,,	17 : iii : '13	4	0	1 (sr)	3 (1sr, 2r)
,, ,,	5 : v : '13	10	3	2 (1s, 1r)	5 (2sr, 3r)
,, ,,	19 : v : '13	6	5	1 (sr)	0
,, ,,	2 : vi : '13	6	3	0	3 (r)
,, ,,	9 : vi : '13	7	1	3 (1sr, 2r)	3 (sr)
$8\frac{1}{2}$,,	27 : v : '13	6	2	2 (1sr, 1r)	2 (sr)
9 ,,	11 : iii : '13	6	3	1 (s)	2 (sr)
,, ,,	18 : iii : '13	3	1	1 (sr)	1 (s)
,, ,,	13 : v : '13	6	6	0	0
,, ,,	20 : v : '13	6	6	0	0
,, ,,	3 : vi : '13	6	2	3 (1s, 2r)	1 (sr)
10 ,,	12 : iii : '13	4	2	2 (r)	0
,, ,,	19 : iii : '13	6	3	2 (1s, 1r)	1 (r)
,, ,,	14 : v : '13	8	7	0	1 (r)
11 ,,	13 : iii : '13	2	1	1 (r)	0
12 ,,	29 : vi : '10	1	1	0	0
14 ,,	1 : vii : '10	2	2	0	0
Grand total . . .		609	230	223	156
Percentage . . .		100	37·77	36·62	25·61
Total of six days and over .		118	65	28	25
Percentage . . .		100	55·08	23·73	21·19

* Stomachs only examined.

containing flagellates would have been far higher than is shown by our tables, in fleas exposed to infection by T. lewisi.[1]

(c) Notes on the Histological Structure of the Stomach of the Flea.

We shall have occasion, when describing the developmental cycle of the trypanosome in Part II below, to relate how the trypanosome penetrates into the epithelial cells of the stomach of the flea and goes through a process of multiplication within them. It is a necessary preliminary, therefore, to understanding the effects of the parasites that we should preface our description of their development by some remarks upon the structure and contents of the flea's stomach; and in the following section we give an account of our observations upon these matters, without claiming to have added anything to the scientific knowledge of insect histology.

The histology of the digestive tract of insects has been the subject of

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[1] Nöller (1912), discussing the question of the leptomonas-infection of the fleas, remarks, p. 398, that since the larva of the flea acquires the infection, adult fleas bred in a cage can be infected, and that consequently "the arrangement of the experiments ('Versuchsanordnung') of Minchin and Thomson, who used fleas bred in a rat-cage, does not correspond to the requirements ('Anforderungen')." We are at a loss to understand to what this criticism applies or what are the "Anforderungen" to which Nöller refers. At the time Nöller wrote we had published only our three preliminary reports. The first two of these (1910, 1911, 1) refer only to the transmission of T. lewisi by fleas, and it is sufficiently obvious that the presence of leptomonads in the fleas could not affect in any way the value or significance of positive results obtained in experiments on the transmission of the trypanosomes, since, ex hypothesi, the trypanosome and the leptomonad parasite are in no way connected. Our third report (1911, 2) gave an account of the intracellular multiplication of T. lewisi in the flea's stomach, a discovery which Nöller himself has confirmed, and which also would be quite unaffected by the presence of leptomonads in the fleas. Nöller's criticism appears to us, therefore, both premature and superfluous; premature, because our stock of fleas was not, as a matter of fact, infected with leptomonads; and superfluous, because, even if the fleas had been infected with leptomonads, it would have made no difference to the experiments and observations which Nöller criticises.

numerous memoirs, and its general characteristics are very well known. It would be beyond the scope of this memoir to attempt to discuss this subject in detail or to cite the very copious literature dealing with it; but of recent works we may refer more especially to the very excellent monograph of Léger and Duboscq (1902), who have studied the intestinal epithelium of Tracheata from the same point of view as ourselves, that is to say, with the object of describing the changes produced in the epithelium by parasites (gregarines) attacking the cells, None of the insects studied by Léger and Duboscq, however, were of blood-sucking habit and the stomach-epithelium of the flea differs in a number of points from any of the epithelia described by the French authors.

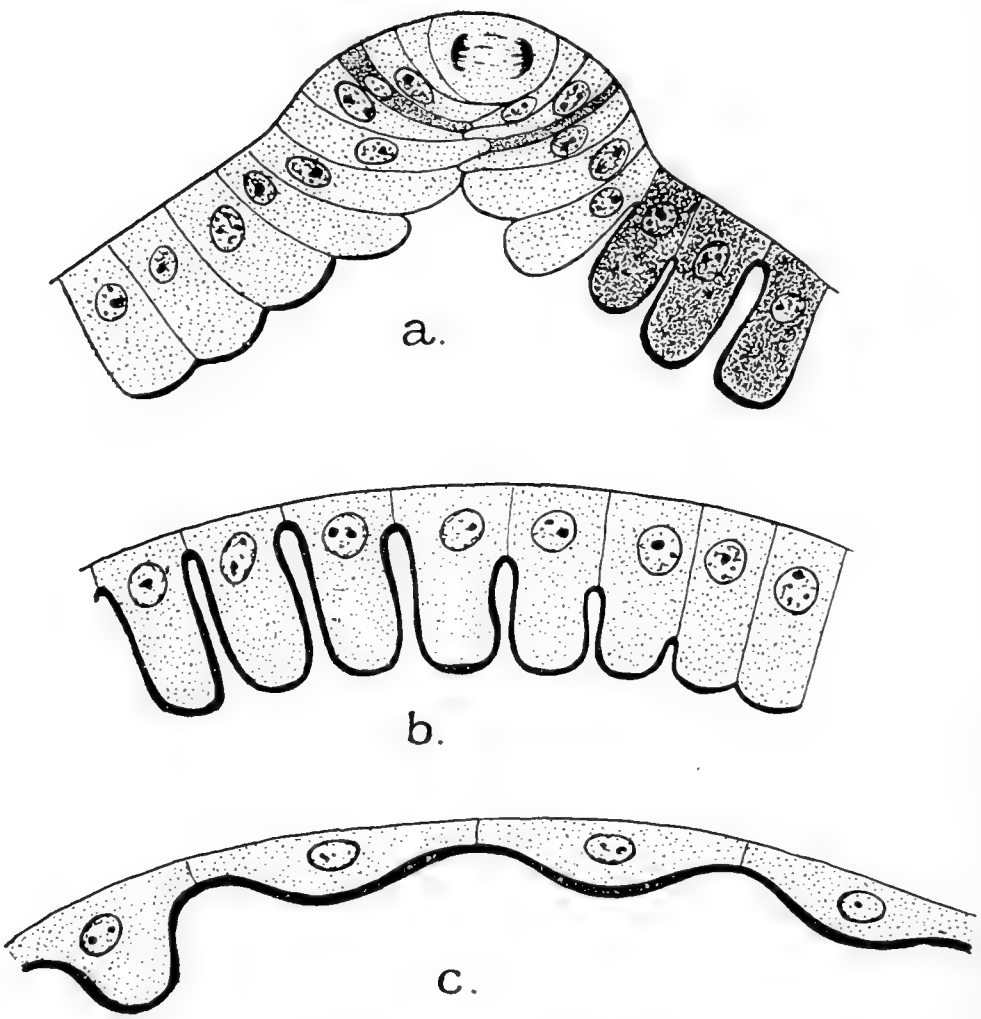
The wall of the stomach consists of the following principal layers, counted from within outwards (Pl. 39, fig. 126): (1) the lining epithelium, (2) a layer of circular muscle fibres, and (3) a layer of longitudinal muscle fibres. In addition to these layers, which are very easily seen, there are to be found also, though by no means in every section, flattened epithelial cells external to, and in contact with, the lining epithelium, between it and the circular muscle-layer; they occur sparingly and far apart, but appear to represent an integral and perhaps primitive constituent of the wall of the mid-gut. Similar flattened cells are found here and there on the Malpighian tubules, the wall of which is similar in histological composition to the stomach-wall if the latter be imagined as reduced to the lining epithelium and the flattened cells alone, without the muscle-layers.

We are concerned here only with the lining epithelium of the stomach, but it may be mentioned in passing that the circular muscle-fibres occur as bands or separate rings with considerable intervals between them, and consequently do not appear in every transverse section of the stomach. The longitudinal muscles are also separated from one another by intervals, a fact at once apparent in the transverse section, in which the muscles are seen cut across and in which it can further be seen in a well-preserved section, that each longitudinal muscle-fibre is connected to its neighbours by a delicate membrane, appearing as a fine line running between each adjacent pair of muscle-bands and forming a delicate sheath or investment round the whole stomach. The circular muscle-layer is continued on into the intestine, where it forms a continuous investment without intervals between the bands; the longitudinal muscles end at the pylorus posteriorly and start anteriorly behind the proventriculus, which has its own system of musculature, running for the most part in oblique bands arranged symmetrically right and left. Each muscle-band in the stomach-wall is a single, transversely-striated fibre, in which an occasional elongated nucleus is seen, embedded in a small quantity of protoplasm.

The following account of the epithelium and contents of the stomach

applies, unless otherwise stated, to sections of the stomach fixed with Flemming's fluid and stained with iron-hæmatoxylin followed by

TEXT-FIG. 2.



Diagrammatic representations of sections of the epithelium of the flea's stomach, to show the various conditions: *a*, to show a section through an epithelial crypt from which clear, regenerated epithelium is arising on all sides, while on one side three black, degenerated cells are seen (compare fig. 316, Pl. 44); *b*, to show the manner in which the border of the cells arises in relation to the gradually-developed separation of the cells from one another; *c*, to show the transition from the ordinary, columnar type of epithelium to the flattened type.

Lichtgrün-picric (see the following section dealing with technique). If such a section through a number of stomachs—all taken from a batch

of fleas dissected at the same sitting and at the same interval of time after having fed on the infected rat, all preserved in the same way, all stuck on the same slice of liver, cut and stained simultaneously—be examined even in the most cursory manner, very considerable differences are seen between the stomachs in one and the same microtome-section. These differences affect both the epithelium and the contents. The epithelium varies, in the first place, in the form of the cells, from flattened to columnar, and secondly, in the staining reactions of the cells. The contents of the stomach, that is to say the blood-débris, vary greatly in colour, staining in some cases deep opaque black, or less deeply in various shades of grey, in other cases, however, bright yellow.

The variations in the form of the epithelial cells are to be ascribed to the differences in the degree to which the stomach is dilated by the ingested blood. In a gorged flea the distension of the stomach stretches the epithelium until the cells become thin and flattened; but when the flea is hungry, or has taken in a small quantity of blood, or when the quantity ingested has become reduced by digestion and absorption, the epithelium resumes what may be considered its normal columnar form. Every gradation between the flattened and columnar conditions can be found in different sections or in different parts of one and the same section.

The variations in the staining reactions of the epithelial cells depend, in the first place, on the age or senescence of the cells. It is a matter of common knowledge that the lining epithelium of the mid-gut of insects is continually being thrown off and regenerated. The ordinary epithelial cells do not multiply and no mitoses are ever found in them; the centres of regeneration are the so-called epithelial crypts, each representing morphologically a small diverticulum of the epithelium in which the approximation of the cells usually obliterates the cavity and produces a solid, bud-like mass of cells (Text-fig. 2, *a* and Pl. 44, figs. 314, 316). When a flea's stomach, containing a certain amount of ingested blood, is plunged into a fixative, the epithelial crypts are very easily seen with a hand-lens or with the naked eye as little opaque white spots in the semi-transparent stomach-wall, very conspicuous against the reddish-brown background of the stomach-contents. In the sections it is common to find mitoses in such a crypt, especially towards its fundus (Text-fig. 2, *a*). As the cells multiply they are pushed upwards to the general level of the epithelium and outwards from the crypt to replace the old epithelial cells which, having degenerated, are cast off from the wall into the lumen of the stomach, and are digested there.

The young, freshly-regenerated epithelial cells have the cytoplasm clear, staining light-grey, and are relatively poor in granulations; the older cells, on the contrary, have the cytoplasm full of granules that stain very deeply, until finally the whole cell, including its nucleus

becomes black and opaque. Consequently, the epithelia of different stomachs show very varied appearances. A recently regenerated stomach will show clear epithelium all round, and, according to the time that has elapsed since regeneration, there may be no detached cells in the lumen of the stomach, or there may be a certain number of detached black cells, or there may be still, here and there, isolated black cells or patches of such cells in situ in the epithelium or in process of being cast off from it. On the other hand, a stomach which is about to be regenerated shows very dark epithelium all round, and in places this may be in process of rejection and replacement from the crypts, in which the cells have clear cytoplasm. The condition of the epithelium may vary in different parts of the same stomach, and from what we have observed we have gained the impression that the regeneration proceeds usually from before backwards, so that the anterior part of the stomach is further advanced in degeneration or regeneration, as the case may be, than the posterior region. We find in our preparations all possible conditions of the epithelium in different stomachs in one and the same microtome-section, and we have not been able to establish any definite relation between the feeds of the flea and the regeneration of the epithelium, but we have not paid sufficient attention to this point to be able to state positively that no such relation exists; the differences seen in the epithelia of flea-stomachs examined at the same interval of time after feeding may be due to inequalities in the rate at which digestion proceeds.

In addition to what appears to be the normal process of senile decay, in which the cells take up the iron-hæmatoxylin stain very deeply and become black and opaque, we have observed a second mode of degeneration, which we are inclined to ascribe to the action of the trypanosomes, since in all cases where it occurs in our preparations there are trypanosomes to be found in the stomach, and frequently in the degenerated cells themselves. In this second type of degeneration the black-staining granules in the cell diminish in quantity, without, however, disappearing entirely, while the cytoplasm of the cell stains yellow (Pl. 39, fig. 133; Pl. 40, fig. 140); hence we have generally referred to this condition in our notes as "yellow necrosis." In all the stomachs in which we have found it the blood-débris is also stained yellow, and it is often very difficult to make out the precise boundary of the necrosed cell-body, or to distinguish the cells from the débris when they lie free in it (Pl. 39, fig. 125), except by the presence of the nucleus and of a certain number of black granules in the cytoplasm of the necrosed cell. Indeed, our first impression was that the yellow colouring matter of the blood had in some way penetrated into the cell and stained its cytoplasm, but there can be no doubt that this idea is an illusion and that the yellow colour, both of the blood-débris and of the necrosed cells, is due to the picric

acid in the Lichtgrün-picric staining combination, though it is, of course, possible that the substance, whatever it may be, which stains yellow in the blood-débris may have become infiltrated into the dead cells and given them their peculiar staining properties.

The variations in the staining reactions of the contents of the stomach are more difficult to explain. There appear to be two types of staining after the use of the iron-hæmatoxylin-Lichtgrün-picric combination, one in which the contents stain in various shades of grey up to black, with a greenish tinge, and one in which they stain a bright lemon-yellow. It is not possible to bring these two types of staining into one series, as there is no transition between them; the grey-black and the yellow types occur side by side in different stomachs in one and the same microtome-section, and each stomach shows either the one or the other condition through the whole series of sections. So far as our observations permit us to generalise, the grey-black series represent the normal stages of the digestion of the blood; the yellow reaction appears to be due to some abnormal condition.

The blood ingested by the flea is very soon affected by the digestive action of the stomach, and the red corpuscles cease to be recognisable within a few hours after digestion. In the middle period of digestion, that is twenty-four hours, or thereabouts, after feeding, the blood has become thick, viscous and brick-red in colour, and contains immense numbers of irregularly shaped grains of all sizes, but for the most part coarse and large. Towards the end of digestion, forty-eight hours or so after feeding, the stomach contents are fluid and watery, dark brownish-black in colour, and the grains are much diminished in number and in size.

In the sections, taking first the grey-black series, the blood in the earlier phases of digestion (eighteen to twenty-four hours) usually consists of densely-packed grains and spherules, varying in size from very coarse to very fine, and staining intensely black. Between the grains there is visible a coagulated albuminous matrix, stained greenish with the Lichtgrün-picric combination. The stomach-contents fill the whole section and adhere closely to the epithelial cells, penetrating down between them when they have the columnar form, but in the centre of the section there is generally a clear patch, circular in outline, which is seen to owe its clear appearance to the fact that coarse grains are absent, and it consists only of the albuminous matrix with finer granules. Hence the digestion, or more probably the passage backwards towards the rectum of the indigestible remnants, of the blood-débris appears to proceed from the centre of the section—that is to say, from the axial region of the stomach—towards the periphery.

As the digestion proceeds, the grains in the débris become smaller

and stain less deeply; consequently the stomach-contents stain grey, in varying shades of darkness, while the matrix still shows the greenish hue. In stomachs thirty-six hours after feeding the contents of the stomach are generally greatly diminished in quantity, and are absorbed in the centre of the lumen, leaving a clear space of variable form, while round the periphery the greenish-grey débris adheres close to the epithelium. Leucocytes, especially the polymorphonuclear forms, can be recognised in the blood twenty-four hours after feeding, but at thirty-six hours we have not found them. Owing to the lighter tints of the stomach-contents at thirty-six hours, the trypanosomes free in the blood-débris can be seen more easily, in contrast with the earlier state of affairs.

In the yellow stomachs the contents appear at first almost uniform, but on close examination they are seen to consist of closely-packed granular substance, all of which, both granules and matrix, is coloured by the picric acid in the staining combination used. The first point that strikes one immediately is that the contents in such stomachs are large in quantity and fill the whole stomach, or show but a slight amount of absorption towards the centre of the section, even at thirty-six hours, when the contents of the grey-black stomachs are considerably diminished. The epithelium of the yellow stomachs may vary from the flattened to the columnar form, but the normal cells stain grey or black, in sharp contrast with the yellow contents.

It seems obvious from these data that the yellow stomachs represent an abnormal condition; we have endeavoured, not very successfully, to find a relation between this condition and either the presence of trypanosomes, on the one hand, or the state of the epithelium on the other.

In the yellow-staining stomachs which we have studied we have found trypanosomes to be present in the stomach in every case except one, and in that case there were attached clumps of crithidial forms immediately behind the pylorus, showing that the stomach-phase was over. But on the other hand, we have found the grey-black condition of the contents in well-infected stomachs also, showing at least that, if the yellow condition is in any way due to the parasites, they do not always produce that effect. On the other hand, in those cases in which we have found no trypanosomes at all, either in the stomach or outside it, the contents are always in the grey-black condition. A significant circumstance is, perhaps, the fact that we have only found the "yellow necrosis" of the cells in stomachs with yellow-stained contents.

As regards the condition of the epithelium, we have found the yellow condition of the contents associated (1) with epithelium black all round and in process of being cast off, or (2) with epithelium mostly clear, but with black patches of cells in situ or detached; in one such stomach the

first condition is found in the anterior half, the second in the posterior. We can state, therefore, that in our experience the yellow-stained contents occur only in stomachs about to be regenerated, or in process of regeneration, or very recently regenerated. But, again, we have found the grey-black condition in stomachs that appeared also to have undergone regeneration very recently, which makes it difficult to correlate this condition with the process of regeneration. The question of the significance and cause of the yellow-staining condition of the stomach-contents must be left an open question at present; the data to hand do not suffice for drawing decisive conclusions, and it would lead us too far to attempt further investigations upon this problem. On the whole, however, it seems at least probable that we are dealing with an abnormal state of the digestive processes towards which the trypanosomes are a contributory cause, if not the sole one.

As already stated, the different conditions of the stomach-contents described above are those seen after staining with iron-hæmatoxylin and Lichtgrün-picric. After the use of Giemsa's stain the colour of the contents differs considerably in different cases.

Most of our sections stained with Giemsa were fixed with Maier. In those in which the trypanosomes were best stained and show the flagella clearly and sharply the grains and spherules of the débris are coloured for the most part orange-pink, especially in those stomachs in which the digestion of the blood is further advanced (figs. 109, 113, Pl. 38); in the earlier stages of digestion many of the larger grains and masses in the débris are stained deep purple, making the contents of the stomach more opaque. In one of our series preserved in Flemming, consisting in all of seven slides, the first six were stained with the iron-hæmatoxylin-Lichtgrün-picric combination, the seventh with Giemsa's stain; on this seventh slide there are sections of four stomachs, two of which, on the other six slides, show grey-black contents, while the remaining two have the contents yellow in colour. In the Giemsa-stained slide the blood-débris shows a coloration very different after Flemming to that which it shows after Maier, being stained a bluish-green tint. The stomachs of the yellow type are slightly more blue in tint than those of the grey-black series, but otherwise the difference between them is but slightly marked.

Having now described the chief variations in the conditions of the stomachs and their contents, or at least those differences which are obvious upon the most cursory inspection of the sections, it remains to give a more detailed account of the epithelial cell. In any given stomach the cells show great individual variation in form and structure, but, nevertheless, it is not possible to divide them into distinct classes. There are no special glandular or secreting cells, as described by Léger and Duboscq in other insects, and all the cells of the general epithelium

of the stomach of the flea are to be regarded as equipotential, the differences visible between them being merely the expression of varying physiological conditions in relation to their changing environment, on the one hand, or to their constitutional vigour or senescence, on the other. Hence it is possible to give a generalised description of the cells, beginning first with the normal, healthy cell and dealing afterwards with the changes it undergoes in the process of degeneration.

The epithelial cells are produced, as already stated, in the "crypts of regeneration," which have been described in various insects by Léger and Duboscq. In the flea these structures appear usually as solid, bud-like cell-masses that often project outwards from the wall of the stomach to a considerable extent (Pl. 44, figs. 314, 316) beyond the level of the muscle-layers, which pass on either side of them. Internally the crypts do not rise up beyond the general level of the epithelium. The closely-packed cells of the crypts show distinct limits, and do not form a syncytial mass of protoplasm, as described by Léger and Duboscq (l. c., p. 410) in the larva of Anthrenus verbasci, for example. At the fundus of the crypt mitoses are often found, sometimes in two cells simultaneously in the same crypt; in other cases all the nuclei are in the resting state. Doubtless the crypts have periods of active multiplication, alternating with periods of repose, as in other insects. The crypts are often seen to be marked off from the general epithelium by slender dark cells, the "cellules de recouvrement" described by Léger and Duboscq (l. c., Pl. II, fig. 2, *c. r.*; p. 388). The crypts appear to have the monopoly of cell-production in the stomach of the flea. We have not found basal cells, "cellules de remplacement," in the general epithelium.

By multiplication and increase in their numbers the cells are pushed outwards on all sides from the crypt to take their place in the general epithelium (Pl. 44, fig. 316, and Text-fig. 2*a*). The young epithelial cells seen in the immediate neighbourhood of the crypts are columnar cells, roughly rectangular in form, and generally about twice as high as they are broad. The lateral boundaries of the cell are approximately parallel, and each cell is in contact with its adjacent neighbours for its whole length. The free, apical surface of the cell is convex, and on this side is developed a very distinct, thick border, at first covering only the upper surface of the cell, which projects like a dome towards the lumen of the stomach.

The further development in the form of the cell consists in an extension of the upper free surface, brought about by the cells becoming free and separated from one another at their sides, first at their apices and then downwards along almost the whole length of the side of the cell, till finally each cell is connected with the adjacent cells only by a narrow isthmus at its base (Text-fig. 2*b*). As the cell

becomes free the border develops also on the exposed surface, so that, instead of being confined to the apex of the cell, it extends down the vertical sides also (Pl. 40, fig. 136). This process of separation between the cells has an obvious significance in connection with the process of flattening which they undergo when the stomach is dilated after feeding; it can be regarded as an adaptation to the blood-sucking habit. When the flea gorges itself each cell is so stretched that its tallest part in the vertical direction is scarcely thicker than the nucleus, which bulges out the middle part of the cell in an even curve towards the lumen of the stomach, while towards the periphery the verticle height of the cell diminishes to the isthmus connecting it with its neighbours (Text-fig. 2*c*). As the cell resumes the columnar form the nucleus remains at or near the base, as a rule, and the cytoplasm of the cell is heaped up over it. In the extreme columnar form the apex of the cell is generally slightly expanded, the middle region more narrowed, so that spaces are left between adjacent cells, into which a considerable quantity of blood-débris penetrates (Pl. 39, fig. 126). The nucleus is usually situated at the base of the cell, but occasionally towards the apex (Pl. 40, fig. 136). The border clothes the whole free surface of the cell, whether flattened or columnar, and is of considerable thickness over the apex and the sides, becoming thinner as it approaches the isthmus, but in the columnar form of the cell, when its apical region is expanded, the border may be thinner, as if stretched, at the apex of the cell (Pl. 40, fig. 144).

The blood-débris has a great tendency to adhere closely to the border, so much so that the border is often more sharply marked off from the cell-contents within than from the blood-débris without, in the sections, but places can be found occasionally where the blood-débris has split away from the epithelium, leaving the border distinct and sharp. The border appears usually homogeneous and refringent, though in some preparations indications are seen of a vertical striation, as if it were composed of little darkly-stained rods, placed at right angles to its two limiting surfaces, and separated by intervening substance of lighter colour (Pl. 39, fig. 129, and Pl. 40, fig. 142). After sublimate-fixation the border is colourless, but when stained with iron-hæmatoxylin the blood-débris adhering to it often hinders the extraction of the stain and at these spots it remains black; when the hæmatoxylin is extracted it tends to take up the green from the Lichtgrün-picric mixture (Pl. 40, fig. 147). With Giemsa after sublimate-fixation it stains a pinkish-yellow. After Flemming-fixation the border is yellowish, as if tinged by the chromic acid in the mixture, and when this fixation is followed by the Giemsa-stain the border is coloured green (Pl. 38, figs. 99–103). There is no "bordure en brosse," or palisade of stiff rod-like cilia, external to the border, as in many insects. The condition in the flea

more resembles that figured by Léger and Duboscq for Scolopendra (l. c., pl. vi).

The border is evidently a fairly tough structure since in teased up stomachs examined fresh, the borders of cells are often seen quite empty, but retaining their shape, like shells.

The nucleus of the epithelial cell calls for no special comment: as can be seen in our figures, it is rounded or oval, with the typical structure seen in tissue-cells, namely, a distinct membrane, a reticulum containing chromatin-grains of various sizes, and one or more nucleoli which stain black, like the chromatin, after iron-hæmatoxylin. Mitoses of the usual type are found commonly in the crypts of regeneration, but we have never seen the slightest evidence of nuclear division in cells forming part of the general epithelium outside the crypts.

The cytoplasm of the epithelial cell varies at different ages. In the youngest cells bordering the crypts the cytoplasm appears more or less homogeneous and finely granular in all parts of the cell; it stains light purplish-grey or grey-black after iron-hæmatoxylin, bluish-purple after Giemsa, and no coarse granulations are to be seen. In the fully developed cell the cytoplasm has undergone local differentiation; round the nucleus, in the basal half of the cell, it has a denser texture, but above the nucleus, in the apical region, it has become of looser consistence, more spongy, so to speak, in appearance, with irregular spaces (Pl. 39, figs. 126, 127, Pl. 40, figs. 136, 144), containing fluid in the living condition, and transversed by strands of protoplasm disposed irregularly. The more the apical part of the cells is expanded the more watery its contents appear. Sometimes the apical region appears almost empty in the sections, with only a few traces of cytoplasm close under the border and at the sides. It is in this region in which the stages of the trypanosomes are most often found, and into which the parasites first penetrate.

In addition to these changes in the cytoplasm, numerous grains and enclosures of various kinds make their appearance in it. A detailed study of these granulations would require a lengthy investigation, an expenditure of time and trouble, that would go beyond the scope and objects of this work. We must confine ourselves to a brief summary of the appearances seen in our sections, without attempting to give physiological explanations of the various conditions seen. It is obvious that the bare observation that a granule is stained black by iron-hæmatoxylin or red by Giemsa's stain does not permit very far-reaching conclusions as to its nature or function in the cell; bodies of most diverse properties might agree to this extent in their reactions.

The first granulations to appear are minute grains which, whatever the fixation, Flemming or sublimate, stain black after iron-hæmatoxylin and red after Giemsa. They are seen at first chiefly at the sides of the

nucleus, between it and the cell-wall and extend up the sides of the cell close under the border. Scanty at first in the apical spongy part of the cell, they are soon deposited in this region also, appearing often in considerable numbers and varying in size from small granules to conspicuous grains, and even large masses (Pl. 40, figs. 136, 137.) The larger grains are seldom homogeneous, but appear as rings, black or red, as the case may be, with clear centre, apparently hollow (Pl. 38, fig. 99). Those of still larger size show, especially after Giemsa, darker and lighter parts disposed in various ways; inside the peripheral deeply-stained shell there may be darker grains or patches. After iron-hæmatoxylin, however, the whole mass may be opaque black, but usually shows lighter inner portions. The extent to which these granulations are developed varies in different stomachs, doubtless in relation to their secretive or absorptive activity at the moment of preservation. When a number of stomachs are cut in the same block, one stomach all through the series may show the cells clear and very free from granulations, while another stomach shows nearly every epithelial cell loaded with coarse grains in its apical region.

The red grains, as they may be termed from their distinctive reaction to Giemsa's stain, appear to be always present in greater or less quantity in the fully-developed cells of every stomach. In addition there are often found, lodged in the apical spongy region of the cell, masses of relatively large size which do not retain the iron-hæmatoxylin stain firmly throughout their substance, and consequently appear for the most part light grey in colour after this stain (Pl. 40, figs. 145, 146); after Giemsa they are either scarcely stained at all, appearing a sort of neutral tint, or they are coloured bluish-purple in various shades, sometimes very deeply, with streaks and blotches more reddish in tint (Pl. 38, fig. 102). These masses vary considerably in size and contour, and show differentiation of their substance into lighter and darker parts. With superficial examination they often simulate the intracellular stages of the trypanosomes to a remarkable degree, especially in the living condition, when they are often very conspicuous; for a long time we confused them with the spheres in the freshly teased-up stomachs, and spent much time watching them in the expectation, never of course fulfilled, of seeing them perform the characteristic movements. After we had made smears of stomachs in which these bodies were abundant, without finding any intracellular stages of the trypanosomes in such preparations, we came to the conclusion that these motionless spheres (as they appeared to be) were merely cell-products, and referred to them in our notes as "pseudospheres." Even in sections the pseudospheres often mimic the true spheres and might be confused with them at first sight, but only by an inexperienced observer who had never seen the actual intracellular stages of the trypanosome in the epithelium. The

idea occurred to us at one time that some of the pseudospheres might possibly be degenerated stages of the trypanosomes, destroyed, and in process of absorption, within the epithelial cells into which they had penetrated: but we have found no decisive evidence for this. It is most probable that the pseudospheres represent secretion-masses formed by the cell itself.

In some of the stomachs, especially in those preserved about twenty-four hours after feeding, there are to be seen dense and very conspicuous accumulations of coarse grains in the epithelial cells immediately below the border (Pl. 38, fig. 98, Pl. 40, fig. 147). The grains in question are especially distinct after fixation with Maier's fluid; they are more difficult to make out in the stomachs fixed with Flemming. The grains resemble very closely those of the blood-débris adherent to the border external to the cell, so much so that the first impression gained is that the débris has been absorbed into the cell through the border. It is easy to imagine this after iron-hæmatoxylin, which stains both these granules and the débris very black after sublimate-fixation (fig. 147); but the Giemsa-stain colours the grains within the cell differently from the débris (fig. 98), and when the digestion of the blood has gone beyond a certain point the grains inside the cell may be stained much darker with iron-hæmatoxylin than the grains in the blood-débris. It is improbable that the coarse grains of the débris would pass bodily through the border, which is to all appearances a dense, tough structure; but it is probable that these grains are formed in the cell in direct relationship with the process of absorption of nutriment from the blood.

Amongst the enclosures of the epithelial cell must be mentioned finally peculiar yellow grains which occur with great frequency in some stomachs, not at all in others. Their presence or absence is in no way connected with that of the trypanosomes, and they occur both in normal as well as in degenerating cells, though perhaps more abundantly in the latter. In the Flemming-iron-hæmatoxylin sections these grains have a brownish-yellow tint, often with a darker shell (Pl. 40, fig. 141). They vary in size from small granules up to the large grains reaching as much as 13 μ in diameter (fig. 142). Their tint also varies in depth, being usually much lighter in the larger grains. With Giemsa, after Flemming, they are stained bright green (Pl. 38, fig. 103), probably as the result of a blue stain (azure) imposed upon their original yellow tint.[1] These yellow bodies are very similar to, probaby identical

[1] A similar result is seen in the chitinous spines of the proventriculus in sections stained with Giemsa; the cuticle at the base of the spine is stained red, but that of the spine itself, from near the base to the tip, is coloured emerald green. The unstained spine is yellow in tint. Com-

in nature with the enclosures characteristic of the pericardial cells. As one of us has described elsewhere (E.A.M., 1910), the pericardial cells of the flea may be so crammed with yellowish-brown grains and spheres that the cell becomes visible with the naked eye as an opaque black spot through the integument of the living flea. In some of our stomach-sections there are also casual sections of pericardial cells which have been pulled out of the flea together with the stomach, so that we have had the opportunity of making a direct comparison between the yellow grains in the epithelial and pericardial cells. The yellow grains are probably an excretory product, eliminated by the flea under certain physiological but apparently normal conditions, and elaborated either in the epithelial cells, to be cast out into the lumen of the stomach, or in the body-cavity, to be taken up by the pericardial cells.

We come now to the process of cell-degeneration which occurs in the effete, senile epithelial cells. This process is very different in the flea's stomach from that described by Léger and Duboscq in various insects, none of them of blood-sucking habit. It is described by these authors as a "Dégénérescence mucoïde," an infiltration of the cells with mucoid substance. In the flea's stomach the process appears to be more of the nature of a fatty degeneration, combined perhaps with a mucoid infiltration.

In our sections fixed with Flemming's fluid and stained with iron-hæmatoxylin the intensely black, often perfectly opaque, degenerated cells, which are seen frequently detached completely or in process of detachment from the epithelium, are very distinct from the clear, lightly-stained cells originating from the crypts of regeneration and taking the place of the degenerated cells (Pl. 44, fig. 316). In some of our sections the stain has been over-extracted: the trypanosomes have become ghosts, faintly visible only to the practised eye, the nuclei of the epithelial cells are pale, and even the blood-débris has had its usually intense black stain reduced to a shade of brown; but the black grains and masses in the epithelial cells remain as black as ever, showing that they do not owe their colour to the stain but to the fixation, that is to say, to the osmic acid in the Flemming's fluid. Such preparations, spoilt for other purposes, are very useful for showing the gradual process of deposition of the blackened grains. First they appear as fine granules round the nucleus, near the base of the cell (Pl. 40, figs. 143, 144). Next, other, and for the most part larger masses, are deposited in the cytoplasm above the nucleus. The cell then becomes gradually filled up with black grains from below towards the apex; often an

pare also the green stain of the border, mentioned above, after Flemming and Giemsa, evidently due also to the super-position of a blue dye upon a yellow ground.

empty space is seen at the apex, immediately below the border (Pl. 40, fig. 138), but finally this, too, is filled up and the whole cell becomes an opaque black mass (fig. 139).

Very instructive is one of our series preserved in Flemming, in which there is one stomach in which nearly all the epithelium is degenerate. The sections of this stomach are spread over seven slides, six of which were stained with iron-hæmatoxylin, while the seventh, on which are sections through the hindmost region of the stomach, was stained with Giemsa. On this slide the degeneration is not so far advanced as in the more anterior region of the stomach, and in different parts even of the same section the following conditions are to be found: (1) Cells of normal type, with clear cytoplasm containing a few red granules (Pl. 38, fig. 99); (2) cells with cytoplasm of a darker bluish-purple tint, with many more red granules and amongst them a few coarser grains intensely black in colour (Pl. 38, fig. 100); (3) cells in which both the red and the black grains, but especially the latter, are greatly increased in number, leading up to (4) opaque black cells in which nothing can be focussed clearly. The black grains, it is obvious, can only owe their colour to the action of the osmic acid in the fixation, and must therefore be of a fatty nature. On the other hand there is also a marked increase of the red grains in the degenerating cells, indicating, perhaps, that in addition to deposition of fat, there is also a tendency to mucoid infiltration, as described by Léger and Duboscq. The darker tint of the cytoplasm, in so far as this is not an optical effect due to crowding of the grains, indicates that it becomes impregnated with the substances produced in the process of degeneration.

The deposition of the fat round the nucleus in the first instance indicates that the nucleus takes an active share in the process, and this is borne out by the fact that the nuclei themselves become very dark in the degenerating cells and are sometimes quite opaque.[1]

In sections of stomachs fixed with sublimate mixtures the blackening of the degenerating cells seen in the Flemming-fixed sections is conspicuously absent, so that at the first glance it is difficult to pick out the senile portions of the epithelium. More careful study of the sublimate sections shows that here the degenerated epithelium is distinguished from the regenerated by its pale, empty appearance, owing to the fat-grains having entirely disappeared, leaving empty spaces to mark their former position. This is best seen in stomachs fixed in sublimate-acetic, since, after sublimate-alcohol mixtures (Maier's and Schaudinn's) the cells are often much deformed and shrunk. In a favourable spot it is seen that the young cells, freshly produced from

[1] Léger and Duboscq have noted also that the mucoid substance is deposited first in the nucleus.

the crypts, have denser cytoplasm filling the cell throughout, except in the apical expanded portion of the cell; the cytoplasm stains deep grey or neutral tint after iron-hæmatoxylin and shows relatively few enclosures. The senile cells, on the contrary, are full of cavities, so that the cytoplasm has a spongy appearance throughout the cell and not merely in its apical region; and scattered through the spongy cytoplasm are grains, fine or but moderately coarse, which are stained black after iron-hæmatoxylin, red after Giemsa.

The difference between the senile cells after the two methods of fixation is easily explained if the grains deposited in them are principally fat. In all the sections alike the fat has been dissolved away during the process of imbedding in paraffin. In the Flemming-fixed sections, however, each fat-grain has reduced the $Os O_4$ to metallic osmium, and consequently is represented in the sections by a black mass, a model of the fat-globule in metallic osmium. In the sublimate-fixed sections no such reduction takes place, and the fat-globule is represented by an empty space; only the mucoid grains (if we are right in calling them so) remain in the cytoplasm, stained red or black according to the stain used.

It should be mentioned finally that after sublimate-fixations the blood-débris is stained very much blacker by iron-hæmatoxylin, and holds the stain much more tenaciously than after Flemming-fixation. This is especially true of that part of the débris which penetrates down between adjacent epithelial cells, and which often remains jet-black after all the rest of the débris has become pale in tint. In consequence the cells of the columnar epithelium in sublimate-fixed sections are often seen to be separated by black masses, which careless observation might confuse with the black stain of the degenerated cells after Flemming-fixation, especially when, as often happens in such sections, the main mass of the débris has shrunk away from the epithelium into the centre of the stomach-lumen. Such a mistake could only be made, however, with powers too low to discern that the black masses are between the cells and not in them.

The degenerated cells are thrown off bodily into the lumen of the stomach, which often contains great numbers of them in the blood-débris. There they are doubtless digested and absorbed along with the other contents of the stomach. Léger and Duboscq described a process of mucoid degeneration in which the entire cell, having a remarkable and deceptive resemblance to a gregarine, is engulphed by a basal cell; ultimately the latter also degenerates, and is thrown off with the cell it has taken in (l. c., p. 451). We have seen nothing of this sort in the flea, in which basal cells do not occur in the epithelium of the stomach.

(3) Technique.

We have already described above our methods of dissecting the flea and extracting from it the organs which it is required to examine for the presence of stages of T. lewisi. Here we propose to describe the methods by which the trypanosomes, when found, were preserved as permanent preparations for microscopic study.

The organs of the flea, extracted in the manner described above, are at once examined carefully under the microscope for the presence of trypanosomes in their various phases of development. When trypanosomes were found in any of the internal organs, after note had been taken, or sketches made, of their forms, position, and other points of interest, we proceeded to make permanent preparations of them. For this purpose the coverslip is carefully raised up, by means of the pair of fine needles that were used in the dissection of the flea, lifted off, and dropped at once with wet surface downwards into a suitable fixative. The slide is then handed to the collaborator or to an assistant, who places it bodily into a tube containing a small quantity of four per cent. solution of osmic acid. In the tube the slide remains about ten to fifteen seconds, tightly corked up, in order to fix the trypanosomes with the vapour of osmic acid. Subsequently the slide is fixed with absolute alcohol for about fifteen minutes and stained with Giemsa's stain in the usual manner.

For the fixation of the coverslip-films we used, in the earlier periods of our investigation, either Schaudinn's fluid (corrosive sublimate, saturated solution in distilled water, 100 c.c.; absolute alcohol, 50 c.c.; glacial acetic, a few drops) or sublimate-acetic ($HgCl_2$ saturated in H_2O, 95 volumes; glacial acetic, 5 volumes). Both these fixatives gave results about equally good; it is difficult to choose between them. Latterly, however, we used only Maier's modification of Schaudinn's fluid (distilled water, 200 c.c.; absolute alcohol, 100 c.c.; sodium chloride, 1·2 grm.; $HgCl_2$, 10 grm.), since this appeared to us to give better preservation, and, in particular,

less shrinkage of the bodies of the trypanosomes, than the others. The fluid being put into a large watch-glass, the coverslip is dropped into it with the film downwards. The coverslip usually sinks in the fluid and then rests on its corners on the rounded bottom of the watch-glass, so that the film itself escapes any friction or injury. The coverslips are left in the fixative from ten minutes to half-an-hour or longer (the exact time appears to be immaterial), and are then passed through 50 and 70 into 90 per cent. alcohol, where they can be kept until it is convenient to stain them.

The coverslip films were stained almost invariably with Heidenhain's iron-hæmatoxylin, using $3\frac{1}{2}$ per cent. iron-alum solution and $\frac{1}{2}$ per cent. hæmatoxylin-solution, both in distilled water. The film, after having been brought down through graded strengths of alcohol (80, 70, . . . 10 per cent.) to water was left about twenty-four hours in the iron-alum, then as long in the hæmatoxylin. Immediately before using the hæmatoxylin-solution a few drops of a saturated watery solution of lithium carbonate was added to it, drop by drop, until the solution, when shaken up, was a bright claret-red colour. After the film had been twenty-four hours in the hæmatoxylin-solution the differentiation of the stain was carried out under control by the microscope in a weak (light brown) watery solution of iron-alum. When differentiation was complete the film was washed in a current of tap-water for at least twenty minutes, then rinsed in distilled water and brought up through graded strengths of alcohol to absolute alcohol. At this stage the coverslip was usually dipped for a moment into Lichtgrün-picric solution (Lichtgrün, 1 grm.; picric acid, $\frac{1}{2}$ grm.; absolute alcohol, 100 c.c.), then washed again in absolute alcohol, passed through xylol, and mounted in pure xylol-balsam on a slide. The Lichtgrün stain must be used very rapidly, as it stains intensely.

In this way two preparations were obtained of the contents of each organ—one on the coverslip, the other on the slide—and as a rule trypanosomes were found more or less

abundantly on both of them, so that it was possible to compare corresponding phases of the development prepared by distinct methods of technique. It is very important, however, that the operation of removing the coverslip and fixing the films should be performed very rapidly and expeditiously, in order to avoid any drying taking place. The coverslip is particularly liable to dry, since the film of liquid that adheres to it is very thin; the slide, on the contrary, does not dry so quickly. A coverslip that has dried before fixation is quite useless for staining by the iron-hæmatoxylin method; the trypanosomes acquire a characteristic shiny appearance, as if they had been glazed, and when the stain is extracted in order to differentiate the preparation, it does not come out of the cytoplasm evenly, but gives a blotchy appearance, with no sharp differentiation of the nucleus or flagellum. It sometimes happens that a coverslip-film may be otherwise satisfactory, but may have dried slightly at or near the edges, thus affording opportunities for comparing the effects of desiccation on the trypanosomes with the condition of others that have never been dried. It is then seen that, in addition to the defective staining already described, the trypanosomes are flattened and distorted in various ways.

The fragments of tissue in the dissection adhere, for the most part, to the coverslip; it is not possible, however, to make out anything of trypanosomes which remain within the organs in film-preparations, and it is therefore necessary to tease up the organs well, after dissecting them out, in order to set free the trypanosomes. In the case of those phases which are attached to the gut-wall many remain so attached even when the wall is teased up, but a certain number are usually set free. When such forms are seen in the fresh film they should be dislodged, as far as possible, by tapping gently on the coverslip with a needle.

In some cases a coverslip-film which had been stained with iron-hæmatoxylin was unmounted by dissolving the Canada balsam in xylol, after the trypanosomes on it had been

studied and drawn, and the hæmatoxylin-stain completely extracted by placing it for twenty-four hours in a 3½ per cent. solution of iron-alum. The coverslip was then washed for an hour in a current of tap-water, and could then be re-stained by some other method—for example, Twort's stain. Trypanosomes that had been already drawn after the hæmatoxylin-staining could then be drawn again after being stained in a different manner. This double staining did not seem to injure the trypanosomes in any way, but it is noteworthy that after re-staining with Twort's stain they always came out a little smaller, when re-drawn with the camera lucida, than they had done previously after the hæmatoxylin-stain (compare figs. 260–63, Pl. 42, with figs. 260*a*–263*a*, Pl. 38).

When, as sometimes happened, the trypanosomes were so scanty on the coverslip as to require prolonged searching to find them, it was often very difficult to judge the right amount of extraction of the hæmatoxylin in the process of differentiation by means of iron-alum. Morever, a degree of differentiation which is sufficient for trypanosomes in the thinner parts of the film is insufficient for the thicker parts. Hence it was often necessary to unmount the preparations and differentiate them further, perhaps two or three times, before the right degree was attained. It is difficult to judge of the required differentiation by the fragments of tissue in the films, since the minute bodies of the flagellates give up the stain much more quickly than the relatively thick tissue-cells, and in a preparation in which the latter are satisfactorily differentiated the trypanosomes become mere ghosts, requiring to be re-stained altogether. The counter-stain with the Lichtgrün-picric mixture was found to show up the cytoplasm and flagellum of the trypanosomes more clearly.

However carefully the preparations have been made, it is often difficult to make out clearly and with certainty the structural details of some of the minuter phases of the life-cycle, and for this purpose the best optical apparatus was required, both as regards the objectives and the illumination

used. All trypanosomes in the permanent preparations were drawn by Miss Rhodes, under our supervision, with the camera lucida at a constant scale of magnification which was as nearly as possible 3000 diameters in the case of the film-preparations, 2000 diameters in the case of sections.

Our study of the development of T. lewisi in the flea was based principally upon the examination of films, made as described above, but it was found necessary also to cut sections both of the stomach and rectum of the flea. The following is an account of the technique employed by us in preparing sections of the stomach; the same applies to sections of the rectum, the only difference being that the stomachs were cut transversely, the recta longitudinally.

The stomachs of which sections were cut were taken from fleas fed eighteen, twenty-four, or thirty-six hours previously on an infected rat; the fleas themselves had been collected from the non-infected breeding-cage and kept hungry for about three days before being put on the infected rat. The stomach in each case was carefully dissected out from the flea, if possible without puncturing or injuring the stomach, in a drop of salt-solution on a slide, and then plunged into the fixative by inverting the slide in such a way that the stomach alone, all other parts of the flea having been removed, was in a hanging drop. If the stomach was ruptured or punctured in the process of extraction it was not, as a rule, preserved, except perhaps as a smear after teasing it up.

A number of different fixatives were tried, but the best results were obtained with Flemming's fluid[1] and Maier's modification of Schaudinn's fluid, and especially with the former. After Flemming the histology of the stomach is extremely good in all details; the blood fills the whole section

[1] The strong solution, made up as follows: a gramme tube of osmic acid is broken into a clean bottle, and to it is added distilled water, 50 c.c.; 1 per cent. solution of chromic acid in water, about 187·5 c.c.; and glacial acetic about 12·5 c.c.; the whole allowed to mix and dissolve.

and is not shrunk away from the wall, and the trypanosomes, both free and intracellular, are well-preserved both in structure and form, and they stain well either with iron-hæmatoxylin or Giemsa, especially the former. After Maier's fluid the histology of the stomach-tissue is not so good; the cells are shrunk and the minute structure of the nuclei is deformed. It is evident from a careful study of the preparations that the defects of Maier's fluid are due to unequal or differential penetration of its constituents; the alcohol evidently diffuses into the tissues first and produces the shrinkage and deformation of the nuclei; the sublimate does not get to the various tissue-elements until they have already been fixed in a defective manner by the alcohol. The blood-débris is also much shrunk after the Maier; while the greater part, sometimes the whole of it, contracts to form a central mass in the section, a certain amount remains usually adherent to the epithelium at the periphery, leaving an irregular empty ring-shaped space between the central and peripheral zones of the blood-débris. But to compensate for these disadvantages, the trypanosomes are extremely well-preserved and stain admirably with Giemsa's stain; some of our stomach-sections prepared in this way are as clear and demonstrative, so far as the trypanosomes are concerned, as any smear or film-preparation; in fact more so in the case of the large "spheres," which do not suffer so much from the tendency to opacity which is so disagreeable a feature in the smears. One is here confronted with the extraordinary difference, familiar to everyone who has worked at trypanosomes, between the reaction of these parasites, and that of tissue-cells, to the ordinary fixatives and stains used in cytological technique.

Whatever the fixative used, it was allowed to act for about an hour. The stomachs preserved in Flemming were well washed in tap-water and then brought up through a series of alcohols of gradually increasing strength; those preserved in Maier were transferred from it direct to 50 per cent. alcohol. In either case the objects were brought up to 90 per cent.

alcohol and there fixed on liver preparatory to being imbedded for section-cutting. Amyloid human liver was used. A moderately thin slice of a block of liver preserved in alcohol was cut by hand with a razor wetted with alcohol, and floated into a shallow glass vessel with a flat bottom, placed on the stage of the dissecting-microscope, and containing 90 per cent. alcohol to the depth of about a centimeter. The stomachs, taken up in a pipette of suitably coarse calibre, were placed on the slice of liver and carefully arranged side-by-side, their axes parallel to one another and similarly orientated, with their proventriculi all at the same level and all pointing in one direction, their pylori in the opposite direction. Then a tiny drop of glycerine and albumin solution, such as is used commonly for sticking sections on slides, was taken up on the point of a needle and caused to touch the surface of the alcohol immediately above the stomachs. The dense albumin-solution falls at once through the alcohol and spreads out over the stomachs on the liver; at the same time the glycerine is extracted and the albumin coagulated by the alcohol, with the result that the stomachs are stuck to the slice of liver. From six to nine stomachs were thus attached side-by-side on a slice of liver. As the stomachs, before being stuck on, are very liable to roll about or become shifted in position with the slightest disturbance or touch of the microscope, it was found best in practice to put them on not more than three at a time; that is to say, three stomachs having been arranged and fixed upon the liver, three more are then put on beside them. When the required number of stomachs have been stuck on, the slice of liver is trimmed with a scalpel into a rectangular form, in such a way that the longitudinal axes of the stomachs are parallel to the shorter sides of the rectangle; so that by cutting sections of the liver parallel to the longer sides of the rectangle the stomachs are all cut transversely at the same time.

We have thought it worth while to describe the method of fixing the stomachs on liver, although no novelty is

claimed for it,[1] in some detail, as it may not be familiar to some investigators working on similar objects, and because it is a procedure which saves much time and trouble. In the first place, it is much easier to imbed a relatively large block of tissue than a number of separate tiny little stomachs, and the orientation of the objects can be made much more accurate. In the second place, a great economy of labour in the section-cutting and of space in the slides and preparations is effected. To have a number of stomachs cut in the same section diminishes the labour of looking through the preparations under the microscope, and the presence in the section of the slice of liver makes it much easier to go from one section to the next under the high power. Thirdly, with a little experience the liver itself furnishes useful guidance in staining the sections, especially by the iron-hæmatoxylin method; one soon learns what degree of extraction of the stain from the liver-cells gives the best results for the trypanosomes, so that the process of differentiation can be carried out under low powers of the microscope—a great advantage. And finally, since it may be assumed that all the stomach-sections contained in one and the same microtome-section have received exactly the same treatment, it is legitimate to ascribe the very considerable differences seen in different stomachs in the same section to constitutional or functional differences in the stomachs themselves and not to varying local effects of the stain.

The stomachs, after being fixed to the liver in 90 per cent. alcohol, were imbedded in the usual way in paraffin, with a melting point of about 54° C. Methods of celloidin-imbedding were tried, but yielded no advantages to compensate for the extra trouble, especially that of extracting the celloidin from the sections—an indispensable preliminary to staining them. The best thickness for the sections of stomachs was found to be 6 μ; with less than that the trypanosomes are too

[1] One of us (E. A. M.) first became acquainted with this method in 1891 from fellow-workers in the Zoological Station at Naples, and has practised it constantly ever since.

fragmentary. The recta may with advantage be cut thinner than 6 μ, since the crithidial forms are very minute.

Various methods of staining were tried on the sections, but the results of the trials were that we kept finally in practice to two methods only, namely, iron-hæmatoxylin (Heidenhain), followed by Lichtgrün-picric in absolute alcohol as a counter-stain, and Giemsa's method. For the iron-hæmatoxylin method the sections were treated first as has been described above for the coverslip-films. The Lichtgrün-picric, which stains very rapidly, was merely washed over the sections for a moment and then washed off again with absolute alcohol. Giemsa's stain was used, according to the published prescription, as follows: The sections have their paraffin removed, and are brought down to water in the ordinary way. They are then washed in tap-water and put into dilute Lugol's solution (1 c.c. of Lugol to 25 c.c. of distilled water) for ten minutes. After this they are rinsed quickly in tap-water and put into a 0·5 per cent. watery solution of hyposulphite of soda for ten minutes. Next they are washed in a current of tap-water for five minutes or longer, and then put into the stain. The distilled water used to dilute the Giemsa-stain has to be neutralised in the way prescribed by Giemsa.[1] The sections were first placed in fairly strong stain—say, 1 drop of Giemsa to 1 c.c. of neutralised distilled-water—for about an hour, and then were left overnight in a weaker stain—1 drop of Giemsa to 4 or 5 c.c. of neutral distilled-water. The excess of stain is removed by rinsing in water, and after the excess of water has been drained off differentiation of the stain is carried out with

[1] A measured volume of the distilled water to be neutralised is taken, and to it are added a few drops of hæmatoxylin-solution (5 per cent. in distilled water), sufficient to tint it. Then a very weak solution (1 per cent. in distilled water) of potassium carbonate is added drop by drop, the water being well shaken after each drop has been added, and left for a minute or two, until the colour of the tinted water changes from yellowish-red to reddish-purple. In this way the number of drops of the carbonate-solution required for neutralising a given volume of the distilled water is known.

different strengths of acetone mixed with xylol, beginning with 95 per cent. acetone used for a very short time, in order to dehydrate the sections and extract the stain, and ending with pure xylol, after which the slides are mounted in dammar or Canada-balsam.

Of the two staining methods principally used, iron-hæmatoxylin gave admirable results after Flemming, especially for the intracellular stages; for the extracellular trypanosomes this stain is not so satisfactory, owing to the fact that the blood-débris, especially in the earlier stages of digestion, stains very intensely with it and refuses to give up the stain—at any rate not until after it has been all extracted from the cells and parasites. Consequently trypanosomes free in the blood may be entirely obscured by the opaque, deeply-stained débris, and hence quite invisible. The black stain of the blood-débris is even more intense after Maier than after Flemming; sections of stomachs fixed in Maier less than thirty-six hours after feeding are hopeless for the iron-hæmatoxylin stain, so far as the free trypanosomes are concerned, and those fixed in Flemming are not much better. By Giemsa's method, on the other hand, the trypanosomes in blood-débris are sharply differentiated and admirably shown; in the cells they are also good, better, perhaps, as "show" preparations, but not so precise in minute cytological details as by the iron-hæmatoxylin method.

To sum up the results of our experience in the technique of stomach-sections, we recommend: (1) Flemming's fluid, followed by iron-hæmatoxylin and Lichtgrün-picric; and (2) Maier's fluid, followed by Giemsa's stain. These two methods, supplementing each other, may be relied upon to reveal all essential details of the intimate life of the trypanosome and of the disturbances produced by it in the tissues of the host.

PART II.—THE DEVELOPMENT OF TRYPANOSOMA LEWISI IN THE FLEA.

(1) General Introduction.

From the results of experiments, described further below, it is shown that fleas fed on rats infected with Trypanosoma lewisi do not become infective to rats again until a period of at least five or six days has elapsed from the time that the fleas first ingested blood containing trypanosomes. From these experimental data it may be inferred that the developmental cycle of T. lewisi in the flea requires a minimum of five days for its complete course. The conclusions drawn from the experiments are confirmed by direct observation, since it is found, as will be described presently, that the little stumpy trypanosome which is the final form of the development in the flea, makes its first appearance in the rectum of the flea about five days after the development begins.

During the entire course of its development the trypanosome is confined to the alimentary canal proper of the flea, and is found in the stomach, intestine, and rectum; it is never found in the body-cavity (hæmocœle), and by a series of observations and experiments, which in our opinion are exhaustive (see below), we have convinced ourselves that the trypanosome does not penetrate into the salivary glands. It may, however, occur in the Malpighian tubules exceptionally, as the small crithidial form, characteristic of the rectal phase, attached to the wall of the tubes at or near their proximal opening into the proctodæum.

The developmental cycle can be divided conveniently into phases characteristic of the parts of the gut in which the trypanosomes are found, and we can thus distinguish a stomach-phase and a rectal phase. These distinctions are useful and natural, but their sharpness is blurred by not infrequent variations in the course of events; thus forms belonging normally to the rectal phase may sometimes be found in the

pyloric region of the stomach, though the converse case of the typical stomach-phase occurring in the rectum is not found. We may consider these phases first in their normal and typical modes of occurrence, and deal with the variations subsequently.

The stomach-phase (Pls. 36–39) is the first period of the development and is characterised by a peculiar mode of multiplication on the part of the trypanosomes, which penetrate into the epithelial cells lining the stomach and there reproduce themselves by a process of multiple fission. Hence in this period of the development free and intracellular forms can be distinguished. The stomach-phase is of short duration, perhaps in some cases lasting not more than twenty-four hours, in others two or three days, in rare cases four or even five days, but probably always terminated by the second feed of the flea, counting as the first feed that by which the flea became infected.

In the intestine the trypanosomes find, as a rule, no resting place, but merely pass through it on their way to the rectum. Hence, the forms found in the intestine are usually active, migratory forms which have completed the stomach-phase and are on their way to the rectum to initiate the rectal phase. Occasionally, however, forms similar to those characteristic of the rectal phase may be found attached to the wall of the intestine, especially near the pyloric opening.

The rectal phase (Pls. 41 and 42) consists chiefly of small, often minute individuals, which are crithidial in structure and are attached by the tip of the flagellum to the wall of the rectum, where they keep up a continual multiplication by binary fission. The crithidial form of the development takes origin in the rectum and is first established there, but may migrate forward to the pyloric region of the stomach later on. When once established in the flea, the crithidial phase endures, probably, as long as the flea lives, and thus constitutes a permanent stock of the parasite, enabling the infectivity of the flea to be maintained without renewal of the infection. From the crithidial phase arise by modification of individual

crithidial forms the small trypanosome-forms by which the infection of the rat is brought about, and which are the final forms of the developmental cycle in the flea.

By no means all the trypanosomes, however, which are taken up from the rat by the flea undergo the course of development sketched out briefly in the foregoing paragraphs. By experiment it is found that only a relatively small number of the fleas fed on infected rats become infective, apparently not more than one flea in four, on an average (see below); and these results are confirmed by direct observation. If a number of fleas are fed on a well-infected rat, trypanosomes will be found in the gut of all the fleas dissected and examined a short time after feeding; but the longer the interval between the feeding and the examination of the fleas the larger the proportion of the fleas in which the trypanosomes have disappeared or become very scanty, until finally trypanosomes will be found in but few (see Tables A and B (2) above). Probably the percentage of fleas in which the trypanosome succeeds in establishing itself permanently may be taken, on the average as about 25 per cent. (see p. 663 below). It follows that in about 75 per cent. of the fleas which digest blood containing T. lewisi the parasites die out altogether, and it is probable that in all the fleas a certain number of the ingested trypanosomes die off, since fleas that have been fed on a rat with trypanosomes swarming in the blood may exhibit a very scanty infection of the gut at any subsequent period.

From these data it is to be expected that together with developmental forms of the trypanosomes, various stages in their degeneration would also be found in the fleas, at least during the first few days after the parasites were ingested by them, and this expectation is fully realised. It is necessary, therefore, to recognise a degenerative series of forms (Pl. 43) as well as a developmental series in the gut of the flea, and to distinguish carefully the two series from one another. Any particular flea, when dissected and examined, may present an extraordinary medley of different forms of the trypanosome. To distinguish between the different forms and to refer each

form to its proper position and sequence in the series, whether developmental or degenerative, is our task, and it is no light one. When we were at an earlier stage in our investigations we did not recognise sufficiently the importance of the degenerative series, and consequently tried to interpolate degenerative forms into the developmental series, greatly to our own confusion. On the other hand it is necessary to steer very clear of a tendency to explain any form as degenerative, of which the developmental position is not immediately clear; thus we were at first inclined to regard the peculiar recurved forms in the stomach as degenerative, until we discovered the intracellular multiplication and were thereby enabled to refer the recurved forms to their true position.

In the problem of piecing together and reconstructing the sequence of the two series, developmental or degenerative, there is, to begin with, a known and fixed starting point for each, namely, the ordinary form of T. lewisi as it occurs in the blood of the rat. Further clues are obtained by linking together, through gradual transitions, the forms seen in the fleas, but more especially by the study of "time-fed" fleas, that is to say, fleas dissected and examined at known periods of time after they have been fed on the infected rat. The part of the gut in which a given form occurs is a further guide as to its significance; and all data and conclusions obtained from observation are controlled and checked by the results of experiment, especially useful in determining the final form of the development. Guided by these various considerations we have arrived at the conception, set forth below in fuller detail, of the changes undergone by the trypanosome in the flea (see especially Pl. 45 and description). In our account we describe separately the two series which we regard as developmental and degenerative respectively; but it must be pointed out that while these two series are very distinct and easily recognisable as a whole, certain forms or stages of the one series are sometimes very difficult to distinguish decisively from very similar forms belonging in reality to the other series. Consequently it is impossible to

be free from doubt, occasionally, with regard to the place to be assigned to a particular specimen or type of individual.

It remains only to be stated at this point that we adhere to the following nomenclature for the parts of the body of the trypanosome or crithidia: Blepharoplast for the basal granule of the flagellum; kinetonucleus for the smaller, trophonucleus for the larger, of the two nuclei. In order to save space we shall, however, use for the kinetonucleus the symbol *n* (plural *nn*) and for the trophonucleus the symbol *N* (plural *NN*).

(2) The Developmental Series.

(A) The Stomach-Phase.

The blood ingested by the flea passes in the first instance into the stomach, that portion of the digestive tract which is derived from the embryonic mid-gut or mesenteron, and which is lined by a layer of epithelium representing the true hypoblast or endoderm of the embryo. In the post-embryonic stages of the insect, this part of the gut is characterised by the absence of the chitinous cuticular lining secreted by the ectodermal epithelium of the parts anterior or posterior to it, namely, the stomodæum, comprising the pharynx, œsophagus, and proventriculus, and the proctodæum, comprising the intestine and rectum. The boundary between mid-gut and hind-gut is further indicated by the origin at this point of the Malpighian tubules.

In what may be called a normal feed, the flea fills the stomach and proventriculus alone. It is not an infrequent occurrence, however, for some fleas to gorge themselves to such an extent that the freshly ingested blood not only fills the stomach completely, but overflows beyond it into the intestine and rectum; we have observed this to happen most frequently in the case of female fleas, rarely in the case of males. In such cases some of the ingested trypanosomes

may be carried on at once into the proctodæal regions of the gut, but all such trypanosomes degenerate, and need not be reckoned with in the developmental series, the first phases of which take place always in the stomach alone.

(*a*) The Extracellular Trypanosomes.

The trypanosomes introduced into the stomach very soon begin to undergo changes (Pl. 36, figs. 1 and 2 ; Pl. 37, figs. 47 and 48). The first change is probably purely physiological, since long before any alteration is observable in form or structure these ingested trypanosomes seem to have lost their power to infect when injected subcutaneously into clean, susceptible rats (see below, p. 634). The next change observed in these ingested trypanosomes may be seen on examining microscopically the contents of a flea's stomach four to six hours after the first feed on an infected rat. A certain number of trypanosomes will then be seen to pass rapidly in a straight course across the field of the microscope with their flagella directed anteriorly. The posterior third of the body is held more or less straight and appears more rigid, as it does not share in the rapid undulations of the anterior end of the body. The movements of these trypanosomes thus contrast strongly with the sinuous, serpentine and wriggling rather than progressive movements characteristic of the trypanosomes in the blood. When not actively progressing, the trypanosomes in the stomach have a tendency to attach themselves by the tips of their flagella to pieces of débris, to the wall of the stomach, or to the surface of any other firm body. The stiffening of the trypanosome-body is probably due to increased tension of the cytoplasmic contents produced by absorption of fluid from the ingested blood as it undergoes alteration in the process of digestion. As a result of absorption or imbibition of fluid, the body of the parasite, previously more or less distinctly flattened, acquires a cylindrical and more rigid contour. If this explanation be correct, it follows that the first stimulus to developmental change is

to be ascribed to differences of osmotic tension in the fluid medium, as has been shown experimentally by Miss Robertson (1911) to be the case in the development of the trypanosomes of fishes.

In stained preparations most of the ingested trypanosomes show at first little modification from the ordinary blood-trypanosomes. In rare instances the nuclei may be approximated (Pl. 36, fig. 2). Some show a darker staining-reaction of the posterior third of the body, which appears, from the backward position of *n* in such forms, to be a sign of degeneration beginning to set in (compare Pl. 43, figs. 289, 290).

In their free active state the trypanosomes in the stomach are never found to be undergoing multiplication by any form of fission, and it is doubtful if they undergo any developmental changes further than those described above, until after they have multiplied within the cells of the lining epithelium of the stomach. The multiplication of the stomach-phase takes place solely within these cells, and although, in strict chronological order, we should now describe the intracellular stages of multiplication, it is more convenient for purposes of description to divide the stomach-phase into "free" and "intracellular" stages, and to describe all the free developmental forms before describing the intracellular multiplication.

It can be established by direct observation that the process of intracellular multiplication produces a long, free type of trypanosome which may be characterised by the term "crithidiomorphic," because while externally similar in form and movements to a large crithidial type of flagellate, it lacks, as a rule, the diagnostic structural feature of a true crithidial form, since only exceptionally is *n* found actually beside or in front of *N* (Pl. 36, figs. 3–11, Pl. 37, figs. 49–57). We shall now proceed to describe in more detail this late, free form of trypanosome. It must, of course, be understood that as several generations of the intracellular stage may follow each other in succession (see below), free and intracellular forms in all stages of development can be found

together in the same stomach. We believe, however, that the typical crithidiomorphic type of trypanosome always follows an intracellular stage, that in its less developed form it is the direct product of intracellular multiplication, and that though in this form it may again enter an epithelial cell and multiply, it is, in the more advanced form, the highest developmental type of the stomach-phase and is destined to pass down the intestine into the rectum where, after undergoing further modification, it initiates the characteristic crithidial rectal phase to be described below.

In the living condition the crithidiomorphic form progresses at a great pace in a straight line with the flagellum directed anteriorly ("mouvement en flêche"), in much the same manner as does the early stomach-form above described. It is, however, considerably longer and the posterior end is more rigid and swollen, often distinctly clubbed. Owing to the rapid motion and imperfectly straight body, the clubbed appearance is exaggerated in the living condition, but stained preparations also show that some of the trypanosomes are distinctly clubbed in shape. Like the early stomach-form, when not actively progressing the crithidiomorphic type has a strong tendency to attach itself by the tip of its flagellum to cells or débris, etc. Apart from its size the distinguishing characteristic between this and the earlier form is the marked approximation of the two nuclei, best seen in stained preparations (Pl. 36, figs. 7, 8, 10; Pl. 37, figs. 50, 51).

It has been mentioned that while, as a rule, the long, free stomach-trypanosomes have *n* behind *N*, it is found in a few cases that they have the typical crithidial structure with *n* in front *N*. We have observed altogether but three instances in which such forms occurred in sufficient abundance to make them worthy of special note. The first and most striking was the case of a flea taken from a bell-jar in which a number of fleas had been kept for some time with an infected rat, so that the length of time since the flea had ingested the parasites was not known. The body-cavity of the flea contained a cysticercoid of Hymenolepis diminuta (vide Nicoll and Minchin, 1911). The intestine of this flea showed a peculiar malformation in the form of a globular pouch-like appendix, distended with red fluid, and due apparently to an obstruc-

tion or strangulation of the intestine. The stomach, examined fresh, was seen to contain a great number of active trypanosomes, some of which were adhering together in couples, and in the intestine a clump of attached forms was seen near the origin of the Malpighian tubules.

In the preparations of the stomach of this flea a great number of trypanosomes were found showing every possible gradation of structure, from forms similar to the ordinary blood-trypanosomes to a long crithidial type with *n* far in front of *N* (Pl. 36, fig. 11 and Pl. 37, figs. 60–66). Many of these were found closely adherent in couples, just as had been seen in the fresh state, each such couple being composed of two crithidial forms in most cases, but sometimes of two ordinary forms (Pl. 36, fig. 12, and Pl. 37, figs. 67, 68). In every couple seen the two individuals appeared quite distinct and showed no signs of actual fusion; one couple was found attached tête bêche (as in Pl. 43, fig. 310). In the preparation of the rectum and intestine (preserved together) a few similar large trypaniform or crithidial individuals were seen, and also a fair number of dwarfed, degenerative forms, but no couples.

Special mention has been made of this flea because we were at first, and remained for some time, under the impression that the couples seen represented a true sexual fusion, and that we had discovered the sexual phase of the trypanosome. We have been quite unable, however, to confirm this notion or to find a similar state of things in any other flea of all those examined by us, and we now regard the state of things found by us in this particular flea as exceptional and abnormal, in relation probably to the malformations noted by us in the flea itself. It is possible that the malformed condition of the intestine prevented, to some extent, the passage onwards of the trypanosomes from the stomach, and so caused an arrest of development in the parasites, in which the tendency towards the crithidial type of structure became realised to its fullest extent. The coupling of the trypanosomes must then be regarded as agglomeration due to abnormal and unfavourable conditions, though in no case were more than two trypanosomes seen adhering together.

The second case in which the long crithidial forms were prominent was in a flea of a batch which had been fed on an infected rat three days before being examined and dissected. The fleas had been kept in an incubator at a temperature of 25° C. after the infective feed, and had not been fed again. In one of the fleas long crithidial forms with *n* in front of *N*, were fairly numerous, together with intracellular multiplicative stages, in the stomach (Pl. 37, figs. 56, 57); in the rectum one active trypanosome of the long stomach-type and a clump of degenerative forms were seen in the fresh state.

The third case to be noted was in a flea of a batch which had been fed twenty-four hours previously on an infected rat. There was nothing

special to note about this flea; the stomach contained many long active trypanosomes, with *n* and *N* closely approximated, or with *n* in front of *N* (Pl. 36, figs. 9, 10), and also some dwarfed degenerative forms, but no multiplicative stages. In the rectum a few clumps of degenerative appearance and some developmental forms were seen.

Besides these three cases which have come under our observation, in which the long crithidial type was conspicuously abundant in the stomach, we have noted the occasional occurrence of this type at various ages—twenty-four hours, forty-eight hours, and sixty hours—after the flea had fed on an infected rat. It is evident that it must be regarded as exceptional for the trypanosomes to reach the complete crithidial condition in the stomach, and that no special significance can be attributed to the crithidial form in this part of the life-cycle, although in rare instances and under special circumstances it may be abundant.

It will be clear from the foregoing remarks that we are quite unable to agree with the statements of Swellengrebel and Strickland (1910), who, having examined two fleas one day after feeding on the infected rat and five others two days after the infected feed, describe the transformation of the long crithidiomorphic type of trypanosome into the long crithidial type as the normal and usual method of development in the stomach. On the strength of somewhat more extended experience, we consider the long crithidial form to be of highly exceptional occurrence, both in the stomach and elsewhere, at so early a period of the development, as already stated; we can only explain the results of Swellengrebel and Strickland on the supposition that they were so unfortunate as to have chanced upon abnormal fleas, similar to the three cases described by us above, or that they may have regarded as crithidial forms the very commonly-occurring recurved forms, which they do not describe at all.

In view of what is known with regard to both the later development of T. lewisi in the flea and the life-cycle of other trypanosomes in their invertebrate hosts, it is evident that the crithidial type of form and structure is the principal and most characteristic phase of the development, and that there is a pronounced tendency for the trypanosome to assume crithidial characters when taken up by the flea—a tendency which asserts itself more strongly after the trypanosomes have undergone multiplication in the cells of the lining epithelium of the stomach. So long, however, as the trypanosome remains in the stomach the atavistic tendency towards

the assumption of the crithidial form $\left(\frac{n}{N}\right)$ does not normally (or, at all events, usually) get beyond the crithidiomorphic form $\left(\frac{N}{n}\right)$. Occasionally, nevertheless, the crithidial form asserts itself, as it were, even during the stomach-phase; more especially, perhaps, under the influence of any circumstances which tend to retard the development of the trypanosome and retain it in the stomach after it is ripe for passage into the proctodæum, but not infrequently even under conditions which cannot be asserted to be in any way abnormal.

(*b*) The Intracellular Multiplication of the Trypanosome.

As already stated, the multiplication never takes place in the free, active condition of the trypanosome, but only after it has penetrated into one of the large epithelial cells lining the stomach, within which it goes through a process of multiple fission to produce a number of daughter-individuals which escape from the cell and pass back into the lumen of the stomach as free trypanosomes again. The whole process of intracellular multiplication, so far as it could be made out by observation of living trypanosomes in the stomachs of freshly-dissected fleas, was described by us in our preliminary report (1911); we had not then had sufficient time or opportunity to make detailed studies, which present peculiar difficulties, of the multiplication in preserved and stained material. The ordinary smear-methods seldom permit any finer details to be made out of the trypanosomes within the cells, on account of the large size and thickness of the cells and consequent opacity of the preparation. It is only possible in smears to study the stages of multiplication set free by the rupture of the cells; but even of such specimens it is difficult to get perfectly satisfactory preparation for microscopic study of detail. With the method of fixation by vapour of osmic acid and subsequent coloration with Giemsa's stain or other

modification of the Romanowsky method of staining we have obtained occasionally very clear preparations of the later stages of the multiplication, but as a rule, the "spheres" with many nuclei take up the stain with such intensity that they become opaque masses showing nothing of the internal structure, although in the same preparations the free trypanosomes may be stained to perfection. If in such preparations the stain be cautiously extracted by means of acetone or other suitable media, it is possible to obtain specimens showing the nuclei satisfactorily, but then, as a rule, the flagella are invisible, having lost the stain completely, while the free trypanosomes or early stages of multiplication on the same slide have become mere ghosts or have vanished altogether, beyond the power of visual resuscitation by the most delicate and refined methods of microscopic illumination. Very often in such preparations only the kinetonuclei can be seen, the trophonuclei having disappeared. In the study of Romanowsky-stained preparations it was generally found necessary to begin by drawing all that could be seen, general outline, projecting flagella, in the opaque, untouched preparations of the spheres, and then to perform a number of successive operations of cautious extraction of the stain, examining the preparations after each such operation and adding to the drawing any fresh details of structure brought to light. It was difficult, however, to control the extraction of so sensitive a stain with sufficient exactness to avoid losing the whole of it in an instant. The last state of the preparation was generally one which left it useless for purposes of demonstration; always a disappointment to the microscopist and his friends. We have never succeeded in re-staining satisfactorily preparations in which the Romanowsky stain has been over-extracted.

By far the best and most instructive preparations of the intracellular multiplication were obtained in the coverslip preparations fixed in Schaudinn's or Maier's fluid and subsequently stained by the iron-hæmatoxylin method, as described above. Only in such preparations was it found possible to

control the stain so that in the largest spheres both nuclei and flagella were visible; even then, however, the trophonuclei were sometimes faint and difficult to make out clearly when the flagella were still sharp and distinct. Of one film in which the smear was thickly crowded with spheres of various sizes, some free, others still in the tissue, a very satisfactory preparation was obtained by staining with Mann's hæmatoxylin, carried out with the friendly help of the inventor of the stain himself. The result was a very good "show" preparation of the multinucleate spheres, sharp and clear, even in the thick parts of the smear, and especially suitable for moderate magnification; the flagella, however, could not be made out.

While the ordinary smear-methods presented special difficulties, very convincing and beautiful preparations of the intra-cellular phase were obtained in sections of fleas' stomachs extracted carefully from the body and preserved in various ways; a full account of the technique employed is given above. Such preparations have the immense advantage of exhibiting the exact relations of the trypanosomes to the cells; it is possible to look through every section of each series, to note every trypanosome, free or intracellular, occurring in each stomach, and to observe what each parasite was doing at the moment the stomach was preserved. On the other hand, for the study of the stages of multiplication, sections have the disadvantage that the parasites themselves are often halved or mutilated, so that any given specimen may be only a part or fragment of the whole body. Both smears and sections are therefore indispensable, and supplement each other in obtaining a complete picture of the course of events.

So much for methods and technique; we proceed now to give an account of our observations.

From Nöller's investigations on the development of T. lewisi in Ctenocephalus canis, it appears that the intracellular multiplication begins about six hours after the ingestion of the trypanosomes by the flea. In our prepara-

tions of a batch of fleas, of which the infection could not have been more than nine and a half or less than seven and a half hours old, we have found the recurved forms fairly commonly and also some of the rolled-up forms characteristic of the early intracellular stages (Text-fig. 23, p. 635). We have no other records of intracellular multiplication in our fleas earlier than twelve hours. The stages of the intracellular multiplication are to be found in all parts of the epithelium of the stomach, from close behind the proventriculus to the pylorus.

Our investigations upon the intracellular multiplication contain, unfortunately, one gap which we have been unable to fill; we have not succeeded in observing the actual penetration of the epithelial cell by the trypanosome. Nöller, however, has been so fortunate as to observe the process, and gives the following account of it. In a dog-flea which had sucked infected blood five hours and fifty-five minutes previously, he saw "a trypanosome, of which the pointed hinder end had already penetrated into an epithelial cell. The flagellum-bearing anterior end beat violently and incessantly, whereby the trypanosome penetrated further and further into the cell. After I had watched this spectacle for about five minutes the trypanosome, which had so far penetrated into the cell as far as the middle of its body, shot suddenly into the cell and stirred up the granular cell-contents by its lively movements. Since, however, the cell was torn on the opposite side, the trypanosome soon shot out of the cell again." Nöller thus confirms the suggestions we made in our preliminary report (1911) with regard to the probable method in which the penetration of the cell is affected.

We have frequently seen trypanosomes, not distinguishable in the living state from the ordinary type, singly within cells; the first time we ever discovered the trypanosomes within the cells was just such a case, a single trypanosome of quite ordinary appearance, wriggling and squirming actively in the cytoplasm of an epithelial cell, in a flea which had

been fed twelve hours previously on an infected rat. Careful examination of the cell, at different foci of the microscope, convinced us, greatly to our astonishment, that the parasite was really within the cell and not above or below it. This, and other observations, repeated subsequently upon trypanosomes of ordinary appearance, contained singly within epithelial cells, suggest that the trypanosomes in each such case had but recently penetrated into the cell; but the observation might also be interpreted to mean that the trypanosome seen was the last of a batch produced by multiple fission within the cell from which its sister-trypanosomes had already escaped. In the latter case, however, the trypanosome would probably be within a vacuole, as will be described presently.

Observation of the free trypanosomes in the living state shows that, as already stated, they are extremely active, but have a great tendency to attach themselves by the tip of the flagellum to firm objects; to the wall of the stomach, to pieces of débris, even to the glass surface of the slide or coverslip when under observation. The study of sections of the stomach confirms this observation in an unmistakable manner; many trypanosomes of the long, stiff type are seen in the sections attached to the epithelial cells by their flagella. The attachment is not, as a rule, to the outer projecting ends of the cells, but to their sides; the trypanosomes put their long flagella down between the epithelial cells and often adhere to the cell close to its base; it would appear as if the side of the cell, at least in its columnar form, is its vulnerable region. A still more striking point is that many of these trypanosomes attached to, but still quite outside the cell, have already assumed the recurved form. These observations make it very probable that in some cases the trypanosome may first attach itself to the cell by its flagellum and then bore its way into the cytoplasm in some way.

Several trypanosomes may penetrate independently into one and the same cell. We have frequently observed numbers of the parasites, from five or six up to a dozen or

more, in different stages of multiplication, side by side in a cell both in the living condition and in sections (Pl. 38, figs. 112, 113; Pl. 39, fig. 130). The parasites lie in the cytoplasm usually in a distinct vacuole, produced, apparently, by the liquefactive action of the parasite on the cytoplasm of the host-cell. The trypanosomes are nearly always in a state of movement, a point to which we shall return again. It is not infrequent to observe a number of trypanosomes in the same vacuole, wriggling actively one over the other.

The infected cell may become reduced simply to a bag containing fluid in which large numbers of trypanosomes, generally with their multiplication completed or far advanced, move actively. Such cells are found commonly in sections (Text-fig. 3); they are generally thrown off from the epithelium and lie quite free in the blood-débris, sometimes even in the centre of the lumen of the stomach; nothing remains of the cell-contents except a thin superficial layer of cytoplasm, under the cell-membrane, and the nucleus, adherent usually to the wall at some point. This condition obviously represents the last stages of the exhaustion and death of the cell, from which the trypanosomes will escape either by their own activity or by disintegration of the cell. We shall consider the effects produced by the parasites on the cells in more detail subsequently; at present it will be more convenient to confine our attention to the development of the trypanosomes themselves.

The study of the trypanosomes in the living cells, checked by the examination of preserved material, permits readily enough of the recognition of a number of well-marked stages in the process of multiplication:

(1) Trypanosomes of quite ordinary appearance, which have apparently but recently penetrated into the cell, as already described.

(2) Pear-shaped forms, with the flagellum continuing the stalk of the pear; the body of the pear is distinctly flattened, and therefore presents a contour which differs according as it is seen from the edge or from the flattened surface; as the

parasite is in constant motion within the cell it presents continually different views to the observer. The body of the parasite also shows in life incessant "metabolic" changes of form, movements of an active protoplasmic body imperfectly restrained by the thin, yielding envelope or periplast. It is very easy to see in the living condition that these pear-shaped forms are produced by the body of the trypanosome being doubled upon itself, an interpretation confirmed by the examination of preserved specimens. When the stomach is teased up and examined fresh, many of the recurved pear-shaped forms are found swimming freely, with the flagellum forward, in the salt-citrate solution used in the dissection. If such a form be watched attentively, it is often seen to uncurl itself, straightening out the body and thus passing from the pear-shaped form to that of an ordinary trypanosome. In some cases a trypanosome which had been seen to unbend itself in this manner can be observed to curl up again, while swimming freely, and thus to assume or to lose the pear-shaped form several times in succession.

In the fixed and stained preparations it is seen that the trypanosome is bent upon itself in such a way that the posterior part of the body, containing the kinetonucleus, is closely applied to the anterior half of the body. Thus a pear-shaped body results in which *N* is lodged in the thickest part of the pear, at (Pl. 36, fig. 15) or near (fig. 13) the blunt end of the body; while *n* is usually well in front of *N*, that is to say, nearer to the pointed end of the body (Pl. 37, fig. 70). In some cases, however, *n* is close beside *N* (figs. 13–17), or even, exceptionally, behind *N* (fig. 16). The variations in the positions of *n* and *N* are easily explained by their variability in this respect, already described, in the free trypanosomes, on the one hand, and on the other by variations in the exact region of the body at which the bending takes place. As a general rule, the body appears to be bent between *n* and *N*, so that *N* lies a little way from the extreme posterior end and *n* in front of it (figs. 13, 17); but the point of greatest curvature may be in the region of *N*, which is

then at the hindermost extremity of the body (fig. 15), or even in the region of *n*, which is then at the extreme blunt end (fig. 16). In exceptional cases the bending takes place in front of *N*. In all cases the flagellum runs backwards along one side of the body, round the blunt posterior end, and forward, for a variable distance, to the basal granule or true blepharoplast situated close beside *n*. Thus the course of the flagellum, as a whole, may be compared to the letter U modified by making one arm of the letter much longer than the other.

In some cases the distinction between the two limbs of the recurved body can be seen plainly in the fixed specimens (Pl. 36, fig. 15; Pl. 37, fig. 70), but in other cases no line of demarcation can be made out, and the applied portions of the body appear to have formed completely into a compact pear-shaped mass, leading on to the stage next to be described.

The recurved forms differ remarkably in size, and from a comparison of these forms with one another, with the free trypanosomes, and with later stages of the intracellular development, there can be no doubt that the initial stages of the life of the trypanosomes within the cells is accompanied by a pronounced diminution in the size of the flagellates (see especially Pl. 36, figs. 13–17, and compare them with figs. 1–10 of free trypanosomes, and figs. 21–34 of later stages, on the same plate). How this shrinkage takes place it is difficult to say; probably the cytoplasm of the flagellate gives up a large amount of watery fluid and so diminishes in bulk, while becoming at the same time correspondingly denser in texture, a change which would account for the intensity with which the intracellular forms take up the stain and the consequent opacity which they acquire, as already noted. It is necessary, however, to exercise caution in estimating the size of the forms in preparations, since there is no doubt that they vary owing to differences in fixation. Thus, Pl. 36, fig. 14 shows a specimen from the same slide as fig. 13, but the former is from a part of the film which appeared to have dried before it was exposed to the action of the osmic vapour;

its large size, light colour and the elongation of *N* are all indications that the soft body had become flattened out by being dried before fixation. The specimens may also become deformed in other ways; Pl. 37, fig. 72 is probably to be explained as representing a recurved form in which the flagellum has become torn away from the side of the body and so projects freely from the rounded posterior end.

We were at first under the impression that the pear-shaped, recurved trypanosomes found free in teased-up stomachs examined fresh were forms that had been originally intracellular and had been set free by rupture of their host-cells. As stated above, however, examination of sections proved that these recurved forms may be extracellular in occurrence, attached to the epithelial cells or even free from them. It is evident, therefore, that the recurved form is not simply an adaptation to life within the confined limits of the cell.

(3) Forms with rounded or oval body, derived from the pear-shaped recurved forms by a further contraction and rolling up of the body; these are the forms which we described in our preliminary account as "block-like" since the body often shows during life irregular contours, changing continually owing to the active metabolic movement. The flagellum, which runs in a U-shaped course in the recurved forms, acquires now an additional bend (compare especially Text-fig. 23, p. 635, *h* and *i*; it usually runs round the outer contour of the rounded body and protrudes from it to a variable extent. In some cases a very considerable length of the flagellum is free (Pl. 37, figs. 77, 78), in other cases a very little (Pl. 36, fig. 28; Pl. 37, fig. 82), while in other cases again the flagellum is simply wrapped closely round the body (Pl. 36, figs. 29, 30, 35; Pl. 37, figs. 81, 84). The extreme length of the free flagellum seen in Pl. 37, fig. 76 is possibly due in part to its having become artificially detached from the body in the process of making the smear.

The data in the foregoing paragraph have been obtained chiefly from the study of preserved specimens. In the living condition this stage appears as a small, rounded or oval body

within the cell, usually in motion and showing a distinct flagellum. Sometimes, however, these bodies are quite motionless with no flagellum visible. In our preliminary communication (1911), we were unable to decide whether a flagellum was always present, and were prepared to admit that in some cases this stage might be a non-flagellated leishmania-like form. We had observed in one case that a body which had been for some time quiet and motionless within the host-cell became suddenly active, showing a distinct flagellum. In all our permanent preparations, however, whenever flagella can be made out in the other stages or in the free trypanosomes, they can be seen to be invariably present at this stage also, and there can be no doubt that the motionless forms are those in which the flagellum is wrapped round the body. We are now convinced that non-flagellated leishmanial forms do not occur. We have the impression that the rolled-up trypanosome can wrap its flagellum round the body and pass into a resting, quiescent condition for a time, after which it can become active again by uncurling and setting free its flagellum, or at least a certain length of it, probably never quite the whole length.

The body in this stage, as in the last, is actively metabolic, with constantly changing contours in life. When the rounded forms are set free by rupture of the host-cell they swim actively in the liquid, progressing with the flagellum directed forwards; they then resemble ordinary flagellate monads, and the observer might easily have the impression that he was watching some intruding flagellate derived from contamination of the salt-solution or from some extraneous source.

In a few rare instances the rolled-up forms have been found in preparations to exhibit a central perforation or fenestration (Pl. 36, figs. 24, 30, 34), evidently produced by the trypanosome curling itself round so as to leave a central space. This condition, when it occurs, is probably quite transitory, the plastic cell-body of the trypanosome fusing into a compact lump sooner or later.

It seems probable that some of the rolled-up forms degene-

rate at this stage and are absorbed; that is, at least, the only explanation we are able to offer for such minute forms as those shown in Pl. 36, figs. 43–45, which appear to be undergoing degeneration.

The smallest of the rounded forms have each a single kinetonucleus, trophonucleus and flagellum. Now they begin to grow in size, with concomitant multiplication of their nuclei and formation of daughter-flagella. The division of these various parts appears to go on much as in other trypanosomes, independently, but more or less synchronously. The division of *n* may be slightly in advance of that of *N*, or slightly after it: thus, stages are found in which *n* and *N* appear to be both in the same stage of division (Pl. 37, fig. 79); or in which *N* appears to be in advance of *n* (Pl. 36, figs. 28 and 37); or with two distinct *nn* and *N* still in division (Pl. 37, figs. 80, 82); or finally with *n* and *N* both completely divided (Pl. 36, figs. 33, 36). The division of *n* is dependent on, or connected with, that of the basal granule or true blepharoplast, which may be regarded as representing the centriole or division-centre for *n*. The original flagellum does not divide, however, but remains attached to one of the daughter-blepharoplasts, from which it arises in close proximity to one of the daughter *nn*; and from the other blepharoplast a new flagellum grows out, at first a very fine and delicate structure and consequently very difficult to make out clearly or with certainty in the opaque body. In many cases in which *n* is divided completely no second flagellum can be seen, but it would not be safe, in view of the difficulties of technique presented by these objects, to conclude in all such cases that the formation of the new flagellum had not begun, since in other cases a very delicate line can be seen plainly growing out from the daughter-blepharoplast—that is to say, from the blepharoplast other than that from which the original flagellum arises (Pl. 37, figs. 79–81). As a rule the daughter-flagella can be made out in the preparations stained with iron-hæmatoxylin, but not in those stained by Giemsa's method; in the latter case, as already mentioned, the body

usually stains so intensely as to obscure completely the delicate daughter-flagella, which are imbedded in the mass of the cytoplasm, while the original flagellum runs for the most part on the exterior of the body; and if the stain be extracted sufficiently to make the body clear, it comes out of the growing flagella and leaves them invisible.

We may infer, therefore, that as the division of the nuclei proceeds the formation and growth of new flagella follow hard upon the division of the blepharoplast and kinetonucleus, and that a new flagellum grows out from each blepharoplast, in close proximity to *n*, quite independently of the original or parent flagellum, which remains unaltered.

As a general rule the multiplication of the nuclei begins at the rolled-up stage, but this rule is by no means invariable. Sometimes the nuclei are found to have multiplied even before the trypanosome has taken on the recurved form (Pl. 36, figs. 18–20). Such forms might possibly be specimens which, after becoming recurved, have straightened themselves out again, but their appearance is that of trypanosomes in which nuclear multiplication has begun before change of body-form. Attention must also be drawn to the peculiar elongated forms with 2 *nn* and 2 *NN*, such as Pl. 36, figs. 21–23; Pl. 37, fig. 74. At one time we were inclined to suspect that these forms, and also the unaltered trypanosomes with 2 *nn* and 2 *NN*, might be examples of fusion instead of multiplication (see below, p. 604); but we could find no definite evidence of there being fusions of two trypanosomes, and the fact that forms occur with 3 *nn* and 3 *NN* (fig. 18, pl. 36) makes it more probable that they are early stages of multiplication. On the other hand the possibility cannot be excluded that in some cases accidental and purely plastogamic (non-sexual) fusion of intracellular stages may occur, and that such fusions may explain the enormous size of some of the spheres and later stages of multiplication (Pl. 36, fig. 42).

The multiplication of the nuclei proceeds apace, and the duplication of *n* and *N* is followed by a stage in which the

body contains 3 *nn* and 3 *NN* (Pl. 36, fig. 40[1]; Pl. 37, fig. 84). This stage, which is of common occurrence, indicates that after the first division of *n* and *N* one of the daughter-nuclei in each case remains undivided, while the other divides again. This interpretation is supported by fig. 39, showing a specimen containing 3 *nn* and 2 *NN*, one of the latter being in process of division. A later stage is seen in fig. 38, in which both *n* and *N* have multiplied to four in number. The further stages of multiplication are not easy to follow in detail owing to the difficulty of making clear in one and the same specimen the *nn*, *NN*, and flagella, but from a study of various preparations there appears to be no reason to doubt that the *nn* and *NN* maintain their parallelism in division, and consequent equality of numbers, and that as the *nn*, or, to be more accurate, their blepharoplasts, divide, they continue to give rise to new flagella. Since the blepharoplasts are of different ages, as the result of successive divisions, the daughter-flagella given off from them are of different lengths; the original or parent flagellum remains, however, distinct and recognisable, both by its length, its superficial position, and the sharpness with which it stains (Pl. 37, fig. 92, etc.). In some cases the daughter-flagella also project freely from the surface of the body. This fact was observed in a living specimen, in which two small flagella were seen in addition to the principal flagellum, but it appeared to be a temporary condition, since later on only the principal flagellum could be seen, and still later that also disappeared. In one of our preparations also three daughter-flagella are seen in addition to the main flagellum, but unfortunately the specimen was so opaque that no details of internal structure, except the nuclei, could be made out.

With the growth in size and the multiplication of the nuclei the "block-like" stages pass by insensible gradations

[1] The specimen shown in fig. 40 appears to have become dried and flattened out before fixation; its size is altogether abnormal for this stage; compare fig. 38. Fig. 26, Pl. 36, also abnormally large, is from the same slide as fig. 40.

into: (4) The "spheres," a term used by us to denote the final stages of the intracellular multiplication. They appear in the fresh preparation as relatively large masses, more or less spherical in form, within the cytoplasm of the epithelial cells; they can be distinguished generally as "tailed" and "tail-less." At the very last the tail disappears in all cases, but this appendage, though commonly present in stages not full grown, sometimes cannot be made out in specimens that do not appear to be mature. A comparison of fixed preparations and a consideration of facts already discussed in describing the "block-like" stage leaves no doubt but that the tail-less spheres are derived from those earlier stages which have the original flagellum wrapped round the body, the tailed spheres from those which have the flagellum free. It is, however, evident even in the living state that the tail represents more than the original flagellum, since it is often of considerable thickness at the base and tapers gradually to a point. Examination of stained preparations shows that the tail represents in early stages the anterior end of the body, with the flagellum, of the original trypanosome (Pl. 36, figs. 37, 39; Pl. 38, fig. 110); and that in later stages the daughter-flagella may grow out parallel to the original flagellum, and so contribute to the formation of the tail (Pl. 37, fig. 86; Pl. 38, figs. 108, 111). But in other cases the anterior end of the original trypanosome may be retracted into the main mass of the sphere, round which the flagellum is wrapped more or less completely; the sphere then either has no tail (Pl. 37, figs. 90–94) or, if a tail is present, it represents the flagellum alone (figs. 95, 110).

The appearance and behaviour of the spheres in the living condition have been described by us in our preliminary communication. They are in a state of incessant movement, due both to the activity of the flagellum or tail, when present, and to internal commotions. The movements of the flagellum cause them to rotate within the cell in a jerky, irregular manner. When there are several spheres within one cell they can be seen to push and bump against

the cell-nucleus or against one another, the impact of one sphere causing a movement in another, which shows that they come into actual contact. Internal causes of movement are the metabolic form-change, already described, in the earlier stages, and movements due to the independent motility of the daughter-trypanosomes in the ripe spheres.

Even in the living condition it is not difficult to make out that a process of multiplication is going on within the sphere. First the nuclei and flagella can be seen to have multiplied, though the details of the process are naturally not clear in the living specimen. Later it is seen that the whole contents of the sphere have divided up into a number of distinct daughter-trypanosomes, which are seen writhing and twisting over each other within the enveloping periplast like a bunch of eels in a sack. The independent movements of the contained trypanosomes make their separate individuality clearer in the living state than in the preserved specimen. After the daughter-trypanosomes have become fully distinct and separate from one another a number of changes are seen in the sphere. The tail, if it was present, disappears, and the body acquires a perfectly spherical contour; this can only mean that the original flagellum, distinct from the beginning, has been drawn into the sphere and taken over by one of the sister-trypanosomes resulting from the process of multiplication. The metabolic form-changes, so marked a feature of the earlier stages, now cease, and the sphere shows only slight oscillating or trembling movements, due to the activity of the contained trypanosomes, moving restlessly within the comparatively tense envelope of the sphere, representing the periplast of the original trypanosome.

As the final moment approaches, the envelope of the sphere becomes more tense and rigid, its outline in optical section ever more nearly a perfect circle, until it bursts suddenly, setting free the contained trypanosomes. If the sphere bursts in its normal situation, that is to say within the cytoplasm of the host-cell, the liberated trypanosomes are seen moving actively for some time in the cell, from which

they escape one by one, passing out into the lumen of the stomach. But in the examination of stomachs freshly teased up, it is common to find spheres set free by the rupture of their host-cells. The spheres can then be seen to burst with such suddenness that they appear to explode; the impression given by the eye disappoints the ear, which misses the almost-expected report of the explosion; the trypanosomes set free scatter in all directions.

In one case a single cell was observed to contain three spheres, which were seen to burst successively; first one, then another, and then the third became resolved into free trypanosomes until the cell was full of active trypanosomes which appeared to be wriggling in a clear fluid. The cell retained at first its contours, but was apparently reduced to a spherical envelope, with no nucleus visible. Soon after the last sphere had become resolved the trypanosomes were seen to be escaping from a small area of the cell-wall, which then collapsed slightly; all the trypanosomes eventually found their way out at this part of the cell and swam off. In another case we timed our observations of a sphere and recorded the events in chronological order. It is not necessary to repeat the data already published, but they may be summarized as follows: A full-grown sphere was seen within a cell, moving and rotating by means of its flagellum, which was distinctly visible, and also exhibiting active metabolic form-changes; twenty-three minutes after it was first seen both the rotatory and the metabolic movements became much slower, and one minute later they ceased altogether, the alterations in the contour being slight, and due apparently to the very active movements of the contained trypanosomes; the flagellum had disappeared completely from view; the contour of the sphere then became very tense and rigid, and it burst after having been watched for twenty-eight and half minutes, or about five minutes after the disappearance from view of the flagellum and the cessation of the metabolic movements.

The trypanosomes set free are the long free stomach-type

already described, characterised by their great length, their stiff, crithidiomorphic form, and the more or less pronounced tendency to approximation of their two nuclei. The number produced in a sphere seems to vary considerably in different cases. We have attempted to count those which we have actually seen liberated within cells by bursting of spheres, and came to the conclusion that about eight was the usual number of trypanosomes produced. It is, however, very difficult to count accurately a number of trypanosomes which are at different levels in the cell, and, therefore, not all in focus at the same moment, and which at the same time are moving actively and changing their position. Moreover, the full-grown, tense spheres, which can be observed to burst, differ so greatly in size that there can be no doubt that the contained trypanosomes must vary considerably in number, and that if the average capacity of a sphere be eight trypanosomes, some spheres must liberate not more than four, others, perhaps twelve or sixteen, when they burst. These conclusions are confirmed by a study of fixed preparations; in the large spheres we find 8 (Pl. 37, fig. 86), 10 (Pl. 37, fig. 96), 13 (Pl. 36, fig. 41), and 14 or 15 (Pl. 37, fig. 94, and Pl. 36, fig. 42) *nn* and *NN*. From the results of counting the nuclei in a number of preparations, the most frequent number of *nn* in the largest spheres would appear to be 10 *nn*; the number of *NN* cannot always be made out, but where they can be seen their numbers are equal to, or slightly less than, those of the *nn*, indicating that the *nn* divide a little in advance of the *NN*. On the other hand, some spheres with but few nuclei appear to be nearly mature, having daughter-flagella so long that the nuclear division must have become much retarded, or perhaps ended altogether (Pl. 36, fig. 40, and Pl. 37, fig. 95). The permanent preparations confirm, therefore, the conclusion that while the number of trypanosomes produced is commonly eight or ten, it may be less, or as many as fourteen or more. The intracellular growth and multiplication of the trypanosome must depend on an interaction between the host-cell and

parasite, each a variable factor, and it is, therefore, not surprising if the result varies in different cases. Moreover, when a cell contains several parasites in different stages of multiplication, the cell may become exhausted before the younger individuals have time to grow to their full size.

The length of time required for a complete generation of intracellular multiplication is a point we have been unable to determine, as it is impossible to keep the living object alive under observation long enough. Moreover, the living trypanosomes in teased-up stomachs soon become abnormal, however carefully sealed up between slide and cover-slip, so that no data derived from direct observation of this kind could be entirely reliable. Until physicists and opticians have invented some method whereby the epithelial cells of the flea's stomach can be focussed under high magnification without dissecting or injuring the flea, it will not be possible to give a direct answer to such questions. Our observations cited above show that barely five minutes are required for a sphere to burst after the multiplication is complete, but that gives no clue to the length of time required for the growth and multiplication of the original trypanosome after it has penetrated the cell. From the fact, already noted, that the earliest intracellular stages were found by us about eight hours, and the earliest trypanosomes of the long stomach-type about twelve hours, after the flea had fed on an infected rat, it might be inferred that an intracellular generation required about four hours, but this computation cannot claim to possess any exactness.

It is also not possible to state how many successive generations of multiplication take place in the flea's stomach. Nöller states that at least two such generations, probably more, succeed each other. From the fact (to be described in more detail below) that the stomach-phase may be ended in some cases in eighteen hours, and in other cases may persist for four or even five days in unfed fleas (see above), it is evident that the number of generations of intracellular multiplication in the stomach must be a highly variable quantity.

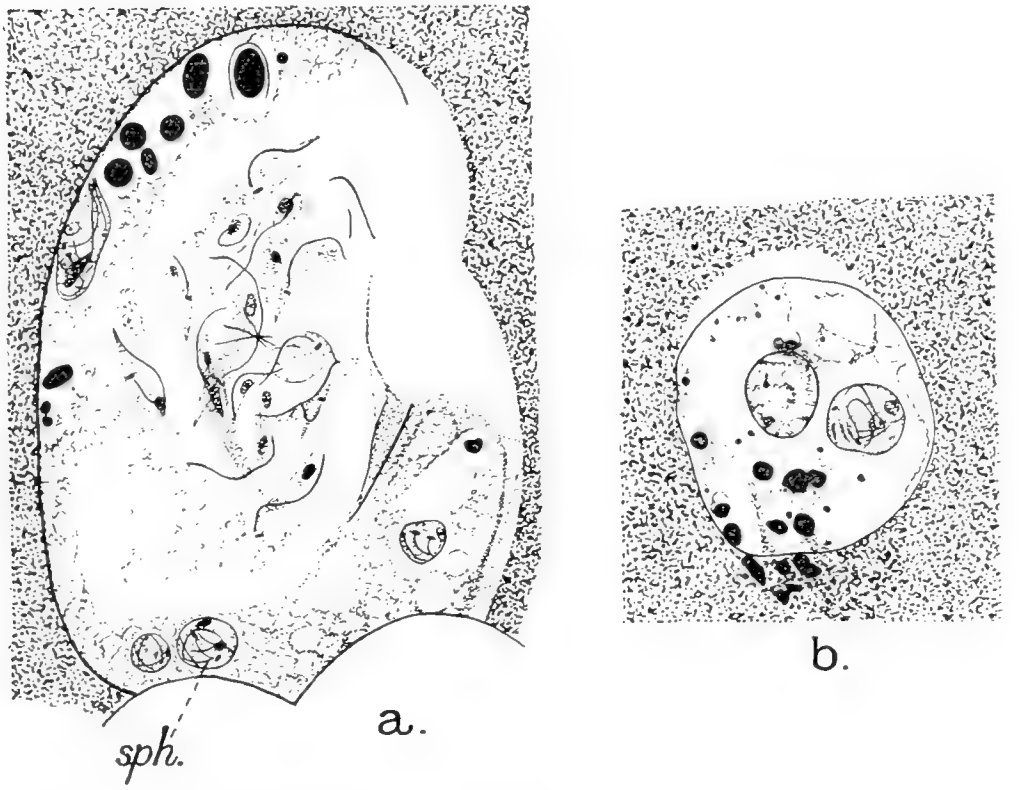
The foregoing account of the stomach-phase has been based almost entirely upon the study of the trypanosomes in the living condition and in smears. It was, in fact, all written, except for a few subsequent additions, before we had studied any sections. It remains now to supplement the description given above with the additional data furnished by the study of sections of the stomach, whereby a much more exact idea can be obtained of the relations of the trypanosome to the epithelium of the stomach.

When sections through a well-infected stomach are examined the trypanosomes are found usually to be both intracellular and extracellular in occurrence; that is to say, both free in the lumen of the stomach and contained within the cells of the epithelium. The extracellular trypanosomes occupy two different positions, speaking generally: they may be scattered throughout the blood-débris, including its most axial region, or they may be found only along the periphery of the sections, close to the epithelium and very often attached to it by the tip of the flagellum.

There can be no doubt that the trypanosomes found scattered through the central part of the blood-débris are for the most part forms in process of migration towards the rectum, their stomach-phase having been completed, but not all of them are to be interpreted in this way, since in many cases they are seen to be in clumps connected together by the tips of their flagella, and are obviously degenerative forms. In many cases also an infected epithelial cell is thrown off into the lumen, and may be seen there, crammed with trypanosomes, the product of the intracellular multiplication, which break out of the exhausted cell, and may possibly find their way into other epithelial cells again. The cell itself may become reduced to a mere sac containing fluid, in which a huge number of trypanosomes are squirming over one another (Text-fig. 3). If any of the protoplasm of the cell still survives it may contain a few spheres and other multiplication-stages. But that the forms free in the débris are for the most part migratory forms is indicated

by the fact that they occur with greater frequency towards the pyloric end of the stomach and sometimes only in this region, which may swarm with them, and in such cases they are always to be found in the intestine also.

TEXT-FIG. 3.



Exhausted cells containing intracellular stages of the trypanosome, from sections through the stomach of a flea thirty-six hours after the infective feed, $\times \frac{4000}{3}$. (*a*) A cell in process of being pushed off from the epithelium; the indented outline of the lower edge shows where it was in contact with epithelial cells in situ. The cell is reduced for the most part to a sac of fluid, in which trypanosomes are swimming, but round the edge some of the cytoplasm still remains, and in it are seen a few multiplication-stages (sph.), and also a few coarse black grains of fatty nature. The nucleus of the cell comes in another section. (*b*) A cell completely detached from the epithelium, the cytoplasm reduced to a few irregular strands and containing: (1) the nucleus at the centre; (2) a sphere to the right of it; (3) some coarse fat-grains, some of which have been extruded into the blood-débris.

On the other hand the trypanosomes that occur in close proximity to the epithelium are for the most part forms about to attack it, as shown by the fact that many of them are actually attached to the cells, and also by the frequency, it might almost be said the predominance, of the recurved form in this situation; at any rate, most, though by no means all, of those that are actually attached to the cells are recurved (Pl. 38, figs. 104, 105; Pl. 39, figs. 120–124). A certain number of recurved forms are also found, however, quite free from the epithelium, though close to it (Pl. 39, figs. 118, 119). The question at once suggests itself whether that represents the state of things as it was in life. It is impossible to extract a stomach from a flea without doing a certain amount of violence to it; and it is quite conceivable that the stresses and strains to which the wall of the stomach is subjected in pulling it out of the flea's carcase might well cause trypanosomes, previously attached, to become detached from the cells. We can but take the evidence as it is presented to us in our preparations, however, where we find that some of the attached trypanosomes are recurved and some not, and that some of the recurved forms are quite free from, though always close to, the epithelium. It would follow from these observations that in some cases the trypanosome assumes the recurved form after, in other cases before, attaching itself to the epithelial cell.

The attachment of the trypanosomes to the cells is peculiar; they are seldom attached to the extreme apex of the epithelial cell, but usually to its side (Pl. 39, figs. 121–124). The long flagellum runs down in the interspaces between two adjacent cells, and its extremity is often seen to be adherent to one of the cells close to the isthmus connecting it to its neighbour; the body of the trypanosomes usually projects into the lumen of the stomach above the general level of the epithelium, but sometimes the whole body is deep in between the cells. Sometimes two or three trypanosomes are attached side by side in such a situation. These statements apply to the usual columnar form of the epithelium with deep spaces inter-

vening between the cells; when the cell is flattened the trypanosomes may be attached at any point, and in the case of the young cells, which have but recently been produced from the epithelial buds and in which each cell is in contact with its neighbours along its side, without interspaces, the trypanosomes are attached to the convex apical region of the cell (Pl. 38, fig. 104), which may even contain early intracellular stages (Pl. 39, fig. 131).

As stated above, we have never been able to observe in the living state the actual penetration of the epithelial cell by the trypanosome; and in our sections we have searched in vain for appearances which could be interpreted as trypanosomes caught in the act of penetration. We have not found any trypanosomes half in and half out of the cell, from which it may be inferred, probably, that the actual penetration is effected very rapidly. But attention must be drawn here to a condition which is probably of significance in connection with this point. Trypanosomes of recurved type are found with the tip of the flagellum attached close to the base of the cell and with the rest of the body so closely applied to the side of the cell that careful focussing is necessary to determine that the trypanosome is still bodily outside the border of the cell and has not invaded it. Such appearances (Pl. 39, figs. 122, 123), suggests powerfully that the parasite, after attaching itself by the flagellum, forces its body into the cell. But the imagination must not be given too loose a rein when interpreting in terms of living activity the appearances seen in the dead fixed preparations.

The next stage is a recurved trypanosome within the cell (Pl. 38, fig. 107). Trypanosomes of the ordinary form (i. e. not recurved) are often seen in cells, but it is doubtful whether these represent individuals that have but recently penetrated the cell; if so, they might be forms which, after having been recurved, have temporarily straightened themselves out again, but it is on the whole more probable that they are trypanosomes which have been produced by the disruption of a sphere and which are about to leave the cell. In

support of the latter conclusion is the fact that we have seen trypanosomes of ordinary type only in cells that appeared more or less exhausted and liquefied, and especially often in detached cells. But, even if such forms represent recent arrivals in the cell, it is certain that they soon take on the recurved form. Consequently the recurved form may be taken in any case as the starting point of the intracellular development.

The growth of the recurved form into the sphere, the multiplication of its nuclei and flagella, and its ultimate fission to produce a bunch of trypanosomes, have been described in detail above, and only a few points in the development of the intracellular phase need be noted here. In the first place, in all preparations satisfactorily stained, it is seen clearly that flagella are present throughout, and that non-flagellated or leishmanial forms do not occur. This is so in all preparations stained with iron-hæmatoxylin and not over-extracted. In preparations stained by Giemsa's method the flagella are sometimes not to be seen, but in successful preparations they are exceedingly clear and sharp; it is always, however, one thing or the other, that is to say, we never find in one and the same preparation some parasites showing flagella and others none; either the flagella are invisible throughout the preparation, or are to be seen in every trypanosome, whether inside or outside the cells.

In sections the parasites are naturally very often in a mutilated condition, especially the large spheres, which may go through two or more sections. Consequently, it is not possible to be quite certain always whether a given sphere is tailed or not. But if the tail happens to lie in the plane of the section it may be shown very clearly, and then it can be seen that in some cases the tail is simply the original flagellum of the parent-trypanosome, projecting freely, while in other cases the tail includes the original flagellum with a prolongation of the trypanosome-body into which daughter-flagella may grow out, alongside of the parent-flagellum (Pl. 38, figs. 108, 110). In some cases, especially when the

host-cell is degenerating and breaking up, the flagellum of a sphere may project out from the cell (Pl. 39, fig. 132).

In concluding the description of the intracellular multiplication, we note that Chatton and Leger (1912), have described what they term a process of "agglutination and cytolysis" in Leptomonas drosophilæ, and suggest that the intracellular multiplication seen by us in the case of Trypanosoma lewisi in the stomach of the flea may be a phenomenon of the same order as that observed by them in Leptomonas drosophilæ. We cannot accept for a moment the suggestion that the behaviour of T. lewisi in the flea's stomach is anything but a quite normal process of multiplication. It is not incumbent upon us to criticise the statements of the authors, otherwise we might be tempted to inquire why they should think it necessary to interpret what they have seen in their Leptomonas as agglutination and cytolysis rather than as multiplication; but however legitimate such an interpretation may be for L. drosophilæ, we cannot admit it for the similar phenomena observed by us in T. lewisi.

Appendices to the Description of the Stomach-phase.

Having given in the foregoing pages an account of the intracellular multiplication of the trypanosomes in the stomach, it remains to discuss the relations of parasite to host and vice versâ. Under this theme are comprised (1) the frequency of occurrence of the intracellular multiplication in fleas exposed to infection, (2) the type of epithelial cell attacked by the parasite, (3) the relation of the intracellular stages to the infected cell, (4) the effects of the parasites on the cells, and (5) the relation of the infection as a whole to the stomach. The first of these problems is a statistical one, and is best studied in fresh or preserved film-preparations; the remaining four questions are best studied in serial questions.

(1) The Occurrence of the Intracellular Multiplication.

The following is a summary of our observations on the occurrence of the intracellular multiplication from twelve hours onwards, not counting those fleas that were used for sections:

At 12 hours approximately, after the fleas had fed on an infected

rat, intracellular multiplication was found in one flea out of ten examined:

At 15 hours,	in	1 flea	out of	15.
At 18 ,,		2 fleas	,,	15.
At 20 ,,		1 flea	,,	2.
At 24 ,,		40 fleas	,,	240.
At 36 ,,		2 ,,	,,	5.
At 48 ,,		5 ,,	,,	20.
At 60 ,,		none	,,	22.

At three, four and five days after infection, we only found trypanosomes in the stomachs of fleas that had been kept isolated and starved; in those that had been allowed to feed again on a clean rat no trypanosomes of any kind, free or intracellular, were found in the stomachs. Counting, therefore, only starved fleas, we found intracellular stages:

At 3 days,	in	5 fleas	out of	16.
At $3\frac{1}{2}$,,		1 flea	,,	7.
At 4 ,,		2 fleas	,,	13.

Later than five days we have never found them.[1]

The total of all these observations is that we have found intracellular stages of multiplication in the stomachs of sixty out of 365 fleas examined from twelve hours to four days after infection, or approximately 16·5 per cent. There is no doubt, however, that this figure falls below the actual percentage of fleas in which the trypanosomes succeed in establishing themselves in the stomach and passing through their intracellular phase when ingested by the flea, for at least two reasons. In the first place, many of the 240 fleas dissected at twenty-four hours after the infective feed were examined very hurriedly and cursorily, the object being to find fleas in which the intracellular stages were swarming, for the purpose of making permanent preparations of these stages, and when they were not found quickly in the teased-up stomachs examined in the fresh state, the search was abandoned; consequently in many cases in which the intracellular stages were scanty they may well have been overlooked, and would probably have been found after more prolonged search.[2] In the second place, as regards the fleas examined

[1] Since this page was written we have found intracellular stages in a flea which had been fed on an infected rat five days previously, and since then had been kept starved.

[2] Compare especially the figures given below (Text-figs. 4–12) of stomachs reconstructed from serial sections; it will be seen that there may be instances in which only one or two small patches of epithelium are infected, and in such cases it would be a lucky accident if the infection were discovered in looking at the freshly teased-up stomachs, even after a prolonged search.

at later periods than twenty-four hours, it is highly probable that in many of these the intracellular multiplication may have been completed and this stage passed. In one of our series of sections of a stomach preserved thirty-six hours after infection the stomach itself contained no trypanosomes of any kind, but two clumps of typical crithidial forms were found attached in the intestine behind the pylorus (Pl. 42, fig. 273); in this case the stomach-phase must have been over, assuming that it is a necessary and essential part of the developmental cycle. In Table M it will be seen that in several cases fleas dissected at two or two and half days after the infective feed had no trypanosomes in the stomach but developmental crithidial forms in the rectum. The absence of intracellular multiplicative forms in the stomach of a flea examined more than twenty-four hours after the infective feed is, therefore, no proof whatever that this phase has not been passed through in that flea.

When due weight is given to both these considerations, namely the probability that scanty infections may be overlooked in hasty examinations of stomachs in the fresh conditions, and the fact that the stomach-phase may be passed through rapidly and ended very soon, there can be no doubt that the true percentage of fleas in which it must take place must be considerably higher than the figure, 16·5, derived from actual observation. We should probably not be far wrong in putting it above 20, a result agreeing fairly closely with the percentage of fleas found by direct experiment to become infective after having fed on an infected rat (see below, p. 663), and thus supporting our belief that the intracellular multiplication is necessary for the trypanosome to establish itself in the flea, and is an indispensable part of the life-cycle ; a belief which is not, however, under the circumstances, capable of direct proof or verification.

It is at least evident from the figures given that the investigator must not expect to find the intracellular multiplication in more than a fraction (1/6 or 1/5) of the number of fleas examined which have fed but once on an infected rat ; and that he is most likely to find them from eighteen to thirty-six hours after the trypanosomes have been ingested by the flea.

(2) The Type of Cell attacked by the Trypanosome.

With regard to this point we have to note, in the first place, that we have never seen intracellular stages of the trypanosomes in the cells of the epithelial crypts. In this we are in agreement with Nöller (1912), who has also studied sections of the flea's stomach. Extracellular trypanosomes are sometimes seen attached to the exterior of cells which are passing out from the crypt, but never inside any cell which is not definitely a part of the general epithelium. It is possible that

the separation which, as described above (p. 494), takes place between the epithelial cells until they are connected only by the isthmus at the base, makes it easier for the trypanosomes to penetrate into them, since the appearances seen in sections suggest that the attack is usually made on the side of the cell (Pl. 39, figs. 120–124); the occasional, though rare occurrence, however, of intracellular stages in quite young cells (Pl. 39, fig. 131), shows that the trypanosomes can penetrate into epithelial cells before the separation between them has developed.

The degenerated epithelium, full of fatty deposits which blacken after treatment with osmic mixtures, is also not attacked by the trypanosomes. It is true that intracellular stages may sometimes be found in a degenerated cell, but in such cases the cell has been thrown off from the epithelium and the parasites are in the condition of large spheres ripe for breaking up into trypanosomes (Pl. 39, fig. 135); or of masses and clumps of trypanosomes that have evidently been liberated by the recent disruption of a sphere (Pl. 38, fig. 114). It may be supposed that in these cases the cell was attacked by the trypanosomes before degeneration had set in; the process of degeneration may have been hastened by the action of the parasites.

The trypanosomes attack by preference the fully-formed, but still young and vigorous cells, which may contain granules of the normal type and even yellow bodies, but no fatty deposits; cells which may be well characterised as adolescent in type, and which stain a clear, light-grey with iron-hæmatoxylin after Flemming-fixation. It is in such cells that the earlier stages of the intracellular multiplication are to be found in a flourishing condition and often in considerable numbers; but if the trypanosomes are numerous the cell soon becomes exhausted.

(3) The Relation of the Trypanosomes to the Cells.

The intracellular parasites are found most frequently in the apical expanded region of the cell, where the cytoplasm is usually of a loose spongy texture (Pl. 39, fig. 126). Sometimes, however, the trypanosomes are seen below the nucleus and occasionally there may be parasites lodged above and below the nucleus in the same cell (Pl. 39, fig. 134). Since the position of the nucleus in the cell is subject, as has been pointed out above, to variation, no special significance attaches to this point. It may be noted, however, that when a sphere is situated, as regards the principal mass of its body, above the nucleus, the tail of the sphere may run down to the base of the cell (Pl. 38, fig. 108).

In epithelium of the flattened form the intracellular parasites are lodged beside the nucleus, which is often pushed to one side of the cell.

(4) The Effects of the Trypanosomes on the Epithelial Cells.

In describing the pathological effects of the trypanosomes, we may begin with the cases where a cell is attacked by a few, not more than two or three, parasites, and has been but little affected by them (Pl. 39, figs. 126, 127). It is seen that the parasite is lodged in a distinct space or vacuole in the cytoplasm, a vacuole which is not to be interpreted as an artefact due to shrinkage from the action of reagents, but as produced by the liquefactive or absortive action of the parasite on the cytoplasm, since the vacuoles containing the intracellular parasites can be seen very clearly in the living cell. Similar effects produced by Toxoplasma gondii in the peritoneal cells of the mouse have been described by Miss Pixell.[1] In the living condition. as stated above, the trypanosomes move freely by means of their flagella in the liquefied cytoplasm, and when there are many parasites in the same cell they can be seen to jostle one another and even to bump against the cell-nucleus. Owing to the liquefaction of its cytoplasm the cell becomes empty and exhausted, and in its final stage is reduced to the condition of a sack, containing fluid, which may be crammed with trypanosomes produced by the process of multiplication within the cell; remnants of the cytoplasm may persist on the wall of the sac, and the cell-nucleus is also to be found at some point, generally adherent to the wall. Often these exhausted cells contain fatty deposits and also the brownish-yellow bodies described above.

The effect produced on the parasitised cell as a whole is a more or less distinct hypertrophy, more obvious when the contained parasites are present in large numbers (Pl. 38, fig. 114). Then the cell often becomes very much enlarged, but this enlargement appears to be brought about in many cases not solely by the hypertrophy of the individual epithelial cell, but also by the fusion of distinct cells; at least this is the only way in which we are able to interpret the large multinucleate cells, containing numerous parasites in all stages of their multiplication, that are frequently to be found. Mitoses, or any other forms of nuclear multiplication are never seen in the epithelium, and the very large size of these multinucleate cells (Pl. 39, fig. 128; compare Pl. 44, fig. 313), certainly indicates cell-fusion having taken place. We have also seen multinucleate epithelial cells not containing parasites: but such cases might very well be those in which the intracellular multiplication was ended and the daughter-trypanosomes produced had escaped from the cell.

As the cells are exhausted and destroyed by the parasites there is a pronounced tendency for them to be thrown off from the epithelium.

[1] 'Proc. Roy. Soc.,' (b), lxxxvii, p. 73, (Pl. ix, figs. 3–6).

In the sections infected patches of epithelium are of frequent occurrence, in which the infected cells are seen bulging far out from the general level of the epithelium into the lumen of the stomach, giving sometimes an appearance like a bunch of grapes (Pl. 44, figs. 313 and 315). In such protuberances every cell, as a rule, contains numerous parasites in all possible stages of multiplication, in progress or completed. In one of our series there is a stomach in which no parasites are to be found within the epithelial cells in situ in any part of the stomach-wall, but in the fore part of the stomach there are very numerous intracellular parasites, all contained in cells completely detached from the epithelium. On the other hand very large patches of epithelium may be found infected without the cell being thrown off; in one of our series there is a stomach, which, in one part shows infected cells the whole way round the section, except in the epithelial crypts (Text-fig. 9), but there is very little detachment of epithelial cells. It is evident that the expulsion of the infected cells from the epithelium is a measure partly of the intensity of the infection, i. e. the number of parasites contained in each cell, and partly of the length of time during which the parasites have been acting on the cells; it is not till the cells are becoming exhausted and incapable that they are ejected from the epithelium.

From a study of the extracellular trypanosomes, which occur in close proximity to the epithelium, and more especially those actually attached to it, it is evident that the extent of the epithelial areas simultaneously attacked may vary very greatly in different cases. Sometimes only an attached trypanosome is to be seen here and there, scarcely so much as one in each consecutive section on the average; this corresponds to the frequent occurrence of solitary epithelial cells, or very small groups of them, containing parasites. Sometimes, on the contrary, all the epithelium on one side of many consecutive sections will be seen to have trypanosomes adherent to it, while on the other side of the same sections not a trypanosome is to be seen (compare Text-figs. 4–12). What determines the extent and distribution of the attacks it is impossible to say, but at least one necessity is probably the presence of regenerated "adolescent" epithelium.

It should be mentioned finally that in one of our series there is a stomach which in its hinder region is almost entirely denuded of epithelium. Vast numbers of trypanosomes are seen in all parts of the stomach-lumen, but swarming most thickly near the wall, on which only the epithelial crypts remain intact. Remnants of the general epithelium occur here and there in the form of broken-down cells containing the final stages of the intracellular multiplication, but for the most part the epithelium is gone altogether. In the anterior region of this stomach, trypanosomes are less abundant and the epithelium is in process of regeneration. It is surely a fortunate circumstance for the

insect host that its epithelial crypts of regeneration are immune to the attacks of the trypanosomes; were it not so, it is hardly credible that the flea could survive the extensive destruction of the epithelium that may occur with an intense infection.

(5) The Relation of the Trypanosome-Infection, as a Whole, to the Stomach of the Flea.

This point can best be made clear by describing a few examples, which will give a more graphic picture of the variations seen in different fleas than can be obtained from a general description. It must be stated, in the first place, that, as already pointed out above, in stomachs of fleas preserved eighteen, twenty-four, or thirty-six hours after having been fed on a well-infected rat, the trypanosomes may have entirely disappeared, having been digested with the blood and failed to establish themselves. Consequently, in many of our series of sections, some or all of the stomachs contain no trypanosomes at all. On account of the great expenditure of labour and time involved in searching through a complete series of sections of a stomach we have not attempted to compile any statistics of the numerical proportion of infected to non-infected stomachs, as we have done in the case of stomachs teased up and examined fresh, the latter being a method much less laborious, though not so exact, for determining whether a stomach contains trypanosomes or not.

In the examples we are about to give we deal only with those stomachs in which trypanosomes have been found, and in order to exhibit at a glance the state of things in each stomach we have made reconstructions of a certain number of stomachs in the following manner:

(a) To show the distribution of the cells containing intracellular stages, the stomach is imagined as cut open from the intestine to the proventriculus along a line which corresponds to the southernmost (lowest) point in the transverse sections, but which is a purely arbitrary line so far as the flea is concerned and does not correspond to any definite anatomical plane of the insect. The wall of the stomach, supposed to have been cut open along this line, is further imagined as laid out flat. Of each stomach reconstructed in this way a diagrammatic sketch was made on paper ruled in millimeter squares to a definite scale, namely, 1 mm. to each transverse section of 6 μ in thickness, and in the sketch three meridians are put in with dotted lines, the middle one to represent the northernmost (uppermost) point of the sections, while the meridians to the left and right represent respectively the extreme western (left-hand) and eastern (right-hand) points in the section. After making a diagrammatic sketch in this manner the series of sections was searched through and all infected cells were mapped out

in the reconstructions, being represented by little circles placed in the line corresponding to the number of the section, and in the relation to the meridians that indicate its position in the section. For instance, if an infected cell is found in the twenty-seventh section, to the north-east of the section, the circle representing it is put into the diagram 27 mm. from the anterior end of the stomach and midway between the meridians N. and E.

In this way a graphic representation, accurate to scale in the longitudinal direction, is obtained of the distribution of the infected cells in the epithelium. It is important to remember that each little circle represents not merely a single intracellular stage of the trypanosome, but an infected cell which may contain many such stages.

(b) To show the distribution of the extracellular trypanosomes the diagram is supposed to represent the unopened stomach, and the trypanosomes that are in close proximity, or attached, to the epithelium are put in along the sides, while those scattered free in the débris are represented dispersed through the diagram, in the position corresponding to the number of the section in which they occur; when very numerous, however, it is impossible to represent them accurately, and they are merely crowded in as thickly as possible in the diagram.

It is very important that the reader should understand clearly that in each of our diagrammatic reconstructions of the stomachs two distinct conventions are combined, as it were superposed one on the other. Originally our intention was to have given two diagrams for each stomach, one reconstructed according to method (a) to show the distribution of the intracellular parasites, the other according to method (b), indicating the occurrence of the extracellular trypanosomes. To have given two diagrams for each stomach, however, would not only have taken up much space, but would not have shown the state of affairs so graphically as when the two diagrams are combined into one and no confusion can arise if it is clearly understood that two different methods of reconstruction are combined in each figure. The fact that the attached trypanosomes are represented adherent to the sides of the diagram, for example, does not mean that they are attached only along the southern meridian; they are attached at any point in the section, but had they been indicated in the diagram in the exact meridian in which they occur the eye would not have distinguished them from the free forms.[1] To indicate the exact position of the attached trypano-

[1] It was suggested by a friend that the attached trypanosomes might have been distinguished from those that are free by drawing at one end of them a little transverse line or semicircle, to indicate the attachment to the cell; but where the trypanosomes are thickly crowded, as in Text-fig. 10, it would have been impossible to distinguish clearly the free and the attached forms in this way.

somes it would have been necessary to have shown the attached and the free trypanosomes in separate diagrams; either to have put in the attached forms in the diagram showing the infected cells, according to method (a), or to have constructed three diagrams showing the infected cells, the attached trypanosomes, and the free trypanosomes on each diagram separately.

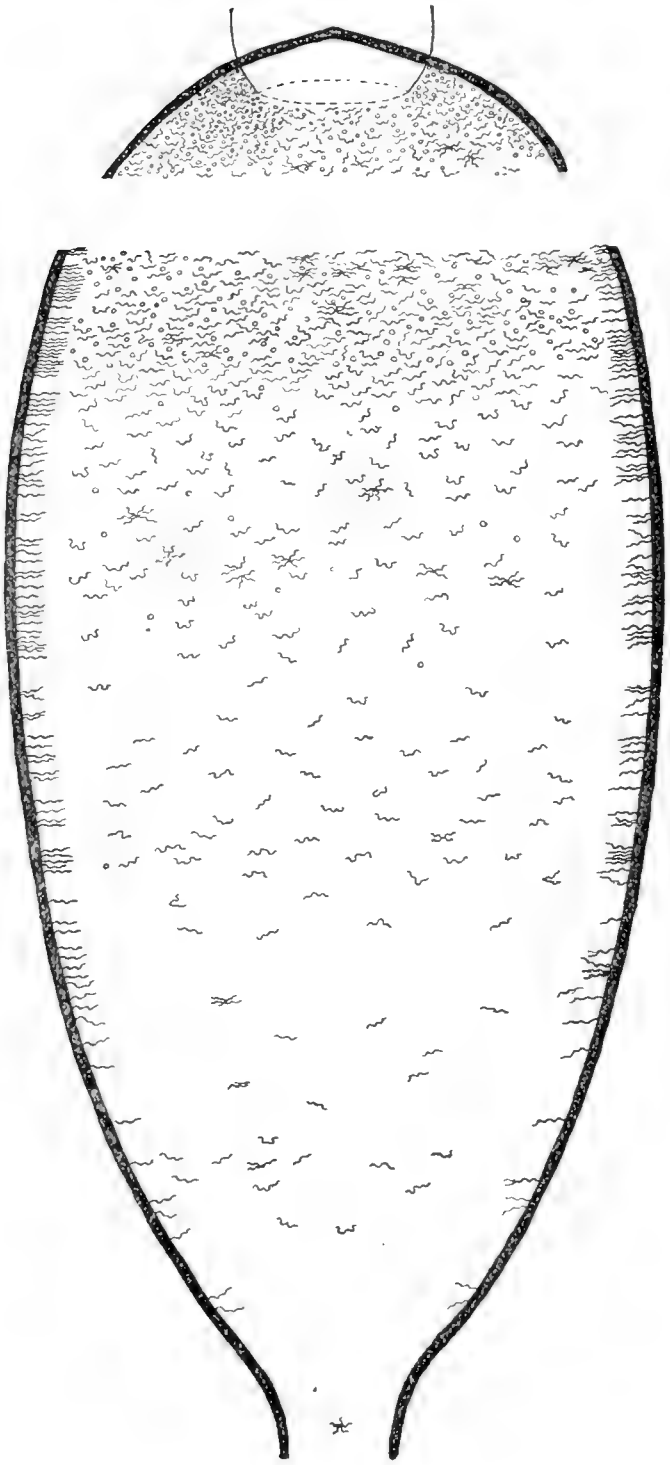
The reconstructions were made, as has been stated, on a definite scale as regards the length of the stomach, namely, on the scale of 1 mm. to 6 μ, that is to say at a magnification of $\frac{1000}{6}$, or 167 approximately. In the reproduction, however, the diagrams have been reduced to two-thirds of their original size, and appear, therefore, at a magnification of $\frac{2000}{18}$, or 111 approximately, with the exception of figs. 3 and 11, which it has been necessary to reduce to one-half on account of the very large size of the stomachs, and which, therefore, appear at a magnification of about 83·5, while fig. 9 has been reduced to one-third. The diagrams show the great variations in the size of the flea's stomach. The small stomachs are males, the large ones females. The number of sections in a series cut through a small stomach was generally about 125, in a large stomach from about 220 to 300. This corresponds in a remarkable way to some observations we had made and recorded long previously to cutting the sections—on the feeding of the fleas. We found that a male flea took about a minute and a quarter to fill its stomach, a female about two minutes and a quarter.

The stomachs reconstructed by us were all from fleas fed twenty-four or thirty-six hours before being dissected and preserved. We will begin with the fleas of thirty-six hours, because we possess a very good series of this period, which has been studied very carefully by us, and which, having been preserved in Flemming's fluid and stained with iron-hæmatoxylin, shows the state of the epithelium and the stomach-contents particularly well.

(a) Thirty-six hours after feeding.—A batch of nine stomachs, all preserved at the same time, stuck on the same piece of liver and cut in the same block of paraffin. Owing to an accident to the block the two stomachs near one end of the liver were damaged; the anterior half of one of these stomachs and a small part of the anterior end of the other were broken away. In what remained of these two stomachs no trypanosomes of any kind were found, and the same was the case with one of the other stomachs in this series. There remain six stomachs to be described.

(1) (Text-fig. 4). The stomach is a large one, going through about 260 sections, corresponding to a length of about 1·56 mm., exclusive of the proventriculus. The blood-débris is large in amount and stained

TEXT-FIG. 4.



Reconstruction of a stomach in the manner described in the text, to show the distribution of the intracellular and extracellular trypanosomes. The portion left blank is where some of the sections were accidentally destroyed. No meridians have been put in because, in this instance, all the intracellular trypanosomes were in detached cells. 36 hours. Magnified about 83·5.

yellow. The epithelium is for the most part flattened and very black, especially at the anterior end, where also there are very many detached cells. There are also a great many yellow necrosed cells, either in the form of extensive patches in the epithelium in situ, or of detached cells loose in the blood-débris.

Extracellular trypanosomes abundant, especially round the detached cells in the anterior region. In the posterior half of the stomach they are much fewer, and almost all peripheral in position or attached to the epithelium.

Intracellular trypanosomes very abundant in the anterior fourth, but only in detached cells, none in the epithelial cells in situ. Passing backwards they become scarcer, and in the posterior half of the stomach none are found.

Since the intracellular parasites in this stomach are found only in detached cells, the meridians have been omitted in the reconstruction, it being impossible to refer the infected cells to their proper position in the stomach-wall.

Interpretation.—This is a stomach in which the epithelium is in the final stages of degeneration, especially anteriorly, but regeneration has not begun. There has been an extensive intracellular infection in the most anterior region, represented now by advanced stages in the detached epithelial cells, and swarms of free trypanosomes are attaching themselves in preparation for a fresh attack. There are also clumps of degenerative forms free in the blood.

(2) The stomach goes through about 220 sections corresponding to a length of 1·32 mm. Blood-débris in moderate quantity, stained yellow; epithelium clear, granular, containing many grains and pseudospheres. No trypanosomes in any stage found in the stomach, but in the intestine, behind the pylorus, in a large clump of attached crithidial forms.

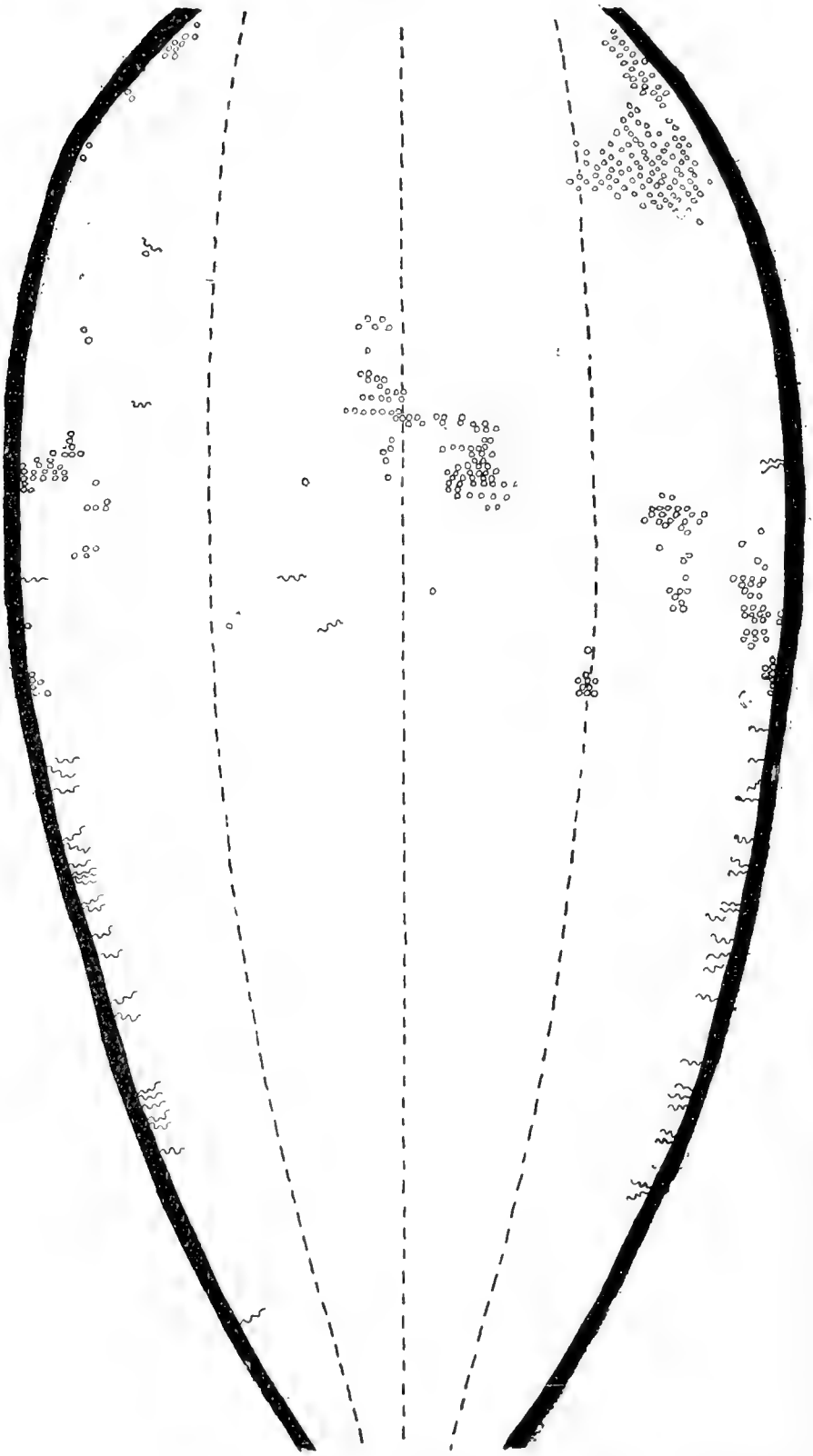
Interpretation.—A stomach which has recently been regenerated and in which the stomach-phase of the trypanosomes is completed and past.

(3) (Text-fig. 5). A large stomach going through about 260 sections. Blood-débris about half absorbed; stains grey. Epithelium for the most part clear, columnar, very granular, with coarse grains and pseudospheres and many yellow bodies. There are a fair number of detached cells, some of them showing yellow necrosis, scattered through the stomach; in the posterior region there are some black degenerated cells still in situ or in process of detachment.

Extracellular trypanosomes very scarce in the anterior half of the stomach; in the posterior half they are more numerous and all attached to the epithelium or peripheral in position.

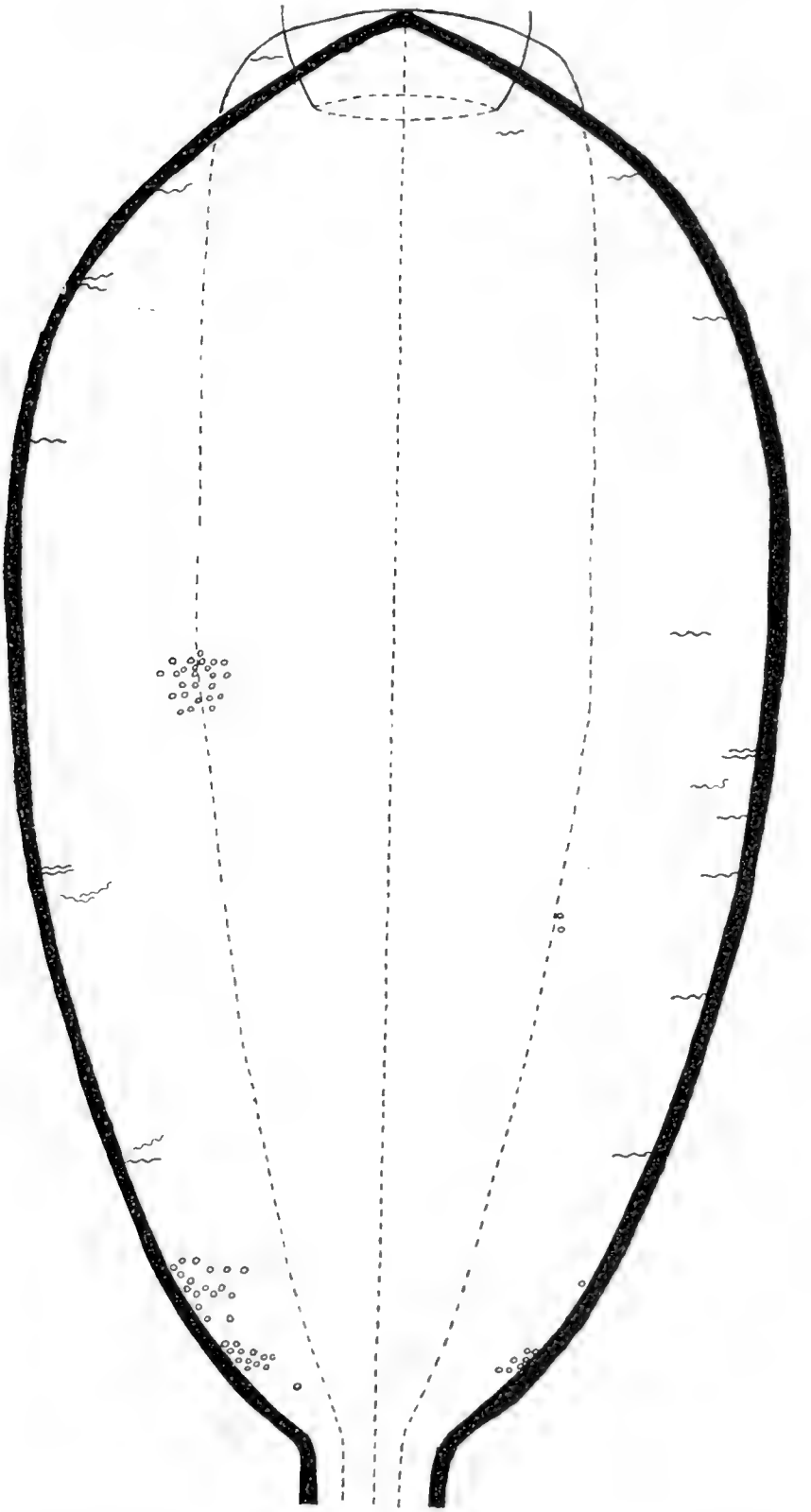
Intracellular trypanosomes are found only in the anterior half, where there are scattered patches, variable in extent, of infected epithelium.

TEXT-FIG. 5.



Reconstruction of another stomach. The series of sections began just behind the proventriculus. 36 hours. Magnified about 111.

TEXT-FIG. 6.



Reconstruction of another stomach. 36 hours. Magnified about 111.

Interpretation.—A stomach very recently regenerated, the process scarcely complete in the posterior region. In the anterior half practically all the trypanosomes have penetrated into the epithelium. In the posterior half an attack is beginning, but all the parasites are still extracellular.

(4) (Text-fig. 6). A large stomach going through 223 sections. Blood-débris as in last. Epithelium as in last, but less granular and with no yellow bodies; no black cells in situ, but a few detached.

Extracellular trypanosomes very few in number, scarcely more than twenty in the whole series; all attached to the epithelium or close to it.

Intracellular trypanosomes in two fairly extensive patches of infected epithelium, one about the middle of the stomach, one close to the pylorus. In one of these patches the cells are badly affected; in the other they are more normal.

Interpretation.—A feebly-infected stomach, recently regenerated, in which almost all the trypanosomes are intracellular.

(5) The stomach runs through 217 sections. Blood-débris large in amount; stained yellow. Epithelium: (*a*) in the first 130 sections mostly very dark, but interspersed with clearer patches of cells; many detached cells, some black, some yellow; (*b*) in the hinder $\frac{5}{8}$, approximately, of the stomach the epithelium is mostly clear, not very granular, with a few patches of black cells in situ, and many detached cells, black or yellow.

Extracellular trypanosomes all confined to the anterior region, in rather scanty numbers for the most part peripheral in position and often attached.

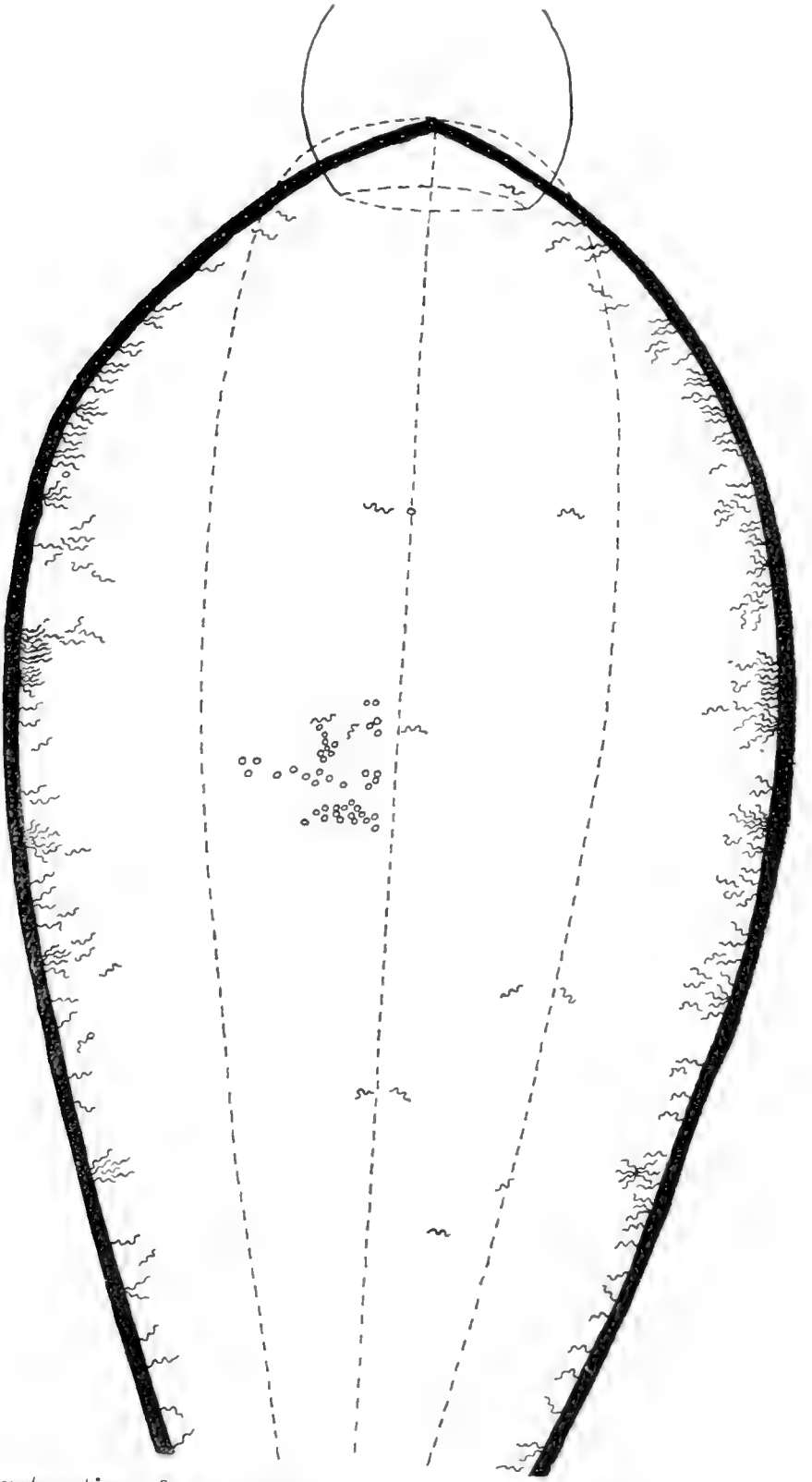
Intracellular trypanosomes found only in the posterior region; three patches of infected epithelium, one fairly large, running through forty-six sections, the cells considerably modified, and two small patches; also a detached cell containing a large sphere in section 132 (rather far forward).

Interpretation.—A stomach in which regeneration is just beginning in the anterior region and is fairly advanced posteriorly. In the anterior region the presence of necrosed cells indicates that there has been a recent attack, but at present there are only extracellular trypanosomes; in the posterior region there are fairly extensive patches of infected epithelium and no extra-cellular trypanosomes.

(6) (Text-fig. 7). The stomach runs through about 240 sections. Blood-débris large in amount; yellow. Epithelium clear, columnar, and with many granules, and in places yellow bodies; interspersed are a few black or yellow cells, singly or in patches, in situ, and detached cells are also fairly numerous.

Extracellular trypanosomes numerous; nearly all close to the epithelium and many attached along the whole length of the stomach.

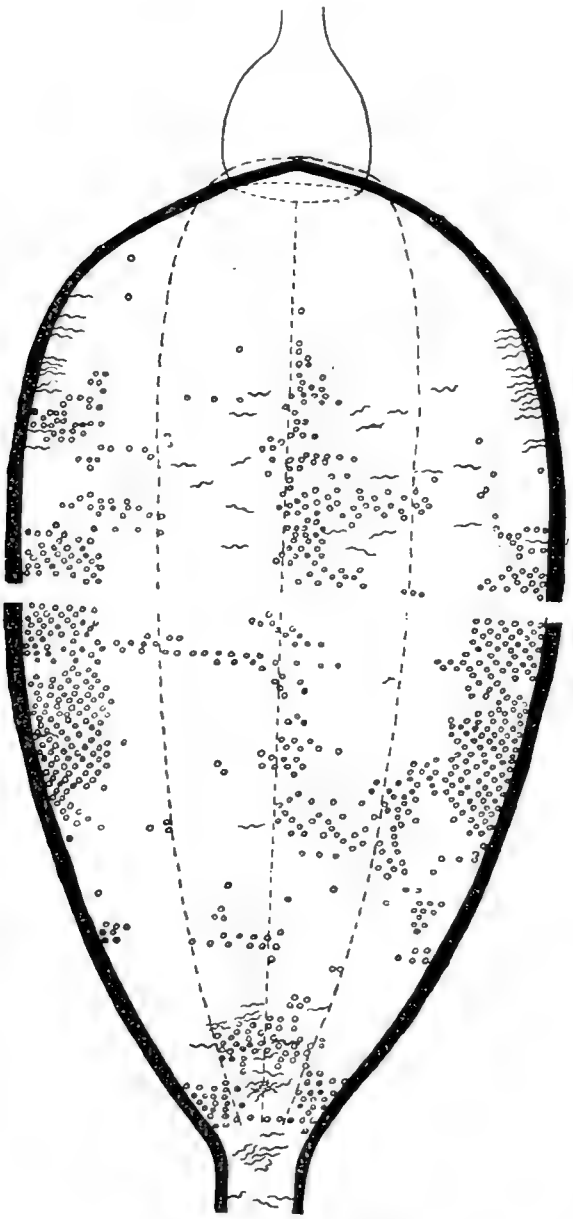
TEXT-FIG. 7.



Reconstruction of another stomach. Owing to the exigencies of space the hinder part of the stomach, in which there were no trypanosomes, has been cut off. 36 hours. Magnified about 111.

Intracellular trypanosomes practically confined to a fairly extensive patch of epithelium about the middle of the stomach.

TEXT-FIG. 8.



Reconstruction of another stomach. The gap in the middle of the figure shows where three sections were accidentally destroyed. 24 hours. Magnified 111.

Interpretation.—A stomach which has undergone regeneration, which is scarcely completed. A previous intracellular generation of trypanosomes, indicated by the presence of necrosed cells, has given rise

to numerous free trypanosomes that are attacking the regenerated epithelium, and have penetrated it and established themselves in the cells in a few places.

(b) Twenty-four hours after feeding.—(7) (Text-fig. 8). From a series preserved in Maier's fluid.[1] A small stomach, running through about 150 sections. In the anterior quarter, or thereabouts, of the stomach are a fair number of attached forms. Behind this region begins a very intense intracellular infection which diminishes somewhat in the posterior fourth, except for an extensive patch close to the pylorus. Through all this region there are practically no attached trypanosomes, but there are a few scattered through the débris, which become more numerous towards the pylorus, and are found passing down the intestine, where some of them are attached to the lining.

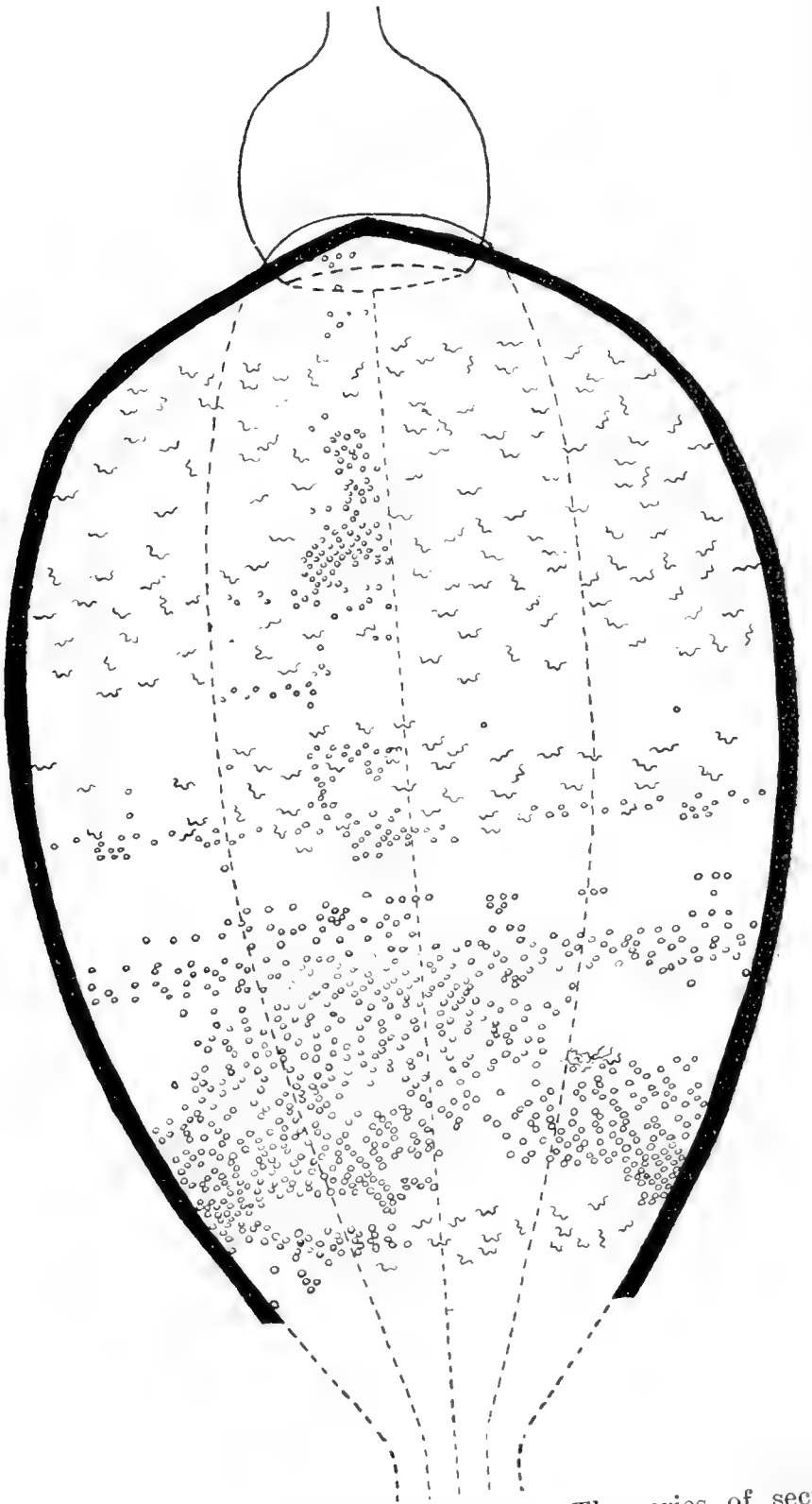
Interpretation.—The intracellular multiplication is at its height and the trypanosomes are beginning to pass on to the rectum, but at the extreme anterior end a fresh attack is developing.

(8) From the same series as the last. A small stomach running through about 154 sections. In the anterior twenty sections the epithelium is columnar, normal, or in places tending to degenerate, with no intracellular trypanosomes, and very few free trypanosomes in the sections. For about forty sections behind this there is an intense infection of the epithelium, which in places is largely destroyed or broken up, and there are great numbers of extracellular trypanosomes, some scattered through the débris, others in close proximity to the stomach-wall. In the next fifty sections, approximately, the epithelium is almost entirely destroyed; only the crypts remain, with here and there a few broken-down cells still adherent to the wall and containing late or completed stages of the multiplication; there are also in this region enormous numbers of extracellular trypanosomes, mostly in close proximity to the wall of the stomach, but also scattered through the débris, in which there are, in addition, masses of detached, broken-down cells, many of them containing large spheres or clumps of trypanosomes. In the twelve sections behind this region the number of trypanosomes and detached cells diminishes rapidly, and in the last thirty-two sections there are no trypanosomes, though still a few detached cells.

Interpretation.—A stomach with an extraordinarily intense infection, which, in the middle region, has destroyed the epithelium completely. The intracellular multiplication has produced vast numbers of trypanosomes, which are not beginning as yet to migrate backwards

[1] In the case of stomachs (7) to (15) the slides of the series were stained alternately with the iron-hæmatoxylin-Lichtgrün-combination and by Giemsa's method.

TEXT-FIG. 9.



Reconstruction of another stomach. The series of sections was not continued quite as far as the pylorus. 24 hours. Magnified about 111.

to the rectum, judging from their absence in the posterior region of the stomach.

(9) From the same series as the last. A large stomach running through about 234 sections. Epithelium almost everywhere columnar, a few flat cells; very few detached.

Extracellular trypanosomes fairly numerous towards the hinder end, mostly peripheral and many attached, a few scattered in the débris. No intracellular typanosomes found.

Interpretation.—A stomach in which one or more generations of intracellular multiplication have been completed and a new attack on the cells is beginning.

(10) (Text-fig. 9). From the same series as the last. A large stomach, but unfortunately not quite enough sections were mounted and the series ends before the pylorus. Blood-débris large in amount everywhere.

In the anterior half of the stomach there are a few extracellular trypanosomes scattered through the blood-débris, very few attached; there are also some intracellular parasites, for the most part scanty, but in one place there is a fairly extensive patch of infected epithelium.

In the posterior half there is a very intense infection of the cells, which in many sections is seen all round the section, or interrupted only by the epithelial crypts. In this region, extracellular trypanosomes are practically absent; one clump, apparently of degenerative forms, was found, and in the hindermost region free trypanosomes, scattered in the débris, begin to appear abundant in the pyloric region.

Interpretation.—Over a large extent of the stomach the intense infection of the epithelium has absorbed, so to speak, all the free trypanosomes except the degenerating forms.

(11) From another series preserved in Maier's fluid. A stomach of moderate size, running through about 180 sections.

Extracellular trypanosomes swarming in the posterior region of the stomach and in the intestine; towards the anterior end they diminish in number, but are to be found right up to the proventriculus. Almost all these trypanosomes are free in the débris, very few are attached.

No intracellular trypanosomes found.

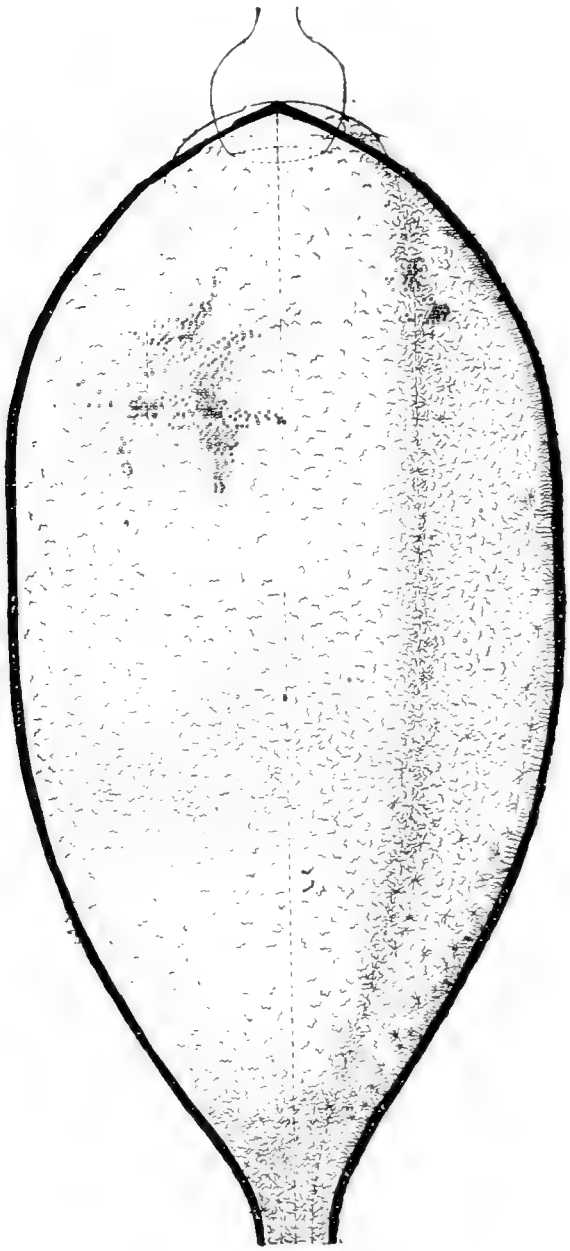
Interpretation.—A stomach in which the intracellular multiplication is practically ended and the trypanosomes, present in large numbers, are passing down to the rectum.

(12) Text-fig. 10). From the same series as the last. A very large stomach, running through about 320 sections, corresponding to a length of nearly 2 mm.

Extracellular trypanosomes occur in vast swarms along the whole

length of the stomach; most of them are peripheral in position occurring all along the east (right-hand) side of the sections, and many

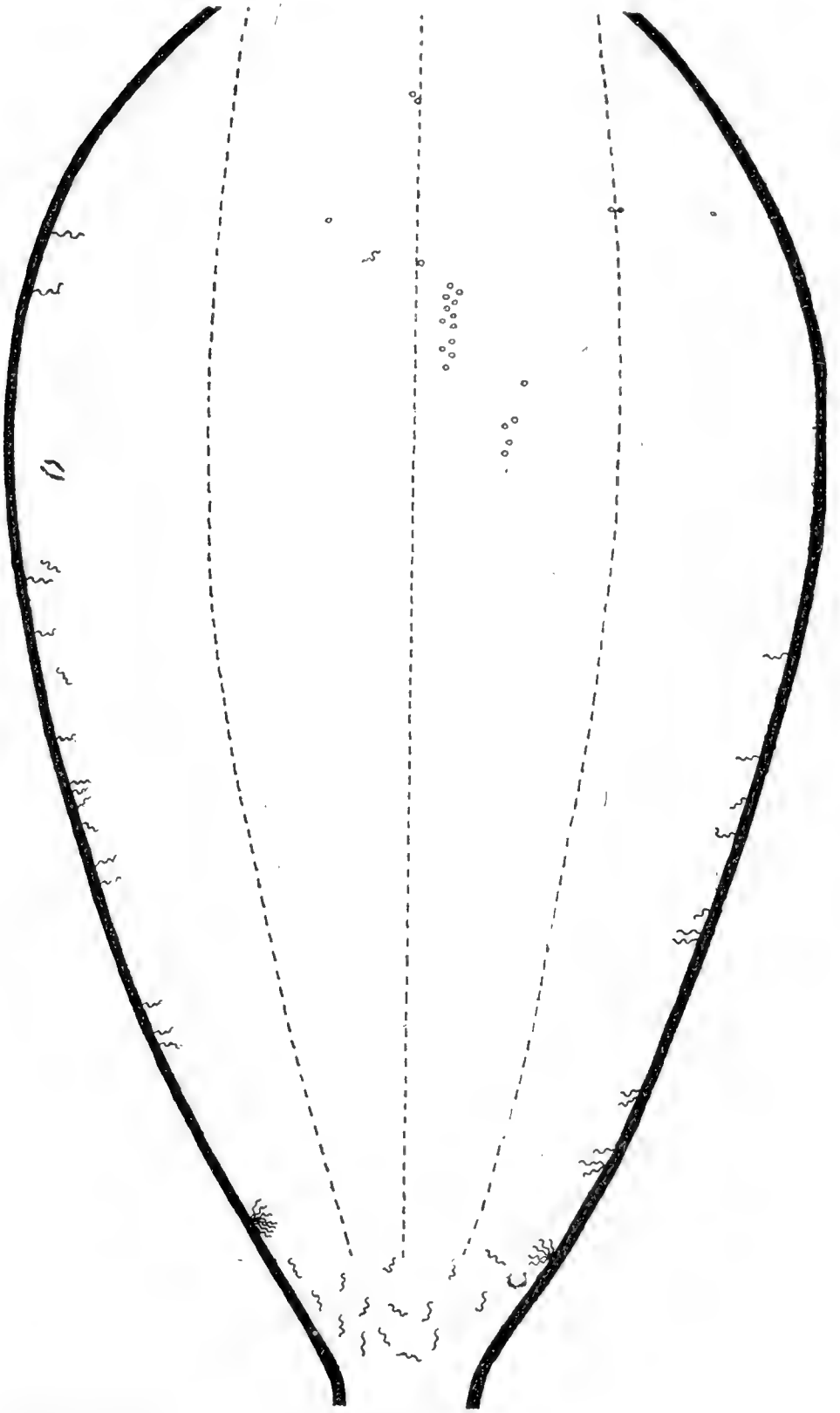
TEXT-FIG. 10.



Reconstruction of a very large stomach. 24 hours. Magnified about 55·5.

of them are attached. Trypanosomes also occur, however, scattered through the débris in all the sections, but more especially in the posterior half of the stomach, and swarms of them are seen in the

TEXT-FIG. 11.



Reconstruction of another stomach. The anterior part of the figure, from just behind the proventriculus, has been cut off owing to exigencies of space, but there were no trypanosomes in the part that has been cut off. 24 hours. Magnified 111.

pyloric region passing down the intestine. Many of these free forms, whether central or peripheral in position, are in clumps and are possibly degenerative.

Intracellular trypanosomes are not found in the posterior half of the stomach, but towards the middle a few detached infected cells are found here and there. In the anterior region there is a very large patch of infected epithelium *in situ* and also two smaller patches.

Interpretation.— Intracellular multiplication is proceeding actively in the anterior region, and a new attack on the epithelium is preparing all along one side of the stomach. At the same time, trypanosomes are passing down in large numbers towards the rectum and there are many degenerative clumps.

(13) (Text-fig. 11). From another batch preserved in Maier's fluid. A large stomach running through 255 sections.

Extracellular trypanosomes very scanty, practically confined to posterior half of stomach, all attached except in pyloric region, where there are a few scattered freely in the *débris*. Many of the attached trypanosomes are in clumps, perhaps degenerative.

Intracellular trypanosomes only in anterior region, very scarce.

Interpretation.—The stomach-phase seems to be maintaining itself with difficulty in this flea; the trypanosomes are few in number and largely degenerative, but a few are passing backwards.

(14) Text-fig. 12). From the same batch as the last. A large stomach, running through about 225 sections.

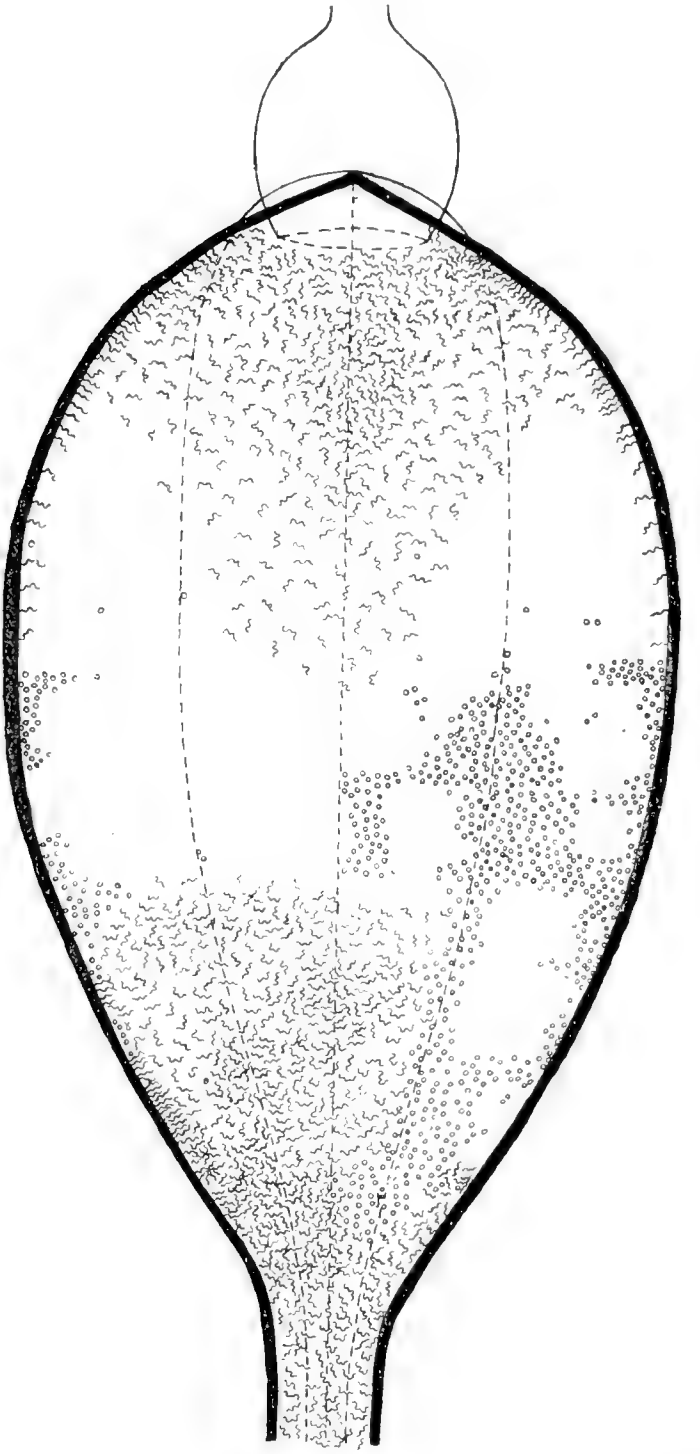
In the anterior third, approximately, of the stomach extracellular trypanosomes are abundant, both free in the *débris* and attached to the epithelium, but there are no intracellular stages.

In the region behind this, approximately the middle third or fourth of the stomach, there are no extracellular trypanosomes, either free or attached, but a very extensive intracellular infection is found, confined for the most part to the right side of the sections, where almost the whole of the epithelium, with the exception of the crypts, has been attacked; the reconstruction shows very well the clear spaces which represent the situations of the epithelial crypts.

In the posterior third of the stomach a remarkable condition of things is found. On the right side of the sections there are still numerous intracellular trypanosomes, but no attached extracellular parasites; on the left side of the sections there are very numerous extracellular trypanosomes, attached or in close proximity to the epithelium, but none intracellular. Scattered through the *débris* are many free trypanosomes which are also passing down the intestine.

Interpretation.—A stomach in which intracellular multiplication is still proceeding actively and from which trypanosomes are passing down towards the rectum.

TEXT-FIG. 12.



Reconstruction of another stomach. 24 hours. Magnified about 83·5.

(15) From a batch preserved in sublimate-acetic. A large stomach which passes through 241 sections without quite reaching pylorus.

About 10 extracellular trypanosomes, most of them attached to the epithelium, are found in the hindermost sections.

No intracellular trypanosomes are to be found.

Interpretation.—A stomach in which the intracellular multiplication is either completed or almost inhibited.

(B) The Migration to the Rectum.

As stated above, the trypanosomes occurring in the intestine are usually in transit from the stomach to the rectum, and only exceptionally attach themselves to the intestinal wall or undergo further development there. We have been able on more than one occasion to observe the actual passage of the trypanosomes along the intestine. In a flea which had fed on an infected rat about twenty-four hours previously, the stomach, with a considerable length of the intestine attached, was dissected out on a slide partly teased up and covered with a glass slip; the posterior part of the stomach, however, with the intestine attached, was left intact. Close to the pylorus the stomach contained fluid in which a great number of brown granules were suspended, coarse granules of fæcal appearance evidently representing indigestible residue of the last meal. These granules could be seen to be in a state of violent commotion, more violent than could be explained merely by Brownian movement, since they were being churned and stirred in every direction; but although there could be little doubt but that the movements were due to the activity of trypanosomes, the flagellates themselves could not be seen clearly in the opaque fluid through the stomach-wall. Soon, however, a bolus of fluid passed through the pylorus into the intestine and passed down it by peristaltic action, pushed onwards like a bead, until it reached the cut end of the intestine and was extruded from it. It was then seen at once that the fluid contained, in addition to the fæcal granules, a swarm of excessively active trypanosomes, long and relatively slender forms with great powers of rapid forward progression.

They began at once to spread in all directions in the fluid; but at this moment the coverslip was picked off with needles and dropped instantly into Schaudinn's fluid; after it had been stained and mounted it was found that, by a piece of good luck, the stomach and intestine had remained sticking to the coverslip, and that round the cut end of the intestine were several trypanosomes of the long stomach-type (Pl. 42, figs. 203, 204), evidently some of those that had been seen to pass down the intestine. In two other fleas, fed respectively twenty-four and eighteen hours previously on infected rats, we were able to confirm our observations on the passage of the trypanosomes down the intestine and to obtain preparations of them (Pl. 37, fig. 50).

It is unnecessary to give a detailed description of the migratory trypanosomes, since it is evident from the figures that they are simply of the long stomach-type already described. They are very active, and in form crithidiomorphic, with the hinder part of the body stiff and straight, sometimes slightly or even markedly clubbed and swollen. The two nuclei are more or less closely approximated, but *n* is almost always well behind *N*. It is seen from this that the trypanosomes resulting from the intracellular multiplication in the stomach may do one of two things; they may penetrate again into epithelial cells and go through another generation of multiplication; or they may collect in the pyloric region and be carried down the intestine (compare Pl. 45). The migration may begin as has been seen, as early as eighteen hours, but this appears to be rather exceptionally early, to judge from our observations on the rectal phase; probably it does not usually begin till twenty-four or thirty-six hours. It continues, doubtless, as long as the stomach-phase lasts, and as already stated, we have found intracellular stages as late as five days in fleas not fed a second time; we may suppose, therefore, that the production of the migratory forms and their passage down to the rectum, may be going on continually, in some cases, until the second feed of the flea. In other cases, however, the multiplication in the stomach probably comes to an end of itself,

before the second feed, judging by the many observed instances in which, in fleas not fed a second time, the stomach may contain many long free trypanosomes, or the rectal phase may be well established, without any intracellular multiplication occurring in the stomach.

(c) The Rectal Phase.

The starting point of the rectal phase is the long, active "crithidiomorphic" type of trypanosomes already described, which migrates down the intestine. During its passage down the intestine the changes of form and structure which may have begun already in the stomach continue, so that by the time it reaches the rectum its posterior end is generally distinctly club-shaped. Arrived in the rectum, it very soon undergoes changes in form, habits and structure, and multiplies by binary fission, giving rise ultimately to the typical forms of the established rectal phase, forms which, apart from other characters, are of much smaller size and bulk than those of the stomach-phase. We will discuss first (*a*) the transition from the initial to the established forms of the rectal phase, and then (*b*) the various types of modifications of the latter, culminating in the little trypanosome-form, which is the final stage of the development of the flea.

(*a*) The Transition to the Crithidial Form.

If the various processes of change in the initial stages of the rectal phase be analyzed, after study of both living and preserved material, and by comparing the starting point of this part of the development with its final result, we may note the following tendencies in the organism.

In the first place it loses its intense activity and becomes more sluggish in movement, with a great tendency to attach itself by the tip of the flagellum; under natural conditions the flagellate attaches itself to the wall of the hind-gut, but when under microscopic examination it can be seen to adhere

firmly to pieces of débris of any kind, or to the surface of the glass slide or coverslip. When not attached in this way it progresses slowly with the flagellum directed forwards.

Secondly, the body shortens and changes in form by the cytoplasm becoming concentrated towards the posterior end of the body.

Thirdly, the flagellum becomes progressively shortened.

Fourthly, the two nuclei, if still in their original positions, become transposed into the typical crithidial arrangement, with *n* close beside or in front of *N*.

Fifthly, the nuclei, the flagellum, and finally the whole body are multiplied by division or reduplication.

The order in which these different processes of change have just been stated is in no way to be taken as indicating their chronological order of succession in the development, since they take place more or less independently and, as it were, at different rates of acceleration in different individuals; the result is consequently the production of a great number of forms which at first are rather bewildering and difficult to arrange in a series. The difficulty of tracing in detail the transition from the initial stage of the rectal phase to the established crithidial condition is increased by the fact that the early transitional stages are extraordinarily rare and difficult to find in the permanent preparations; a fact which indicates that the transition takes place very rapidly and is completed very quickly. One explanation of this rapid change may perhaps be found in the great diminution in size which is brought about in this part of the development. Leaving out of consideration for the moment any structural or other changes, it is at least quite clear that the large individuals which come down from the stomach initiate a series of generations of multiplication by fission culminating in forms perhaps not more than a tenth the size of the initial forms from which they are derived. Consequently it is probable that the successive divisions of the body follow one another at first with extreme rapidity and without intervening pauses to allow the daughter-individuals

to grow to the size of the parent, as would happen in the normal multiplication such as takes place in the established crithidial stage. Another explanation for the rarity of the transition-stages may be the possibility that of the long trypanosome-forms which come down from the stomach to the rectum, only a small number may go through their metamorphosis into the typical rectal phase and the greater number may degenerate or be carried out of the flea. Whether this be true or not, it is not necessary to suppose that all those which pass down from the stomach do so in a single swarm; it is more probable that they dribble down from the stomach, so to speak, in larger or smaller bands or singly, a supposition which would also account for the small number found at any one time in the rectum undergoing their metamorphosis.

A further difficulty which may arise in distinguishing the forms of the transition from trypanosome to crithidia, and in assigning them to their proper position in the series, is the fact that the body may become artificially broadened in preparations which have been allowed inadvertently to dry up the least bit before fixation. Deformed specimens of this kind can be recognized by their flattened appearance and consequent even staining of the body, which in a properly preserved specimen should be thicker and more opaque in the axial region than towards the edges of the body; the trophonucleus in flattened specimens is often transversely elongated; and, further, the process of drying seldom affects a single specimen, unless it is very isolated or near the edge of the film, but, if it has taken place at all, the effects of desiccation are apparent over at least a considerable area of the slide or coverslip. It is, therefore, not difficult with a little practice to detect the specimens which have become broadened artificially; and in any case the process of drying does not affect the length of the body or flagellum to any appreciable extent.

It is a result, doubtless, of the rapidity with which the transition is effected that we have not been able to come to a perfect agreement of opinion between ourselves as to certain points of the development during this transitional period,

namely, the exact stage in the process of change of form at which the first division of the initial rectal form (that is to say, the long club-shaped forms that come down from the stomach) takes place, and consequently the type of binary division, whether equal or unequal, which initiates the whole series of generations in the rectal phase. Before we discuss this doubtful point, we may first classify the various types seen in the initial transitional stages of the rectal phase, for which it is simplest to take the types of body-form as the basis of classification. Bearing in mind the considerations of technique that have been discussed already, and being careful, therefore, to eliminate all cases where there is reason to suspect artificial deformation of the specimens, we can recognise the following series of forms, each of which is a stage in the progressive shortening and broadening of the body by concentration of the cytoplasmic substance in the posterior third of the original slender trypanosome:

(a) Slender forms, differing but little from the long stomach type, and evidently but recently arrived in the rectum. The flagellum is still long, and N is usually in front of n (Pl. 42, fig. 208); sometimes, however, the reverse is the case (Pl. 42, fig. 209).

(b) Forms in which the hinder region of the body, containing the two nuclei, become swollen and club-shaped (Pl. 42, fig. 206), and the anterior part correspondingly attenuated, until the body as a whole becomes more or less tadpole-shaped (Pl. 41, fig. 150; Pl. 42, figs. 205, 207). The flagellum at this stage is usually long, but sometimes distinctly shortened (Pl. 41, fig. 158; Pl. 42, fig. 210).[1] The nuclei are usually still in their original relative positions, but are sometimes crithidial in arrangement (Pl. 42, fig. 211).

(c) Forms in which the concentration of the body-substance

[1] How the shortening of the flagellum takes place is not clear, but it is worthy of note that in smears there are often found broken pieces of flagella near the specimens, as if the flagellum had become brittle and had broken off (Pl. 41, figs. 158, 160).

in the posterior half or third of the body has proceeded so far that the body is nearly half as broad as long, and the anterior prolongation which forms the undulating membrane is much reduced in length and very slender. Examples of transitions from the last stage to this are seen in Pl. 41, figs. 151, 152;

TEXT-FIG. 13.

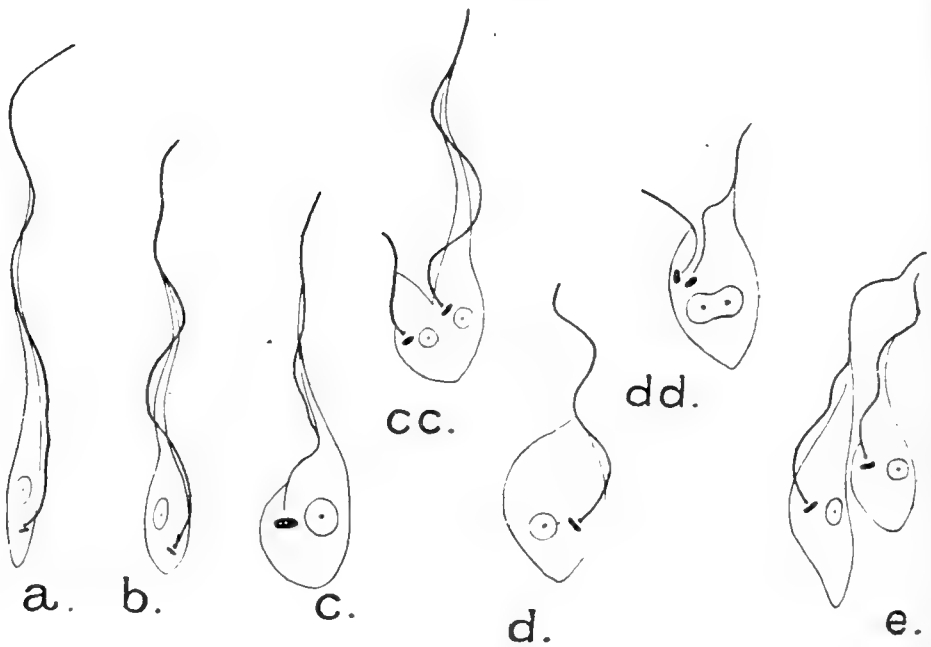


Diagram to show the possible modes of transition from the stomach-phase to the rectal (crithidial) phase: *a*, slender form newly arrived from the stomach (compare Pl. 42, fig. 208, etc.); *b*, early club-shaped form (compare Pl. 42, fig. 205, etc.); *c*, later (more swollen) club-shaped form (compare Pl. 41, fig. 149); *cc*, unequal division of *c* (hypothetical); *d*, large pear-shaped form (compare Pl. 41, figs. 154, 155, etc.); *dd*, division of *d* (compare Pl. 41, fig. 179); *e*, forms resulting from the initial division of the rectal phase (compare Pl. 41, figs. 153, 157, 164, etc.). (× about 2000.)

the complete realisation of this stage is seen in Pl. 41, figs. 149, 154, 162; Pl. 42, fig. 212. From a comparison of the figures it is seen that the position of the nuclei varies considerably; *n* may still be at or near the hinder end (Pl. 41, fig. 162), or may be close beside *N* (Pl. 41, figs. 149, 154; Pl. 42, fig. 212). The hinder end may be pointed, or quite round. The

flagellum may still be a fair length, or quite short (Pl. 41, fig. 162).

(d) Forms in which the body is contracted into a pear-shaped mass, nearly as broad as long. The stalk of the pear is formed by the flagellum together with a slight projection of the body representing the anterior prolongation, now usually reduced to its smallest limits (Pl. 41, figs. 155, 156, 161; Pl. 42, fig. 215). The nuclei are usually transposed, *n* being beside or in front of *N*. The length of the flagellum varies within wide limits represented by Pl. 41, figs. 155 and 156.

(e) Forms which are distinguished from the preceding stage chiefly by their smaller size. Here again, however, caution is necessary in referring a given specimen to its place in the series, since the apparent size in permanent preparations may be affected considerably by technique. In the preparations fixed on the slide with osmic vapour and stained by Giemsa's method the trypanosomes always appear considerably larger than those on the coverslips fixed wet with sublimate solutions, stained with hæmatoxylin and mounted without drying in canada balsam (vide Minchin, 1909). Consequently, different standards of sizes are required for interpreting preparations made by these two different methods of procedure, and preparations made by the one method must not be compared directly, without making due allowance for the differences in result, with those made out by the other method. Nevertheless, after giving due weight to these considerations, it is not difficult to distinguish forms which are about half the bulk of the stages already described (Pl. 41, figs. 153, 159, 160, 164; Pl. 42, fig. 218). Such forms are, without doubt, individuals derived from at least one, possibly more than one, division of the initial form, a conclusion supported by the occurrence of such forms in pairs, possibly as the result of division recently completed (Pl. 41, fig. 157; Pl. 42, fig. 216*a*).

The question which must remain open at present is, in which of the four stages of changes of form (*a*), (*b*), (*c*), or

(*d*) (see Text-fig. 13), does the initial division of the rectal phase take place?

If, as is possible, and as one of us (J. D. T.) thinks probable, the first division takes place in the club-shaped form as soon as the concentration of the protoplasm is completed and when (as in Text-fig. 13, *c*) the two nuclei lie close together, it may well be the case that the products of division would then be markedly unequal in appearance owing to the retention of the old flagellum by one of the two daughter-individuals; that is to say, the division would be of the type found in similar club-shaped forms in the trypanosome of the gold-fish both in cultures and in the leech, and in bird-trypanosomes both in cultures and in the mosquito, and which has also been seen in an early culture of T. lewisi itself. On this view the swollen, pear-shaped forms of stage *d* would have to be interpreted as forms subsequent to, and the products of, the initial division. In spite of much searching, however, through our preparations, we have been unable to find actual examples of club-shaped forms showing division markedly unequal in appearance.

If, on the other hand, as one of us (E. A. M.) believes, it is the most usual state of things for the organism to continue the process of contraction and broadening of the body until it has reached the condition of stage *d*, it is probable that the flagellate would then divide by the type of binary fission characteristic of the subsequent generations of the crithidial phase, that is to say, producing two daughter-individuals that are equal, or not markedly unequal in bulk, and differing only in that one of them has the flagellum temporarily shorter than that of the other. Pl. 41, figs. 179, 180, and Pl. 42, fig. 216, may then be interpreted as examples of the initial division, and Pl. 41, fig. 157 and Pl. 42, fig. 216 *a*, as pairs resulting from the initial division recently completed.

The problem of the initial division of the trypanosomes in the rectum is one which involves more than the question as to the type of form, club-shaped or pear-shaped, in which the division takes place, or the question whether the products of division differ in visible characters of

bulk, structure, or appearance; it raises a much deeper and more fundamental problem, namely, whether the division which initiates the rectal phase is an equating division which gives rise to two equipotential daughter-individuals or a differentiating division which produces inequipotential forms. If the division-products are equipotential, then visible differences between them of any kind would be merely temporary and of no significance for the future behaviour and destiny of the sister-individuals, which would be true twins; all such differences, however pronounced, would be immaterial for the development as a whole, and the same would apply to the parent individual, whether club-shaped or pear-shaped. If, on the other hand, the daughter-individuals are inequipotential, in the sense that the smaller of the two division-products becomes the starting point of an indefinite number of generations of small crithidial individuals, while the larger is merely a "parent" which, though it may divide in the same manner to produce small crithidial forms several times in succession, does not ultimately develop further, but drops out, as it were, of the direct line of the development when its powers of reproduction are exhausted; if they are inequipotential in this sense, it follows that the observed difference between the two daughter-individuals would have an important significance and that they would not then be true twins, and further, that the club-shaped form, assuming that this is the form in which division takes place, would then be a developmental form of special and peculiar significance in the life-cycle and not merely a stage in the change of form leading to the pear-shaped stage. We must be content, unfortunately, with enunciating these possibilities, without being in a position to decide between them; owing to the fact, to which reference has been made above, that the extreme rarity of transitional forms in our preparations has supplied us with insufficient material for a decisive judgment.

The type of the initial division and the exact point at which it occurs in the series of progressive form-changes of the rectal phase must be left at present an open question, unfortunately; but this much may be stated positively about the process of division in the rectal phase in all cases, whether in the initial or subsequent stages. No multiple division occurs henceforth in the developmental cycle, but the parasites settle down to a course of simple binary fission continued indefinitely, and always taking place in the lumen of the gut, never within cells. The process of fission is initiated by division of the blepharoplast or basal granule of the flagellum, but the flagellum itself does not divide; the original or parent

flagellum remains attached to one of the two daughter-blepharoplasts and a new flagellum grows out from the other blepharoplast. The division of *n* follows hard upon that of the blepharoplast, then *N* starts its division, and finally the whole body divides; of the two daughter-flagellates produced, one has the original flagellum, the other has to grow a new one, which it may not do, in some cases, until after complete separation from its twin sister. In any case, one of the two products of division has a much shorter flagellum than the other, irrespective of any difference in bulk between the two.

As already stated, while these changes of form and processes of multiplication are going on the flagellate is also undergoing a change which, though a very small thing in itself, produces nevertheless the characteristic morphological distinction between the trypaniform and crithidial types; namely, the approximation and final transposition of the two nuclei, so that *n* comes to lie beside, or even well in front of, *N*. The approximation of the two nuclei begins with the first alteration and changes of form, but the exact point in the development at which the change of position of the two nuclei is completed is subject to great variation. Very exceptionally, as has been described above, the transposition of the nuclei may be complete in the stomach itself, but more usually, it may be said normally, the change does not take place until the flagellate reaches the rectum. Any of the forms distinguished above as *a*, *b*, *c*, *d* or *e*, may be found with the nuclei, but slightly or completely approximated, and finally in the clumps of developmental forms such as that represented in Pl. 41, figs. 182–184, evidently consisting of forms which have completed several, at least two or three generations of fission since arriving in the rectum, all stages in the process of transposition of *n* or *N* are to be observed. Since the small forms of the established rectal phase show invariably the typical crithidial arrangement of the nuclei, all that can be said is that the change in position of the nuclei is effected earlier or later, but without fail, in the transition from the stomach-phase to the rectal phase.

The successive generations of the transitional rectal phase cannot be distinguished with precision. The size to which the individuals are diminished after a given number of generations is determined by two variable factors during those generations, namely, the rate of individual growth and the frequency with which multiplication takes place. All that can be said is that the rectal forms diminish in size by repeated division until they reach a minimum size which is attained when growth and multiplication balance each other more or less evenly; and that in the rectal infections of recent origin the average size of the individual flagellates is slightly larger, as a rule, than in the old-established infections.

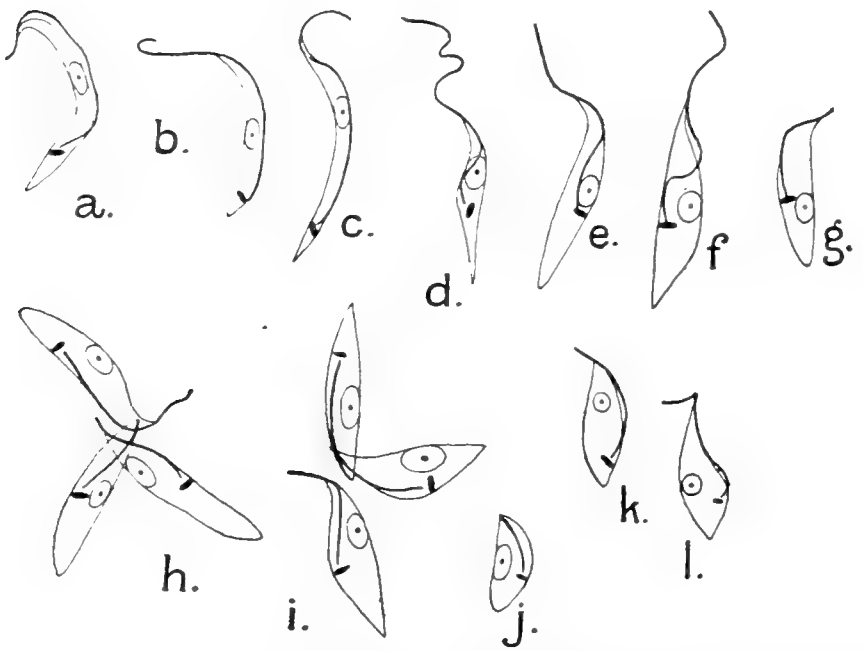
(*b*) The Established Rectal Phase.

In its fully-established and definitive condition the rectal phase consists chiefly of small crithidial individuals which multiply by binary fission, attached to the wall of the gut by their flagella or by the anterior (flagellar) extremity of the body. The crithidial stage appears normally, if not invariably, to take origin in the rectum in the manner described in the preceding section, and this part of the digestive tract is its most usual and characteristic habitat; consequently we have made use of the term "rectal phase" for this part of the development, in spite of the fact, presently to be discussed in more detail, that the crithidial and other forms of this phase may be found not uncommonly in other parts of the gut, more especially in the intestine close behind the pylorus, but also in the stomach itself.

In one flea we have found the rectal phase well-established so early as eighteen hours after the infective feed (Text-fig. 14), and in another flea we found a few typical examples of this phase at twenty-four hours, but both these examples are abnormally early cases of the fully-developed crithidial phase, which, in our experience, is seldom completely established before thirty-six or forty-eight hours after the infective feed.

The extent to which the crithidial infection is developed in different fleas varies greatly, from a swarming infection covering the wall of the rectum like a pile-carpet, especially in the region behind the rectal papillæ, to a condition in which a few scanty flagellates are to be found only by careful searching; in either case, however, the crithidial stage represents

TEXT-FIG. 14.



Various forms from the rectum of a flea eighteen hours after the infective feed, *a*, *b*, *c* and *d* are probably degenerative forms, the rest developmental; *e* and *f*, early rectal forms, *g*–*l*, haptomonads (× 2000).

the permanent stock of the parasites in the flea, multiplying continually and indefinitely and maintaining itself, in all probability, as long as the flea lives. We have succeeded in keeping a single flea alive for nearly three months, and during that time seven rats were infected by it (see p. 640 below). This permanent infectivity can only be explained by the establishment of the crithidial stock in the rectum and its continued multiplication. Each crithidial individual may do one of two things: it may multiply by binary fission to produce two crithidial forms (multiplicative phase); or it may be

transformed gradually into small stumpy trypaniform individuals (final propagative phase). Hence the flagellates of the rectal stock may be classified, broadly-speaking, into crithidial, transitional and trypaniform individuals.

The forms that occur in a well-established rectal infection are very varied, and it is a somewhat difficult task to classify them and to assign to each and every form its due place in the developmental series. To obtain a general notion of the various types met with, it is best to examine fleas some eight or nine days after they have fed once on an infected rat and subsequently on clean rats, so as to obtain the rectal phase in its typical condition, free from admixture of earlier developmental or degenerative forms. Such fleas may have no flagellates at all in their rectum or may present every gradation between an extremely scanty and a swarming infection.

If a rectum containing numerous flagellates be examined fresh after having been dissected out in salt-citrate solution, opened up by tearing it with fine needles, and then covered with a coverslip, the majority of the flagellates are seen attached to the wall of the rectum, chiefly in the region behind the rectal papillæ. Many of them will have become detached as the result of the dissection, and will be seen floating about singly or adhering together in larger or smaller clumps. If the preparation be sealed up carefully and watched for some time many of those that lie about singly will attach themselves to the glass of the slide or coverslip, and it can be seen very clearly in all cases, whether in the forms still attached to the rectal wall or in those set free in clumps or singly, that the attachment is by the tip of the flagellum or by the flagellar extremity of the body in those that have no free flagellum. The flagellates may be attached to the rectal wall in such numbers and so closely crowded together that they resemble a furry lining or pile on it. Seen in profile they appear in serried ranks, each in contact with its neighbours; seen in surface view they present the appearance of a honeycomb, each flagellate in optical transverse section, showing outlines nearly polygonal as the result of mutual pressure. In the living condition the attached

crithidias are motionless for the most part, but occasionally a given individual performs a kind of jerky nutating movement; the body, remaining attached, sways rapidly from one side to the other, with a slight curvature of the axis. After bending, first to one side and then to the other, in this way the animal remains quiescent for a time, but when there are a large number of the flagellates, there is scarcely a moment in which one or another, or several at once here and there, are not performing these movements.

In addition to the attached forms, with the flagellum for the most part very short or wanting altogether, there are usually a certain number of free forms. Some of these are crithidial in form, with the undulating membrane feebly developed and scarcely, if at all, recognisable, and with a distinct free flagellum, often quite long. The function of these flagellated forms appears to be that of migrating in order to colonize other parts of the wall of the gut. When a clump of attached forms has been multiplying actively at one spot it doubtless tends to become overcrowded, at least in its central part. Then probably one of two things may happen; some of the crithidias may become transformed into the final trypanosome-form and detached from the clump; or a certain number will develop flagella, remaining crithidial in type, migrate to another part, and attach themselves again.[1]

Besides the crithidial forms with long flagellum there can be seen free forms, also with a flagellum of variable length, but with a distinct undulating membrane running along the whole or greater part of the length of the body, which appears more flexible and performs sinuous undulatory movements more or less distinctly. These forms are the little trypanosomes, or transitional stages in their development, the forms which constitute the final infective stage of

[1] One of us has seen in the living rectum a crithidia with a long flagellum become detached from the rectal wall, swim actively across the rectal cavity, with its flagellum directed forwards, and attach itself again to the wall on the side opposite to its former attachment.

the cycle in the flea (so-called "metacyclical trypanosomes" of Brumpt).

We can thus distinguish in the rectum of the flea three principal types of individuals, each of which varies considerably in size and details of form, and between which are to be found every possible transition, namely:

(1) The attached or haptomonad[1] form, which is the multiplicative stage of the rectal development.

(2) The free or nectomonad[1] form.

(3) The final trypanosome-form.

The many variations of these three types and the transitions between them cause the rectal phase of the development to present a variety of form which is at first very bewildering, but which becomes easily intelligible if the classification into the three types given above be used as the key. It may be noted here that it is very common, in the development of other trypanosomes, for the crithidial phase to exhibit great variation in size, form, and structure. Compare the works of Chagas on the development of T. cruzi in the bug, and of Miss Robertson (1911, etc.) on the development of the trypanosomes of fishes in leeches.

We will now proceed to describe the variations of these types and the transitions between them in more detail.

(1) The haptomonad or attached type is the phase in which multiplication by binary fission takes place; consequently its variations of form are related mainly to the function of multiplication. Leaving out the forms which are actually in process of division, we find that chief variations in form are seen: first, with regard to the shape of the body, whether relatively slender, with the hinder end sharply or bluntly

[1] Woodcock (1914) has proposed the useful term "haptomonad" for the attached phase of a Crithidia or Leptomonas, the so-called "gregariniform" individuals of Léger, but of which the resemblance to a gregarine is not very striking, not at least in the developmental phases of Trypanosoma lewisi. To denote the locomotor crithidias with long flagella we propose the term "nectomonad," i. e. swimming monad, as a correlative to haptomonad, fixed monad.

pointed, or stouter, with rounded hinder end, or finally ovoid or even globular in form; secondly, with regard to the extent to which the flagellum is developed.

The typical haptomonad, when not preparing for division, has the body spindle-shaped or pear-shaped with the thickest part anteriorly in front of the nuclei, and the hinder end more or less sharply pointed (Pl. 41, figs. 174, 176, 193; Pl. 42, fig. 252). The two nuclei are usually close together, situated either about the middle of the body or nearer to the hinder end; *n* is either just in front of *N* or close beside it. The cytoplasm has a great tendency to stain very dark by any method, especially towards the hinder end. After Giemsa the body has a purplish-blue tinge; after Twort's combination of neutral red and Lichtgrün it is seen to be full of very fine granules, stained red and scattered irregularly (Pl. 38, figs. 260*a*–263*a*); from these reactions it is evident that the opacity of the body is due to the deposition in the cytoplasm of very fine "chromatoid" grains, probably of the nature of volutin. The cytoplasm is usually free from coarse granulations, which are, however, present occasionally.

In preparation for division the hinder end of the body begins to swell and to become rounded at the hinder end, while the nuclei are shifted more posteriorly. In consequence the body becomes pear-shaped, but in the opposite manner to that previously described, since now the thickest part of the pear is the posterior end, while the anterior extremity of the body is narrowed, with the flagellum representing, as it were, the stalk of the pear (Pl. 41, figs. 168, 188; Pl. 42, figs. 231, 247–249). In other cases the body becomes evenly ovoid or even globular in form (Pl. 42, figs. 243–247).

The process of division calls for no special remark (see Pl. 42, figs. 213, 214, 226–230, 249, 253, 266). The blepharoplast or basal granule of the flagellum divides first, one of the daughter-blepharoplasts retaining the old flagellum attached to it, while the root of the new flagellum begins to grow out from the other daughter-blepharoplast (Pl. 42, fig. 252, lowest specimen). Following the blepharoplast, *n* divides

next and after that *N*. Next the body is constricted into two, beginning from the anterior or flagellar end (Pl. 42, fig. 253). We have never seen any but binary fission of the crithidial forms; multiple fission does not occur in any form at this stage. We have also sought without success for multiplication by endogenous budding (the so-called "infective granules" of Balfour and others). In Leptomonas pattoni of the flea we have found very clear instances of apparent endogenous budding (Pl. 42, figs. 281, 282–284), and Pl. 42, figs. 268, 269 are rather suggestive of a similar process occurring in the haptomonads of T. lewisi, but further proof of it is lacking.

The flagellum in haptomonad forms is extremely short, and as a rule is reduced practically to its intra-cellular root or rhizoplast, the projection beyond the limits of the cell-body being very slight or quite imperceptible. At the point where the rhizoplast comes to the surface at the anterior apex of the body there is usually a fairly large and distinct, but often ill-defined patch of substance which stains like the flagellum, that is to say, red, after Giemsa's stain, black or greyish-black after iron-hæmatoxylin, and green with Twort's stain, and which appears to represent a secretion produced by the flagellate, a sort of cement by which the animal adheres to the wall of the rectum (Pl. 41, figs. 174, 178; Pl. 42, figs. 223, 226, 243–245, 260, 262, etc.).

In many cases the rhizoplast fails to reach the surface of the body, or may even, very exceptionally, be absent altogether (Pl. 42, figs. 241–243, 267). The body is then always ovoid or globular in form. In such cases we have true leishmanial forms, which appear to owe their origin to very rapid multiplication of the ordinary haptomonad type; multiplication is so rapid that one of the two daughter-individuals resulting from binary fission has no time to form completely its new flagellum or even the rhizoplast, before being split off from its twin sister and beginning to divide again. This view receives support from the extremely small size which is commonly a feature of these leishmanial forms. In no case have we seen

anything that could be interpreted as encystation or encapsulation of the leishmanial forms; they appear to represent a purely trophic and multiplicative phase.

The haptomonad forms occur usually, as has been said, attached to the cuticle lining the rectum; in surface views of the rectal wall, living or preserved, they may be seen attached singly, in clumps, or in a continuous carpet-like layer, and exactly the same is found in microtome-sections of preserved recta (Pl. 44, fig. 317; Pl. 42, fig. 277). In sections of a well-infected rectum the haptomonad forms are seen in a long, continuous line, like soldiers on parade. But, both in living and preserved recta, in film preparations or in sections, free clumps of haptomonads are also found, in which the individuals all have their flagella directed toward a certain point (Pl. 42, fig. 274). In spite of careful scrutiny it is not possible to detect any body or particle of débris at the centre to which the monads are attached; they appear to adhere simply to one another by their flagellar extremities. The question at once arises whether these free clumps are a natural or an artificial condition. If they were only seen in teased-up recta one could have hesitation in ascribing their detachment to the manipulation, but they are found also in sections of recta. It is, of course, impossible to dissect out and preserve a rectum without subjecting it to great stresses and strains which might detach the monads, but it is remarkable how tenaciously they adhere to the wall. In one of our series of sections of a rectum it can be seen that it has been badly torn in getting it out; part of the torn wall has curled right back and turned inside out. The tear goes right through an attached carpet of crithidias which have nevertheless remained adherent to the wall, even on the part that has curled back, giving at a first glance the erroneous impression that the monads are attached to the exterior of the rectum. They must, therefore, be attached very firmly to the wall, which is intelligible when it is recognised that the crithidial forms are not the ripe, propagative stages of the cycle and that if they were carried to the exterior with the fæces they would be lost. For this

reason alone it seems highly probable that the free clumps of crithidial forms represent either clumps artificially detached by manipulation, or an abnormal condition of the flagellates detrimental to their future welfare.[1]

As transitions between the nectomonad and haptomonad phase we would expect to find both stages of the development of the nectomonad into the haptomonad, and stages of the development in the reverse direction. It is of course almost impossible to say, by simple inspection of a transitional form in a permanent preparation, in which direction it is developing. We are inclined, however, to interpret as transitions from the nectomonad to the haptomonad the more slender forms, with short flagella and hinder ends pointed or but slightly blunted, such as Pl. 41, figs. 175, 176; Pl. 42, figs. 220, 252; and as transitions in the opposite directions the rounded or broad pear-shaped forms with flagella of various lengths, such as Pl. 41, fig. 166, Pl. 42, figs. 231–234, 251, 265. In some forms of the latter type the distal ends of the flagella are very thin, much thinner and more delicate than the proximal portions (Pl. 42, figs. 246, 250, 268, 269), and we interpret this appearance as indicating that the flagellum is in process of growth rather than of regression in length, for the reason that a similar condition is seen in the flagella of forms transitional to the final trypanosome-type (Pl. 42, figs. 255–257), in which the flagellum is not likely to be in process of shortening.

In the series which we interpret as transitional from the haptomonad to the nectomonad type we find globular forms with flagella of considerable length (Pl. 41, fig. 166; Pl. 42, fig. 234), and, since we have not observed such forms swimming freely in the rectum, we conclude that the haptomonad, while attached, first develops its flagellum to a considerable length, and then acquires the elongated form of body, before becoming detached from the wall and set free.

[1] Comparable, for example, to the sponge-larvæ, which attach themselves to the surface-film of the water instead of becoming fixed to a firm object, and which in consequence perish inevitably.—E. A. M.

(2) The nectomonad or free type of crithidial flagellate has a more slender body, in its fully-developed form about five times or more as long as its greatest breadth, but with great variations in its relative proportions (Pl. 41, figs. 190, 194–197; Pl. 42, figs. 217, 235, 236, 254). The body is usually spindle-shaped, pointed at both ends, its thickest part at the level of or slightly behind, the middle point of its length, and *n* is usually well in front of *N*. When it swims the flagellum, directed forwards, is thrown into even sinuous undulations which begin at the tip and run backwards, in contrast to the type of movement so often seen in free-living flagellates, in which the proximal two-thirds, or so, of the flagellum is held stiff and straight, while the distal third performs lashing movements which drag the body forward. We have not found the fully-developed nectomonad type undergoing multiplication by fission, unless Pl. 42, figs. 253 and 266 are to be so interpreted.

(3) The final trypanosome-form appears to rise in most cases from the haptomonad type, with which it is usually found closely associated in preparations; compare Pl. 41, fig. 202, of a section through the intestine close behind the pylorus; the trypanosomes are seen with their posterior ends projecting above the level of the serried ranks of the haptomonad crithidias, as if they were pushed upwards by the development and growth of their flagella. It is possible, however, that the final forms may sometimes arise from the nectomonad type, and that such an origin explains the occurrence of the slender forms of the trypanosomes, the stout forms being derived from the haptomonads. Forms such as Pl. 42, fig. 237, are perhaps to be interpreted as transitional from the nectomonad type to the final trypanosome-form.

The essential feature in the origin of the final form from the crithidial form, of whatever type, is the transposition of the two nuclei, *n*, and *N*. Both nuclei move backwards usually, but *N* only for a short distance, while *n* passes *N* and goes to the posterior extremity of the body (Pl. 42, figs. 238, 239,

255–259, 270). In some cases, especially in the slender forms, *n* stops short of the extreme posterior end of the body (fig. 239), but in the stumpy forms *n* becomes quite terminal in position, as a rule (Pl. 41, figs. 199, 200; Pl. 42, figs. 259, 271). Further characteristic of the final stage is the relatively large size of both *n* and *N*, and the faint stain that *N* usually takes in the permanent preparations. In many cases *N* appears distinctly elongated in the longitudinal direction (figs. 199, 259). With the displacement backwards of *n* and of the attachment and origin of the flagellum, the undulating membrane becomes correspondingly extended and lengthened.

The occurrence of stout and slender forms of the final trypanosomes has been mentioned already, and was pointed out by Swellengrebel and Strickland (1910); but it is a fact somewhat difficult to explain. It may be, as already suggested, that it is simply due to difference of origin, the slender forms arising from the nectomonads, the stout forms from the haptomonads. On the other hand, it may be that the trypanosomes, when ingested by the rat, become exceedingly active in order to find their way from the digestive tract into the blood, and that the slender forms in the flea represent merely the precocious assumption of a type of structure which belongs strictly to a later period of the life-cycle. These are the only suggestions we can offer at present in explanation of the two forms.

We have never in any case seen the final trypanosome-form dividing, but it is stated to do so by Swellengrebel and Strickland (1910), who, after having examined one batch of thirty-seven infected fleas, have been able to figure no less than three examples of a process of division that we have never been able to find in all the many hundreds of infected fleas we have dissected and examined. For our part we agree with Brumpt (1913), that these "metacyclical trypanosomes," as he proposes to call them, are "phases d'attente" which do not multiply further in the flea.

With the development of the final trypanosome-form the

cycle of T. lewisi in the flea is ended. It only remains to say a few words with regard to the occurrence of the rectal phase in regions of the gut situated further forwards than the rectum. It is by no means an infrequent occurrence to find clumps and carpets of various forms characteristic of the rectal phase attached in the intestine and even in the stomach.[1] In the intestine they occur most frequently at the upper end, close behind the pylorus. When they occur in the stomach they are probably always attached towards its hinder end, near the pylorus. Hence the two chief situations of the crithidial forms, when occurring outside the rectum, may be designated briefly "pre-pyloric" and "post-pyloric."

Two possibilities present themselves at once to the mind with reference to these extra-rectal crithidial infections; first, that the infection of the stomach or intestine is a direct one, brought about by forms which have attached themselves there immediately after completing their stomach-phase, without having ever travelled further back in the digestive tract; secondly, that the infection has been brought about in an indirect manner by forms which have migrated forwards from the rectum.

So far as post-pyloric intestinal infections are concerned, we have some evidence that the infection may be sometimes a direct one; in one of our series of sections of the stomach of a flea that had fed thirty-six hours previously to being preserved, there are two large clumps of crithidial forms attached close behind the pylorus. Probably in such cases those attached in the intestine represent but a small numerical proportion of those that migrated backwards from the stomach, the majority having passed on down to the rectum, while a few have stuck, as it were, higher up.

With regard, however, to the pre-pyloric crithidial infections, we have no evidence of direct infection taking place, but all our data indicate that such infections of the stomach are

[1] Since a certain length of intestine was usually cut off with the stomach it is possible that many of the crithidial forms found by us in our stomach-films were really post-pyloric in situation.

brought about indirectly, and the same is probably true, in most cases, of the post-pyloric infections of the intestine. In the first place we have no record of the occurrence of crithidial infections in the stomach (pre-pyloric) earlier than seven days after the first infective feed of the fleas; but at later periods than this we have so many records of such infections in the stomach that, had they been in all cases brought about directly, we should have expected to have found crithidial forms in the stomach during the period when such forms are being established, that is to say, from about thirty-six hours and five days or so, which we have never done. Secondly, the evidence furnished by experiment 39 (see below, p. 634), indicates very strongly that the final infective forms of the life-cycle were first produced in the rectum on the fifth day and were there also on the seventh day, but had migrated forwards to the stomach on the tenth day.

It seems, therefore, most probable that in the majority of cases at least, the pre-pyloric and even the post-pyloric infections are the secondary results of a migration forward from the rectum of crithidial forms previously established there; and since neither the haptomonads nor the final trypanosome-forms appear capable of undertaking such migrations, it must be the nectomonads, which are obviously active locomotor forms, that are responsible for such migration. We have performed some experiments from which it is clear that the migration forwards is dependent on conditions of nutrition in the flea and that starvation favours a forward migration of the nectomonads towards the stomach.

In many cases the forwards migration of the flagellates leads to the rectum being quite deserted by them. This is well shown by the following instance, by no means an isolated one of its kind in our experience, but very typical. A flea was taken from the infected breeding-cage and put by itself on a clean rat for three days, from the 19th to the 22nd of September; it was then recovered and dissected. The stomach-preparations were found to contain a considerable infection of the typical rectal phase (Text-fig. 17, p. 627),

but no flagellates of any kind were found in the rectum. The rat became infected, and first showed trypanosomes in its blood on September 28th. The age of the infection of the flea was not known, but the crithidial stock seems in this case to have died out in the rectum and to have established itself exclusively in the pyloric region.

We have also, though rarely, seen the attached crithidial form in the proximal portions of the Malpighian tubules.

On the other hand we have never seen in our rat-fleas (Ceratophyllus fasciatus) infections such as are described by Nöller (1912), and Wenyon (1913), in the dog-flea, where both the rectum and the intestine are described as being carpeted along their whole extent with the crithidial phase; though we have seen such infections in fleas harbouring the Leptomonas. It is a fact which seems at first strange, but is probably very significant, that, as we have pointed out elsewhere (p. 610), the rat-flea is not so efficient a host for the rat-trypanosome as other species of fleas which do not usually or of choice feed upon rats; from which circumstance it would appear as if the rat-flea has acquired a certain degree of natural immunity to the trypanosome of the rat which other fleas do not possess.

For a general summary of the development of Trypanosoma lewisi in the flea, see Plate 45, and the description of it (p. 691).

(3) The Degenerative Series.

Trypanosomes undergoing degenerative changes may be found in either the stomach or rectum during the first few days after the flea has fed for the first time on an infected rat. They are most abundant in batches of fleas examined during the first twenty-four hours after feeding. After this period trypanosomes may have disappeared altogether from the gut of the flea, and after thirty-six hours degenerative forms are of infrequent occurrence. In some cases, however, degenerative forms may be found in the rectum much later

than the first day, namely, up to three, four, or even five days after the infective feed. The degenerative forms of late occurrence are probably to be interpreted as individuals which have become degenerative after having developed in a normal manner for a longer or shorter period. The trypanosomes which begin to degenerate immediately after being ingested by the flea probably do not last long beyond twenty-four or thirty-six hours, usually not so long. Fleas that have fed on an infected rat whose blood is swarming with trypanosomes often show no trace of the parasites in any part of the gut by twenty-four hours. The majority of the degenerative trypanosomes that are found in the fleas are those that begin to degenerate immediately after being taken up from the rat.

There is no essential difference between the degenerative forms found in the stomach and the rectum. We may, therefore, give a general description of the forms of the degenerative series without taking special note of their provenance.

In direct contrast to the changes undergone by the developmental forms in the stomach, the principal sign of degeneration is a progressive diminution in size, more especially in the length of the body. The trypanosome gradually dwindles and wastes away, beginning at the flagellar end, during which process the flagellum becomes converted progressively into a fluffy mass, which frequently shows a tendency to stain blue or bluish with the Giemsa stain, instead of the normal red (Pl. 43, figs. 294–296, 308). Meanwhile *N* is pushed backwards towards *n*. The displacement of *N* does not appear to be due to any active migration on its part, but to be the purely passive consequence of the dwindling of the anterior part of the body, whereby it is forced backwards. On the other hand *n* shows no tendency whatever to move forwards, but may do one of two things: it may remain where it is, or be shifted backwards only to a slight extent, in which case the hinder end of the body retains the sharp point characteristic of the trypanosome in the blood (Pl. 43, fig. 301); or it may pass back towards the extreme posterior end, and

even become terminal in position, in which case the hinder end becomes bluntly pointed or even rounded (Figs. 290, 291, 308). If at the same time the body becomes broadened out posteriorly, as sometimes happens, the result is a form which may mimic very exactly the small stumpy trypanosome which is the final form of the development (Figs. 299, 306, 307).

The trypanosomes that undergo this process of degeneration show a great tendency to adhere together in clumps attaching themselves to one another by the tips of their flagella (Pl. 43, figs. 304, 308; Pl. 44, fig. 311). The adherence in this way of the degenerative forms must be distinguished clearly from the process of agglomeration which T. lewisi undergoes so readily when placed in unfavourable circumstances.[1] Agglomeration takes place by the hinder ends of the trypanosomes and more especially by their *nn*, as Laveran and Mesnil have shown, and as a result of it the trypanosomes tend to form rosette-like clusters, in which the flagella radiate outwards. True agglomeration of this kind can also occur in the flea under special circumstances, as will be described presently. But in the degenerative clusters the conditions are precisely the opposite to agglomeration, since the flagella are directed towards the centre of the cluster, while the hinder ends of the trypanosomes radiate outwards. The tendency of the degenerative forms to adhere in clumps must be interpreted as an expression of the general tendency (perhaps it might be termed instinct) of the trypanosome to attach itself by the tip of the flagellum to firm surfaces when in the body of the flea, a tendency very pronounced in all developmental forms, excluding the final stage of the cycle.

Clumps and masses of very considerable size are formed by the degenerative forms adhering together in the manner described. Towards the centre the clumps often show a cement-like substance, which stains pinkish-red with Giemsa. The final stages of the degeneration are small forms, which

[1] Manteuffel (1909) has already drawn attention to the distinction between rosettes, with flagella directed inwards, and true agglomeration.

represent simply the hinder ends of the original trypanosomes. They are usually sharply or bluntly pointed (Pl. 43, figs. 302–304), or may be rounded off (fig. 306). The large clumps of these little degenerative forms in the rectum are often very difficult to distinguish in the living state from the clumps of developmental crithidias. The degenerative clumps, however, generally occur loose in the cavity, while the true crithidias are attached to the wall of the rectum, though in the process of dissection the latter often become torn away from the wall. When a loose clump of this kind consists entirely of forms with pointed hinder ends it is probably degenerative. The true crithidial clumps always have a considerable number of forms with rounded hinder ends, especially in the early periods of the establishment of the rectal phase, when the hinder ends of the crithidial forms are almost always rounded. The degenerative forms, carefully examined, show a certain extent of undulating membrane running down the side of the body to *n*, which is situated behind *N*, while in the typical haptomonad phase the flagellum is reduced to the rhizoplast which comes off close to *n* and terminates at the surface of the body, *n* being situated beside or in front of *N*. Finally, it should be noted that the degenerative forms never multiply by division at any time. Nevertheless, in spite of all these distinctions, which are more easily perceived in permanent preparations than in the living state, it is sometimes difficult to pronounce decisively as to the nature of a given individual, whether degenerative or developmental, in preparations of the rectum; but as a rule there is no difficulty at all.

A remarkable fact is the occurrence of recurved forms amongst the degenerative forms in the rectum, of a type essentially similar to the recurved trypanosomes occurring in the normal developmental series in the stomach (Pl. 43, figs. 297, 298). The recurved forms are often seen in the clumps of degenerative trypanosomes. The occurrence of such forms in the rectum may perhaps be interpreted as an abortive effort on the part of the trypanosomes that have

passed on prematurely into the rectum to go through a development similar to that which they undergo normally in the stomach, but which, in all probability, would be impossible in the rectum, where the cuticular lining would doubtless be an effective bar to the penetration of the epithelial cells by the trypanosome. It is possible that some of the recurved forms in the stomach may also degenerate without ever succeeding in penetrating the cells; the curious forms such as Pl. 43, fig. 305, are very probably to be explained as recurved forms in process of degeneration. It would be difficult, however, as a rule, to distinguish between degenerative and developmental trypanosomes in the recurved condition in the stomach; but in the rectum all such recurved forms must be regarded as abortive and destined to degeneration.

As has also been mentioned above, trypanosomes of degenerative type are found in the rectum on the third and fourth days after infection. Such forms may, in some cases, differ but little from ordinary blood-trypanosomes, and are then to be interpreted, probably, as trypanosomes ingested at a later feed, which have passed on to the rectum; but in other cases they may be forms which are undergoing degeneration after having developed normally in the stomach. They are found, not infrequently, mixed with true developmental forms in clumps, into which they have probably intruded themselves (Pl. 41, fig. 183). It is necessary to be careful not to confuse them with early forms of the rectal phase in which n is still behind N; such forms can be distinguished by their greater stoutness and bulk, and by the fact that n has generally migrated forwards to some extent (Pl. 41, figs. 181–187).

True agglomeration very rarely occurs in the flea, but we have found it in its most typical form (Pl. 43, figs. 309, 310) in fleas of a batch, the record of which was as follows: The fleas, twelve in number, had been fed once on an infected rat in the usual way, and three days later they were fed again on a rat, the object being to test the influence of a second feed of clean blood on the persistence of the stomach-phase

(see p. 664 below). By mistake, however, the fleas were fed again on an infected rat instead of a clean rat. The next day (four days after the first infective feed) the fleas were dissected and examined. In every flea the trypanosomes of the second feed could be recognised, quite unaltered from the blood-form, and in most cases agglomerating in pairs, threes, or rosettes composed of many individuals; they were found in the stomach in every flea and in a few in the rectum also, where in one case degenerative forms were noted; in some fleas these trypanosomes were very numerous; in others they were scanty and had evidently undergone reduction in number. The trypanosomes of the first feed had disappeared in nine out of the twelve fleas, while in the remaining three they were represented by developmental forms of the usual type in the rectum. Agglomerating trypanosomes of the second feed were found both in fleas in which those of the first feed had persisted and in fleas in which they had disappeared.

From this observation it would appear that when a flea has once had an infective feed, its digestive tract, and more especially its stomach, acquires properties which cause the agglomeration and probably also the degeneration of trypanosomes taken in at later feeds, alike whether those ingested at the first feed have succeeded in establishing themselves in the flea or not.

Reference has already been made above (pp. 531–532) to intracellular forms which appear to be undergoing degeneration after having penetrated into an epithelial cell (Pl. 36, figs. 43–45).

Appendices to the Development.

(1) Previous Investigations on the Development of Trypanosoma lewisi.

The first who attempted to follow out the development of T. lewisi in its invertebrate host was Prowazek (1905), who studied the development in the rat-louse, Hæmatopinus spinulosus, and since those

who followed immediately after him in similar investigations also made use of the louse, it is simplest to deal first with all those works in which the development in the louse is studied. Since we have not ourselves studied the development of this insect, we are not in a position to controvert the statements made, but it is legitimate for us to compare the forms and stages described with those which we have found in the flea, and, on the ground of such comparisons, to criticise the interpretations given by the authors.

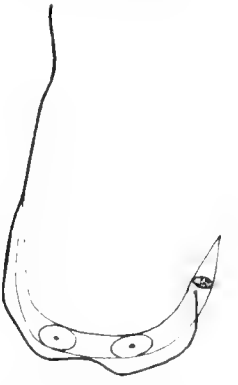
According to Prowazek, the general course of the development in the louse is as follows: The flagellates are first to be found in the stomach, where they do not collect at particular spots, but swim freely everywhere in the ingested blood. In the stomach the processes of maturation and fertilization take place. At the second feed of the louse the parasites are forced down to the end of the mid-gut and finally come to rest in the hind-gut, for the most part near the Malpighian tubules, but also in other parts. Resting stages are to be found on or between the cells of the mid-gut, and more especially at the beginning of the hind-gut. From the fact that the parasites disappear from the hind-gut, it is inferred that they can pass through the epithelium of the hind-gut. [This conclusion is by no means warranted by the observation on which it is founded; it is also, in our opinion, extremely improbable that the flagellates could penetrate through the chitinous cuticle lining the hind-gut.] In this way the parasites are stated to pass into the blood-stream, then into the larynx [sic], and so finally back into the vertebrate host when the louse feeds. [It is not clear whether this statement is founded on observation, or simply on the analogy of the statements made by Schaudinn with regard to Trypanosoma noctuæ; the author never succeeded in obtaining an infection of the rat by means of the louse.] No trypanosomes or their resting stages were found in freshly-deposited fæces.

The author describes in great detail, with figures, various appearances interpreted by him as maturation, fertilisation, and even parthenogenesis. As all this part of the work is in the highest degree unconvincing and appears to consist of forced theoretical interpretations of degenerating forms which were occasionally seen to undergo agglomeration, it is not necessary to do more than refer to the figures given by Prowazek. Pl. II, fig, 32, purports to show the "fertilisation" in the living, and Pl. III, figs. 38 and 39, in the stained condition, while fig. 40 represents the "ookinete," a non-flagellated form with a single nucleus. From the ookinete a crithidial form is stated to arise in the manner described by Schaudinn (Pl. III, figs. 41–43 and 45). An active multiplication follows [but Pl. III, fig. 55, which is given as an example of the division, is simply an unaltered blood-trypanosome which shows the commonly-occurring abnormality of possessing two *NN*; compare our Text-fig. 15].

The author appears to regard most of the crithidias as involution-forms, which form "agglomeration-stars." Such a star is shown in Pl. III fig. 54 [which represents a typical clump of normal developmental crithidias, similar to our Pl. 41, figs. 182–184]. In addition to the crithidial involution-forms there are found other much smaller forms, wedged in between the cells and with the flagella completely absorbed. The crithidial involution-forms may also degenerate into small non-flagellated forms (fig. 50). No special inoculative or final form of the development is described.

Baldrey (1909) professes to have confirmed the development described by Prowazek, including even the process of maturation and fertilisation.

Text-fig. 15.



Trypanosome with two *NN* from the stomach of a flea eighteen hours after the infective feed. Such forms with two and even three *NN* are quite common in the blood of the rat and in the gut of the flea at early stages of the development; compare Minchin (1909), p. 803, Pl. 21, fig. 6, Pl. 22, fig. 74, and Pl. 23, fig. 84; they have nothing to do with reproduction of the trypanosome by fission. (× 2000.)

He gives two text-figures, one showing "male" and "female" forms and "copulation," the other showing "ookinetes" and crithidial forms of the typical nectomonad type. He states that the ookinete reconstructs a flagellar apparatus and divides rapidly to produce crithidial forms, which, by repeated division become smaller and smaller, pass into the body-cavity, thence to the suctorial mouth-apparatus and so infect the rat. The complete cycle takes from eight to ten days.

Rodenwaldt (1909) studied the development of T. lewisi in the louse in order to meet the criticisms of Patton, and succeeded incidentally in proving that Patton's "Crithidia hæmatopini" is a mythical and non-existent species. On the first day of the development he found in the louse both unaltered forms of T. lewisi and long forms,

which he described as "Lanzettformen." [The latter, from the figures given (Pl. 1, figs. 7–12) are clearly the same as our long "crithidiomorphic" stomach-forms.] He also found trypanosomes alleged to be dividing (figs. 5, 6, and 12) [but these, again, are simply forms with two or three *NN*, such as occur frequently in the blood of the rat; see above]. On the second and third days he found the same forms, but a larger proportion of the Lanzettformen. In one louse, however, he found crithidial forms developed on the third day. On the fourth day he found forms with a short flagellum or none at all (as shown in his figs. 13, 14), which he compares with the ookinetes of Prowazek and Baldrey; they are stated to bend their bodies without changing their place. On the fifth day long crithidial forms appear (compare his figs. 15–21), and on the sixth and following days smaller crithidial forms in rosettes, and also, but more rarely, leptomonad forms (figs. 23, 27). From the tenth day there were found (1) small non-flagellated forms (figs. 31–34); (2) stout flagellated forms (figs. 35–39); (3) a few stout, non-flagellated forms, "ookinetes" (figs. 40–47); and also forms regarded as representing copulation of gametes (figs. 48, 49). After twenty days slender, sporozoite-like forms were found (figs. 52–58) in the gut, never in the body-cavity. Rodenwaldt did not succeed in producing infection by means of lice.

Breinl and Hindle (1909) describe the development in the louse mainly as follows. The ingested trypanosomes first show characteristic changes in the nucleus, of which the karyosome divides and the division products move to opposite ends of the nucleus. *N* and *n* then become approximated and a division takes place. Some of the trypanosomes show about this stage a reduction of the cytoplasm, producing tadpole-like forms with a swollen head and the rest of the body reduced to a long flagellum (see their figs. 5–9); at this stage the two nuclei take on the crithidial ("Herpetomonas-like") arrangement. The crithidiæ multiply by division and become "agglomerated in great clusters with the flagellum always directed inwardly" (fig. 18). [These clusters appear to be simply developmental clumps of crithidial forms; as pointed out above, clumps with the flagella directed inwards, whether of degenerative or developmental forms, are not instances of true agglomeration.] Round forms [haptomonads?] are also found (figs. 32–36). The alleged conjugation was not confirmed.

[We cannot help remarking that all the stages of the trypanosome figured by Breinl and Hindle, even the crithidial clumps, present an extraordinarily sickly and degenerative appearance; we venture to think that anyone who compares their figures with ours will agree to this statement.]

Swellengrebel and Strickland (1910), have had the advantage over previous authors that they were able to compare the stages in the louse

with those occurring in the flea; their memoir is illustrated by numerous figures, for which, however, they appear not to claim great exactness, since they refer to them as "diagrams." They find that "the development in the louse is a very irregular one and is not to be compared with that which takes place in the flea." The first changes are that *n* wanders in the direction of *N*, producing finally a crithidial form, slender or club-shaped (diagrams xv and xvi). From the crithidial forms arise large "ovals," some of them without flagella and representing the "ookinetes" of former authors. Later ovals [haptomonads] and crithidiæ [nectomonads] are found singly or in clumps (diagrams xvii and xviii). Degenerative forms were also seen, but no flagellates were found which could be identified with this small, final trypanosome-forms of the development in the flea. No conjugation was observed.

From all these various works, only one positive fact emerges clearly, namely, that T. lewisi can develop in the louse into its typical crithidial phase, with both nectomonads and haptomonads. On the other hand, no intracellular multiplication has been observed, nor has it been proved as yet that the development can proceed so far as to produce the small trypanosomes which end the cycle in the flea. One form, apparently degenerative, occurs in the louse which we have not found in the flea, namely, a large oval form without a flagellum, the zygote or "ookinete" of Prowazek and others.

The first published works on the development of T. lewisi in the flea were those of Swellengrebel and Strickland (1910). We have already noticed above their statements with regard to the development of the stomach-phase and stated that we are quite unable to agree with the account given by them. On the third day of the development they find both long crithidial forms and large "ovals" [stout crithidias of the haptomonad type] in the mid-gut (diagram v). On the fourth day they state that the flagellates had all passed out of the stomach into the intestine. On the fifth day they found only round forms [haptomonads] in the rectum (diagram vii). On subsequent days they found the various forms of the rectal phase, and on the eighth day they found the small trypanosome-forms, the final stage of the development, which the authors were the first to discover. In their diagram xvi, they give a summary of the development showing the following sequence of forms: (1) the normal blood-trypanosomes; (2) a long crithidial form; (3) a stumpy crithidial form with short flagellum; (4) a haptomonad form; (5) the same in process of division; (6) a form transitional to—; (7) a nectomonad; (8) a form transitional to—; (9) the final trypanosome-form. [In view, however, of the great differences seen in the development of the trypanosomes in different fleas, especially prior to the establishment of the rectal phase, it was somewhat rash to attempt to fix the order of events in so few as eighty-three fleas, and it may be

remarked that the entire stomach-phase has practically been omitted from the cycle as summarised by the authors.]

Swingle (1911) gave the following description of the cycle in the flea. He states that the trypanosomes remain but a short time in the stomach, but migrate to the intestine where important changes take place. The first change to be seen is a diminution in size, and at the same time *N* moves towards the posterior end of the body. Occasionally such forms degenerate; in those that do not *n* moves forwards till it is close beside or in front of *N*, thus producing a true crithidial form. The individuals which do not change into the crithidial type curl upon themselves to form an oval rounded mass (figs. 15, 16) [apparently representing recurved forms]. Development of the crithidial forms may proceed along two separate lines which come to the same end; (1) they may "agglutinate" by the anterior ends forming rosettes (figs. 20, 21 [representing typical early crithidial clumps]); or (2) they may form solitary cysts (figs. 22–30) [apparently representing typical examples of the degenerative series]. Other forms [apparently degenerative] are also described; but the haptomonad and other forms of the rectal phase are all referred by the author to the form-series of the leptomonad described by him as Herpetomonas pattoni; a conclusion which Swellengrebel and Strickland (1911–12), justly criticise, though they go too far in the opposite direction in suggesting that H. pattoni is a stage in the development of T. lewisi.

Nöller (1912), studying the development of T. lewisi in the dog-flea (Ctenocephalus canis), confirmed our discovery of the intracellular multiplication in the stomach and added some further details; he observed the penetration of a trypanosome into a cell five hours and fifty-five minutes after the ingested blood had been ingested by the flea and states that the trypanosomes go through at least two generations, probably more, of intracellular multiplication. Whether or not the trypanosomes establish a normal infection in the flea depends, in Nöller's opinion, upon whether they succeed in fixing themselves in the intestine or rectum, or not. As regards the multiplication of the attached forms in the end-gut, Nöller finds that they can always be distinguished from the leptomonads by the possession of a typical undulating membrane and by undergoing a process of multiple fission; neither of these statements accord in the least with our experience of the development of T. lewisi in Ceratophyllus fasciatus.

It remains to mention that Swellengrebel and Strickland (1910) made some observations on the development of T. lewisi in Ornithodoros moubata and Cimex lectularius. In the former they got no development of crithidial forms; in the latter they found large crithidias but no development in the hinder part of the mid-gut.

(2) On the Possibility of the Occurrence of Sexual Phenomena in T. lewisi.

It has been seen from the foregoing summary of previous investigations on the development of T. lewisi that Prowazek first, and after him Baldrey, Rodenwaldt, and Gonder, asserted that the development of T. lewisi in the louse begins with a process of fertilisation, of which the main features are stated to be as follows: Slender male and stout female forms of the trypanosome are differentiated; their nuclei go through a process of maturation and reduction, after which a fusion of the gametes takes place. The zygote is described as an "ookinete" of elongated, oval form, with no flagellar apparatus and with a single nucleus (synkaryon). The nucleus is then stated to divide into two by a heteropolar mitosis to produce the two nuclei of a trypanosome *n* and *N*, and then the locomotor apparatus, flagellum and undulating membrane, are formed. The result is a flagellate of crithidial structure, which proceeds to multiply actively by binary fission.

It must be remarked here that Prowazek's account of the "ookinete" and its development in T. lewisi was modelled in every essential detail on the account given by Schaudinn for Trypanosoma noctuæ. The fertilisation observed by Schaudinn, however, was not that of a trypanosome, but of Hæmoproteus (Halteridium). It is a process of true fertilisation, which was first observed in vitro by Macallum, and its occurrence is not open to doubt. Schaudinn differed from all previous investigators in asserting that the ookinete (zygote) of Hæmoproteus became converted into a crithidial flagellate, a statement which has never been confirmed, and seems never likely to be. There can be little doubt at the present time that in linking together the development of Hæmoproteus noctuæ and of Trypanosoma noctuæ into a single life-cycle Schaudinn fell into error. Prowazek, on the other hand, derived his "ookinete" in T. lewisi from the sexual union and fusion of two trypanosomes, so that in its alleged origin the ookinete of T. lewisi is of quite different nature from that of Hæmoproteus noctuæ. Prowazek is, therefore, the first investigator who claims to have seen sexual conjugation of trypanosomes in the invertebrate host.

Later investigators of the development of T. lewisi in the louse have not confirmed Prowazek's statements as regards the sexual phase, nor has anything similar been found in the flea. Those who have investigated the development of other trypanosomes in their invertebrate hosts have also failed altogether to observe sexual phases or sexual behaviour, in spite of much careful searching for phenomena to which their attention has been strongly directed. As stated above, Prowazek's account of the sexual processes is most unconvincing, and the data he

brings forward are quite inadequate to support the superstructure of theoretical interpretation built upon them. In short, the question of sexuality in trypanosomes may be summed up in the words of Miss Robertson (1912, p. 247): "There is at present no sound evidence of conjugation in any trypanosome life-cycle so far worked out."

We have ourselves searched most carefully, but in vain, for sexual phases and syngamy in the development of T. lewisi. As stated above (p. 519), we found in one flea long crithidial forms adhering in couples in a manner very suggestive of true sexual behaviour, and believed that we had observed true syngamy. We were never able, however, to confirm this observation or carry it any further, and we are now convinced that the phenomena observed on that occasion were simply processes of agglomeration of abnormal forms of the trypanosomes in a malformed flea. When we discovered the stomach-phase we thought it very probable that the sexual processes might take place in this part of the developmental cycle, and we were inclined to interpret as evidence of sexual union some of those stages with two *nn* and two *NN*, such as Pl. 36, figs. 19–23, which are certainly at first sight very suggestive of the fusion of two trypanosomes. We have no evidence, however, of any subsequent fusion of the nuclei, nor of any antecedent processes of nuclear reduction such as should be the preliminary to the process of syngamy. We are not able to arrange the figures of these stages in any series which would suggest a sexual process. In short we are not able to interpret these forms as anything but early stages of the multiplication of the trypanosome.

On the other hand, it has been shown convincingly that the cycle in the invertebrate host effects a marked change in the properties or idiosyncrasies of the trypanosomes that have undergone it. Gonder showed that an arsenic-resistant strain of T. lewisi remained arsenic-resistant so long as it was transmitted from rat to rat by direct inoculation, but lost that property when transmitted by the louse. Miss Robertson (1912) also found that strains of T. gambiense became changed in character when transmitted through the tsetse-fly, and remarks: "It seems clear that the cycle in the fly as a whole, whether conjugation actually occurs or not, has much of the biological significance of the process."

Those who believe that trypanosomes pass through sexual phases in their invertebrate host will be inclined to ascribe the changes in the properties of the parasite to the effects of the sexual process. At the present time it is not possible either to affirm or to deny, with certainty, that sexual processes occur. All that can be said with any approach to verisimilitude is that the change appears to be connected in some way with the metamorphosis of the trypanosome and its passage through a crithidial stage; but proof is lacking that the crithidial stage follows

upon, and is the product of, a sexual process. Attention may be drawn here to another possibility already indicated above, namely, that the crithidial phase may be initiated by a differentiating division into two inequipotential products, one of which is destined to be eliminated sooner or later from the direct line of the life-cycle. If this supposition is correct a possible explanation might be afforded for the renovating effects of the invertebrate cycle. In the present state of knowledge, however, such an explanation must remain hypothetical, and lacking objective foundation.

PART III.—EXPERIMENTAL STUDY OF THE PROBLEMS OF THE TRANSMISSION AND DEVELOPMENT.

(1) Introduction.

Throughout our investigation of the relations of Trypanosoma lewisi to the flea, we have endeavoured, as far as possible, to make experiment and observation go hand in hand, employing the one method to check or throw light upon the results obtained by the other. In the following pages we set forth our results in a number of sections which arrange themselves naturally into two groups. One group (sections i–xv) embraces problems that deal with the complete cycle (including the passage of the propagative forms back into the rat) and with the establishment of T. lewisi in the flea, raising questions that are of general interest in the study of trypanosomiasis. The other group (sections xvi–xix) deals with some further problems that are of interest more especially in relation to the flea Ceratophyllus fasciatus and to T. lewisi itself under more or less special conditions in the flea. Each section is headed by a proposition which it is the object of the experiments cited to establish. If we consider that the proposition is proved satisfactorily by our experiments, it is put in the form of a positive or negative statement; if, on the other hand, the problem stands in need of further proof, the heading of the section is expressed in interrogative form.

The details of each experiment are given when it is cited,

but a few general remarks upon our methods may be made conveniently at this point. We kept going two breeding-cages of the type used by the Plague Commission (see 'Journal of Hygiene,' vi, Pl. iv). In one cage a clean rat was always kept to feed the fleas, in the other an infected rat; these two cages are designated, in the account of our experiments, the non-infected and the infected breeding-cage respectively. From the former we could always obtain a plentiful stock of clean fleas when required, while the latter furnished infective fleas. The rats used were almost always white rats bred in captivity; we found them as a rule docile and good-tempered so long as they were handled with the hands and not with forceps, and the operation of pricking their tails to obtain drops of blood, when required, did not arouse their resentment in the slightest. They live well in captivity, and were none the worse for being exposed to the fleas in the breeding-cages, provided the number of fleas was not allowed to become too great. Many of them suffered, however, from a troublesome itch, caused by a minute Acarine, which is very difficult to get rid of. One of our breeding-cages became over-run by rat-mites, rendering it necessary to destroy it and start a fresh one.

For our actual experiments we used in many cases, especially for experiments with small numbers of fleas, cages of special design in the form of a cylindrical tin-canister with the bottom closed in with tin, the top provided with a lid with a tightly-fitting rim. The canister was 10 in. high and 6 in. in diameter. The top of the lid was made of strong wire gauze, to prevent the rat jumping out, and over that muslin-gauze was pasted to prevent escape of fleas. After these cages had been in use for some time, however, they tended to become rusty on the inside and then the fleas could climb up the tin easily. Consequently, it was found more suitable to use inverted bell-jars, each about 15½ in. in height and 7 in. in diameter. The bell-jars were supported each on a wooden block, or several together in a wooden crate. The open upper end of the bell-jar had a zinc wire cover to

prevent the rat jumping out, but it was not necessary to take precautions against the fleas escaping, because they are unable either to jump so high or to crawl up the smooth glass if kept clean. The bell-jars were cleaned out thoroughly once a week.

The bell-jars were especially suited for experiments with single fleas or a small number of fleas. First of all clean saw-dust is put in the bell-jar to a depth of about 3 in., then the rat and the flea or fleas are put in. Since Ceratophyllus fasciatus is a flea which does not live permanently on the rat but only goes on to it for food, and lives naturally in rat-burrows, the fleas were generally to be found without difficulty in the saw-dust, when it was necessary to recover them, but sometimes they were on the rat itself. In the latter case the rat was held over a deep bowl of enamelled iron and the flea disturbed by blowing on to the fur of the rat, which has the effect of soon making the flea come to the surface of the fur. It was then captured, as a rule, by seizing it gently by finger and thumb, an operation which must be performed rapidly and deftly, otherwise it burrows down into the fur and must be dislodged again. Sometimes the flea drops off the rat and falls into the bowl, where it can be recaptured easily. Our assistant, Mr. George Kauffmann, became exceedingly expert at this job, and if a flea could not be found by him it was safe to assume that it had died or been eaten. In our earlier experiments we used chloroform for recovering the fleas, but later we abandoned this method, often fatal to the rats.

In some cases it was required to expose a large number of fleas—200 or so—to infection on an infected rat for a night or a day. For this purpose the bell-jar was also handy, but it was often found that the rat ate a great many of the fleas, sometimes as many as 100 or more in a single night. To prevent this a cylinder of wire gauze was made, of sufficient length to fit into the bell-jar in such a way that its two ends were closed by the glass wall of the jar, and of such a calibre as to allow the rat to walk forwards or backwards along it,

but not wide enough to permit the rat to turn round or use its paws freely, and consequently hindering it from catching and eating the fleas.

For the purpose of collecting large numbers of fleas from the breeding-cage the following method was found to be the simplest: Two glass capsules were used, each provided with a well-fitting lid, the one smaller, about $2\frac{1}{4}$ in. in diameter and $1\frac{1}{4}$ in. in height; the other larger, about 6 in. in diameter and 3 in. in height. First of all, débris from the breeding-cage containing fleas in all stages of their development is scooped up with the small capsule and the lid at once clapped on. Then the small capsule is placed in the large one; the lid of the small capsule is removed with one hand, and the lid of the large one put on with the other. The adult fleas in the small capsule then begin at once to jump out of it in every direction, and so fall into the enclosing large capsule, in which they soon collect on the side furthest from the light. When the fleas have swarmed out in this way the small capsule is removed and the débris contained in it is returned to the breeding-cage. The fleas in the large capsule can then be emptied through a glass funnel into a suitable receptacle, such as an Erlenmeyer flask. Or, if the large capsule be left to stand until all the fleas have congregated on the side furthest from the light, then by suddenly turning the capsule round through about 180°, so that the side which was furthest from the light is now the most illuminated, the fleas begin at once to move towards the opposite side; and if then the small capsule be held in their way they can be made to jump into it of their own accord, and they can thus very easily be counted and disposed of as required. Ceratophyllus fasciatus is not a very good jumper and its trajectory is low.

The fleas collected can be kept, if required, for a considerable time; we found the best method was to put a little clean white sand, moistened with two or three drops of water, at the bottom of a flask. The fleas burrow down into the sand and appear to live comfortably. If they are to be kept any length of time the sand must be moistened again every

two or three days. Like most blood-suckers, the flea can stand a prolonged fast.

(2) General Problems.

(i) Trypanosoma lewisi is transmitted from Rat to Rat by the Rat-flea, Ceratophyllus fasciatus.

It is not necessary that we should cite experiments specially to prove this proposition, since it is established by the experiments brought forward under the headings that follow, and it has been proved beyond all possibility of reasonable doubt by experiments already published by others as well as by ourselves.

The agency of fleas in the transmission of T. lewisi was first demonstrated by Rabinowitsch and Kempner (1899), who succeeded in infecting clean rats by intra-peritoneal injection of teased-up fleas (species not stated) which had previously been fed on infected rats. In these experiments the trypanosomes appeared in the blood of the rats in six to eight days after the injection. The authors state that they were not able to find any stages of the trypanosome in the flea-débris which was injected. They also obtained positive results by placing fleas, previously fed on infected rats, upon clean rats; the trypanosomes made their appearance in the blood of the rats after two to three weeks. Their experiments with lice gave negative results.

In spite of the experiments of Rabinowitsch and Kempner, the work of Prowazek (1905) on the development of T. lewisi in the rat-louse, Hæmatopinus spinulosus, led to this insect being regarded as the true host of the rat-trypanosome, and no more experiments with fleas appear to have been undertaken until those of Nuttall (1908), who obtained positive infections of rats with fleas, using both Ceratophyllus fasciatus and Ctenophthalmus agyrtes. Two years later we published accounts of a number of experiments, since when the rôle of the flea has been established beyond the necessity of further experiment upon the subject.

We have confined our experiments throughout solely to the common English rat-flea, Ceratophyllus fasciatus, but it has been shown by Nöller (1912) and Wenyon (1913) that the transmission can be effected by other species of fleas, namely, the dog-flea, Ctenocephalus canis; the human flea, Pulex irritans; and the Indian rat-flea, Xenopsylla cheopis. It is indeed noteworthy that other species of fleas appear to be more efficient as true hosts of the rat-trypanosome than the species which in this country occurs habitually

in association with rats, since Dr. Wenyon has informed us that in the fleas with which he experimented, the trypanosomes never failed to establish themselves and to go through their complete developmental cycle, while in Ceratophyllus fasciatus we found that only a small percentage of the fleas became infective (see below), and examination of the fleas showed that the trypanosomes establish themselves in a correspondingly small percentage (see p. 659). It would appear, therefore, that the flea which, more than any other species, is exposed in this country to infection by T. lewisi, has developed a certain degree of natural immunity to the parasite. From the experiments published by the authors cited it is probable that T. lewisi would undergo its development in any species of flea, and would be transmitted by it, provided that the flea could be induced to suck the blood of an infected rat. The natural efficacy of any given species of flea in transmitting T. lewisi depends probably on the habits and tastes of the flea, and not on any specific ability to harbour the trypanosome. Brumpt (1913) has pointed out that all the trypanosomes of small rodents seem to be able to develop in fleas.

A number of experiments have been performed by several investigators on the transmission of T. lewisi by means of the rat-louse, Hæmatopinus spinulosus. The first experiment with rat-lice (species not stated) was carried out by MacNeal (1904), who transferred "several" lice from an infected to a clean rat; trypanosomes appeared in the latter after fourteen days. Positive results in experiments of this kind with rat-lice are reported by Nuttall (1908), Baldrey (1909), Breinl and Hindle (1909), Manteuffel (1909), and Gonder (1911). To judge, however, from the published accounts of these transmission-experiments, positive results were by no means frequent and were obtained in some cases at least with difficulty and by the exercise of great patience and perseverance, or by using large numbers of lice. Nuttall obtained one positive result in an experiment in which sixty lice were used; two other experiments, in which fewer lice were used, were negative. Baldrey reports two experiments in which infection was obtained by means of lice; in the first, 100 lice were used, and the result is regarded by him as a case of direct mechanical infection, but for what reason is not at all clear; the second, in which ten lice were used, is interpreted as demonstrating a developmental cycle in the louse. Breinl and Hindle report three successful transmissions by means of lice, after carrying on numerous experiments for over a year. Manteuffel seems to have been more successful than most other experimenters in this field, though he does not record the actual number of his experiments or the proportion of those which were positive in result, but he states that infections with lice were "prompt and frequent"; his method was to put infected rats, with lice on them, in the same cage with clean

rats, and from his results he concludes that lice do not transmit the trypanosome longer than from three to five days after being removed from the infected rat, and that the transmission is effected by the act of blood-sucking; if the first of these two conclusions be true, it would appear that the trypanosome does not succeed in establishing itself in the louse in the way it does in the flea. Gonder reports that after many fruitless attempts to transmit T. lewisi with definite numbers (80–100) of lice, he obtained six positive results in a series of fifty experiments, and eight positive results in another series of fifty, using greater numbers (grössere Mengen) of lice; and he also brought about six infections by making emulsions of lice taken directly from an infected rat, the lice having been left on the infected rat for five, nine, eleven, thirteen, sixteen, and twenty-one days respectively, in these six experiments. On the other hand, Prowazek, who first described developmental stages in the louse and claimed that this insect was the true host of T. lewisi, was unable to obtain experimental transmission; Rodenwaldt obtained no positive results with numerous transmission-experiments; and we also have obtained only negative results in any attempts that we have made to transmit T. lewisi by means of the rat-louse.

It is evident from the results summarised briefly in the foregoing paragraph that transmission of T. lewisi can be effected by the rat-louse, but only with difficulty, and in a small percentage of cases. This is a great contrast to the ease and comparative certainty with which the trypanosome can be transmitted by fleas. We have always used our flea-cages as the simplest and easiest method of obtaining infected rats when required by ourselves or by our colleagues or friends, and not only have we infected rats with single fleas on many occasions, but we have even succeeded in infecting several rats successively with one and the same flea. We have no hesitation, therefore, in regarding fleas as the usual agency whereby T. lewisi is transmitted from rat to rat in Nature, a result brought about by the louse rarely and exceptionally.

It should be noted that Brumpt (1913) has succeeded in infecting a rat with T. lewisi by inoculating it with the rectal contents of a bug, Cimex lectularius, fed on an infected rat thirty-eight and again six days previously. There is no evidence, however, that this insect transmits the infection naturally.

(ii) The Transmission takes place by the Cyclical Method. Transmission by the Direct Method has not been proved to occur.

These are among the conclusions drawn from experiments described in full detail in our preliminary report (1910). It

is sufficient here to state that experiments "A" (**20**) and "B" (**21**) in our report were devised chiefly to separate "direct" from "cyclical" infection, a matter of primary importance at the time that these investigations were begun, and they show that in the individual cases cited (A_3 and B_2) transmission was effected when all possibility of the direct method was excluded. Experiments "C" (**22**) and "D" (**23**) multiply such cases many times, and show further that fleas once infective retain the infection so as to infect a series of rats without themselves being exposed to fresh infection. Since then a number of experiments have been carried out by us, many of which are enumerated under the different headings which follow, and the sum-total of these experiments not only supports the conclusions in our preliminary report, but establishes beyond doubt that the rat-flea is a true intermediate host, that it can transmit the infection to other clean rats only after the developmental cycle has been completed within itself; that, in short, the infection takes place by the cyclical method; and that there is no evidence whatever to show that the rat-flea is capable of carrying the infection from one rat to another by what is called the "direct" or "mechanical" method.

The term method in the phrase "method of transmission," if used without qualification, should include comprehensively all that happens in the transmission of infection from one vertebrate to another. In the transmission of trypanosomes the natural transmitting agent, when such is known, is a blood-sucking invertebrate of some kind. When the method is said to be "contaminative" or "inoculative," transmission is viewed from the side of the invertebrate in its relation to the vertebrate, and the problems involved are particular, that is to say such as deal with modifications due to special circumstances in those relationships, and are concerned at most with special groups of trypanosomiases rather than with trypanosomiasis in general. On the other hand when the method of transmission is said to be "cyclical" or "direct," transmission is viewed from the side of the trypanosome in its relation to the invertebrate, and the problem becomes a general one, dealing with that phase of the transmission which is concerned with the life-history of trypanosomes as a group of parasites, and with the wider question of their double relationship to vertebrate and invertebrate,

bringing them into line with other known relationships among parasitic Protozoa in this respect.

A great impetus was given to the study of trypanosomes by economic and other considerations arising out of the prevalence of tsetse-fly disease and sleeping sickness in Africa. It was long known that these diseases could be transferred artificially by direct inoculation of blood from a diseased to a healthy subject by means of a hypodermic syringe. Naturally, therefore, before much work had been done in this direction tsetse-flies known to be associated with the spread of these diseases were supposed to transmit them in this direct way. Bruce and others, experimenting with bred-out flies, proved the possibility of this taking place under certain conditions which, in the case of sleeping sickness at all events, were very unlikely ever to be fulfilled in Nature. Only with a swarming infection and by interrupted feeding could the disease be passed on directly from an infected to a clean animal with any approach to certainty, and even under the most favourable conditions in other respects, the longer the interval between interrupting the feed on the infected animal and continuing it on a clean animal, the less the chance of the clean animal becoming infected, until, with the lapse of about half-an-hour, it was just as certain that the infection would not take place. Moreover, however short the interruption between the feeds, an interposed partial feed on a clean animal rendered the fly non-infective to a second clean animal. Later experiments showed that the contents of the stomachs of flies that had fed on an infected animal, if injected into a clean animal, could produce infection only up to about two days after the infective feed. The fly itself, however, could not be shown to act in any way resembling a hypodermic syringe, and the idea of "delayed mechanical transmission" never found support from feeding experiments. The conclusion to be drawn from all the earlier experiments on direct transmission seemed to be that when infection was obtained it was with "fouled proboscis" before the blood in its lumen distal to the entrance of the salivary duct, and perhaps also on its external surface, had had time to dry, and that the conditions under which it was shown to be possible were never likely to be fulfilled in Nature, in the case of sleeping sickness, and in the case of tsetse-fly disease of cattle, far too seldom to account for the spread of the disease, while in no case could such a method of transmission account for the existence of fly-belts through which healthy domestic stock cannot pass. Other things being equal, the efficiency of an invertebrate as a transmitter of trypanosomes would be enormously increased if the invertebrate were a true intermediate host and not merely a "porter" of the parasites from an infected to a clean subject, and to demonstrate beyond doubt that trypanosomes underwent an alternation of

generations was of primary importance in connection with the general trypanosome problem at the time that we undertook this investigation, when it was being maintained by Patton and others that no trypanosomes went through a developmental cycle in the invertebrate, that all transmission of trypanosomes was direct, and that the crithidial forms found in blood-sucking invertebrates were all of them independent parasites of the invertebrate, having no connection with the trypanosomes or other parasites of the vertebrate. It was known that rat-fleas could transmit T. lewisi from infected to clean rats, and although transmission by fouled proboscides seemed quite out of the question, it was necessary to demonstrate beyond doubt that the rat-flea is a true intermediate host of T. lewisi, that it can transmit the infection to other rats only after the developmental cycle has been completed within itself, and that once infected it remains infective for a considerable time, so as to be able to infect a series of clean rats without itself being exposed again to infection. These points, which we believe concern the transmission of trypanosomes in general, and which may be taken as typical of the relations which trypanosomes as a group bear to their invertebrate hosts, as well as other points of more special interest (confined, it may be, to T. lewisi alone or to the lewisi group), are dealt with under different headings in what follows later. Although the practical cannot properly be separated from the scientific, the most interesting problem of the transmission from the scientific point of view is perhaps the way in which the trypanosome becomes established. There are considerable variations in the details of the cycles of different species or groups of trypanosomes in their natural hosts due to special conditions, but arising out of the very meaning of a cycle, and therefore common to all is the fact that until the cycle is completed the invertebrate, though infected, is not infective. This may be of direct practical importance in special cases, and where that is so it is important to ascertain the length of time required for the completion of the cycle in each case. Of more general practical importance in questions connected with the spread of infection is the fact that the trypanosome does establish itself in such a way that the invertebrate remains infective for a long time without requiring to be exposed again to infection.

(iii) The Trypanosomes make their Appearance in the Blood of the Rat Five to Seven Days after Infection; the Multiplication of the Trypanosomes in the Blood of the Rat come to an End Eleven to Thirteen Days after Infection.

In order to establish with exactness the length of the

incubation-period and the multiplication-period in the rat after infection, it is necessary that the rat should have been exposed to infection by the fleas for a short time; long exposure leaves too wide a margin between the possible maximum and minimum deducible from the actual data furnished by the experiment for the mean to be of any value in reckoning the length of the two periods in question. When the rat is removed from contact with the infected fleas it is further very necessary that all fleas should be removed from its skin. The rat is then kept in a flea-proof cage and its blood is examined daily in fresh, living films until trypanosomes are first detected in it, in order to determine the duration of the incubation-period; then smears of the blood are made and preserved daily and examined until the multiplication-period is found to be past and ended. So long as the trypanosomes are multiplying in the rat's blood, they are of various sizes, some of the ordinary, normal size, others very small, and others again much above the normal size. Marked variation in the size of the trypanosomes is a sure sign that multiplication is proceeding, even when actual division-stages are so scarce in the preparation that prolonged search is necessary in order to find them. As soon as the multiplication is ended the trypanosomes are all of one type and size, allowing for slight individual variations that are not perceptible without careful measurement; to such trypanosomes, the normal form of T. lewisi and the sole form occurring in the blood when once the multiplication is at end, we shall refer always as "ordinary."

We cite here a few examples from our series of experiments, choosing first (Table C), those in which the rats were exposed to infection for one day only, so that the periods of incubation and multiplication can be determined within a margin of one day. In our second table (D), we quote those instances in which the rats were exposed to infection for two days, so that a wider margin of possible error must be allowed for in calculating the two periods. In a third table (E), we shall give some results obtained with rats which were infected

TABLE C.—One Day's Exposure to Infection.

No. of experiment.	No. of rat.	Put in.	Taken out.	Trypanosomes first seen.	Multiplication ended.	Incubation period.	Multiplication period.
20 (= A)	122	7 : xii : '09	8 : xii : '09	14 : xii : '09	18 : xii : '09	6–7 days	10–11 days
21 (= B)	123	7 : xii : '09	8 : xii : '09	14 : xii : '09	18 : xii : '09	6–7 „	10–11 „
22 (= C)	129	10 : xii : '09	11 : xii : '09	16 : xii : '09	23 : xii : '09	5–6 „	12–13 „
23 (= D)	138	16 : xii : '09	17 : xii : '09	21 : xii : '09	Rat died	4–5 „	—
40	317	15 : v : '11	16 : v : '11	21 : v : '11	26 : v : '11	5–6 „	10–11 days
45	371	28 : vii : '13	29 : vii : '13	3 : viii : '13	9 : viii : '13	5–6 „	11–12 „

TABLE D.—Two Days' Exposure to Infection.

No. of experiment.	No. of rat.	Put in.	Taken out.	Trypanosomes first seen.	Multiplication ended.	Incubation period.	Multiplication period.
20 (= A)	130	11 : xii : '09	13 : xii : '09	17 : xii : '09	22 : xii : '09	4–6 days	9–11 days
22 (= C)	121	6 : xii : '09	8 : xii : '09	13 : xii : '09	19 : xii : '09	5–7 „	11–13 „
„	124	8 : xii : '09	10 : xii : '09	14 : xii : '09	21 : xii : '09	4–6 „	11–13 „
„	132	11 : xii : '09	13 : xii : '09	17 : xii : '09	24 : xii : '09	4–6 „	11–13 „
„	135	13 : xii : '09	15 : xii : '09	20 : xii : '09	26 : xii : '09	5–7 „	11–13 „
„	141	20 : xii : '09	22 : xii : '09	28 : xii : '09	1 : i : '10	6–8 „	10–12 „
23 (= D)	164	13 : i : '10	15 : i : '10	21 : i : '10	26 : i : '10	6–8 „	11–13 „
„	168	18 : i : '10	20 : i : '10	27 : i : '10	30 : i : '10	7–9 „	10–12 „
24	153	6 : i : '10	8 : i : '10	14 : i : '10	18 : i : '10	6–8 „	10–12 „
„	156	8 : i : '10	10 : i : '10	15 : i : '10	21 : i : '10	5–7 „	11–13 „

TABLE E.—Rats Infected by a Single Flea.

No. of rat.	Flea put on rat.	Flea recovered.	Trypanosomes first seen.	Multiplication ended.	Incubation period.	Multiplication period.
207*	2 : viii : '10	5 : viii : '10	11 : viii : '10	13 : viii : '10	6– 9 days	8–11 days
212*	19 : ix : '10	22 : ix : '10	28 : ix : '10	30 : ix : '10	6– 9 ,,	8–11 ,,
223*	10 : x : '10	14 : x : '10	17 : x : '10	22 : x : '10	3– 7 ,,	8–12 ,,
233	20 : x : '10	25 : x : '10	28 : x : '10	31 : x : '10	3– 8 ,,	6–11 ,,
244	11 : xi : '10	15 : xi : '10	23 : xi : '10	26 : xi : '10	8–12 ,,	11–15 ,,
253*	27 : iii : '11	1 : iv : '11	5 : iv : '11	7 : iv : '11	4– 9 ,,	6–11 ,,
259*	23 : iii : '11	27 : iii : '11	30 : iii : '11	4 : iv : '11	3– 7 ,,	8–12 ,,
263*	20 : iv : '11	25 : iv : '11	1 : v : '11	5 : v : '11	6–11 ,,	10–15 ,,
265*	14 : ii : '11	18 : ii : '11	24 : ii : '11	27 : ii : '11	6–10 ,,	9–13 ,,
272*	14 : ii : '11	18 : ii : '11	24 : ii : '11	28 : ii : '11	6–10 ,,	10–14 ,,
291*	27 : iii : '11	1 : iv : '11	5 : iv : '11	9 : iv : '11	4– 9 ,,	8–13 ,,
292*	25 : iv : '11	29 : iv : '11	1 : v : '11	6 : v : '11	2– 6 ,,	7–11 ,,
296*	15 : iv : '11	20 : iv : '11	22 : iv : '11	25 : iv : '11	2– 7 ,,	5–10 ,,
295*	17 : iii : '11	22 : iii : '11	27 : iii : '11	31 : iii : '11	5–10 ,,	9–14 ,,
315*	29 : viii : '11	left on	12 : ix : '11	16 : ix : '11	14 days (max.)	18 days (max.)
361*	13 : vii : '12	,,	23 : vii : '12	26 : vii : '12	10 ,, ,,	13 ,, ,,
363*	13 : vii : '12	,,	23 : vii : '12	26 : vii : '12	10 ,, ,,	13 ,, ,,

* The rats marked thus were examined only on alternate days. Consequently the dates of the appearance of trypanosomes in their blood, or of the termination of the multiplication period, may have been a day earlier than stated in the table.

TABLE F.—Rats Infected by Injection of Fleas.

Experiment.	No. of rat.	Date of injection.	No. of fleas.*	Trypanosomes first seen.	Multiplication ended.	Incubation period.	Multiplication period.
39	309	29 : iv : '11	10 (r)	8 : v : '11	10 : v : '11	9 days	11 days
39	312	1 : v : '11	10 (s)	8 : v : '11	13 : v : '11	7 ,,	12 ,,
39	314	4 : v : '11	10 (s)	10 : v : '11	15 : v : '11	6 ,,	11 ,,
39	257	15 : xii : '10	6 (s)	21 : ii : '10	26 : ii : '10	6 ,,	11 ,,
34	261	26 : i : '11	6 (s)	1 : ii : '11	6 : ii : '11	6 ,,	11 ,,
34	282	14 : ii : '11	1 (s)	20 : ii : '11	(not noted)	6 ,,	—
32	287	14 : ii : '11	1 (s)	20 : ii : '11	,,	6 ,,	—

* r = rectum; s = stomach.

each by a single flea, in order to show that in many cases, at least, the maximum periods of incubation and multiplication which can be deduced from experiments under these conditions are not greater than those indicated by the experiments in which many fleas were used to obtain infection. In a fourth table (F) we give for comparison, the results obtained by inoculating rats with the stomachs or recta of fleas; in such cases the length of the periods of incubation and multiplication can be determined with exactness, the moment of infection being known.

The determination of the length of the multiplication-period in an infected rat is of practical importance for interpreting other experiments, since, when it has been determined, it furnishes a datum from which approximately accurate conclusions can be drawn as to the time at which the rats become infected, when the point is shown definitely by the details of the experiment. As regards the first appearance of the trypanosomes in the blood, they appear at first in such scanty numbers that it is very easy to overlook them, and they may often be reported absent when a more prolonged search would have detected their presence. Similarly, the trypanosomes at the end of the multiplication-period may sometimes have been reported as "all ordinary" in a smear in which more careful searching might have led to the discovery of a few individuals above or below the normal size. Consequently, the errors of observation are such as tend to over-estimate the length of the incubation-period, and to under-estimate that of the multiplication-period, from the scrutiny of the blood-films. On the whole, however, the results obtained in our experiments are very uniform and indicate an incubation-period of about six days, a multiplication-period of about twelve days. It is interesting to note that these results agree with those obtained in the case of rats infected artificially by inoculation, intra-peritoneal or otherwise, with blood from an infected rat. Since a syringe would inoculate far more trypanosomes than the rat would obtain from even a large number of fleas, it might have been expected that the rat would, so to speak, fill up quicker when infected by means of a syringe, and that consequently the multiplication-period would be correspondingly shorter. In our experience, however, the length of the multiplication-period remains approximately constant in all cases, whether the infection is effected by a syringe, by a large number of fleas, by a few fleas, or even by a single flea; a fact which indicates that the length of time during which the trypanosome multiplies in the rat

is not determined by the number of trypanosomes put into the rat, but by the mutual interaction of host and parasite.

It may be noted here that some rats appear to possess a certain degree of natural immunity to infection with T. lewisi. A single instance which came under our experience will suffice to demonstrate this point. A rat was exposed to infection on June 9th and its blood was examined daily; on June 25th a few trypanosomes were first seen in the blood in scanty numbers, just as they are usually seen at their first appearance between the fifth and seventh days of the infection. The rat was then removed from contact with the fleas and kept apart; but neither on the next day nor on any subsequent day were any trypanosomes to be found in its blood. This rat, therefore, contracted only a transitory infection which was late in its appearance and disappeared after one day; had the trypanosomes been overlooked on that day the experiment would have been returned wrongly as negative in result.

(iv) The Cycle of Development in the Flea requires a Minimum of Five Days for its Completion.

This point was dealt with in our preliminary communication (1910), in which we came to the conclusion that the incubation in the flea was six or seven days. Our method of determining this was, first of all to expose non-infected fleas to infection, by putting them on a well-infected rat, for but a single day, so that if the fleas afterwards produced an infection, the time at which they themselves became infected could be determined within a narrow margin, twenty-two hours in our actual experiment. The fleas were then placed in contact for three days with clean rat (1) which did not become infected; after that for three days with clean rat (2), which also did not become infected; and then for two days on clean rat (3), which showed trypanosomes in its blood six days after being removed from contact with the infected fleas. Clean rat (3) was, therefore, infected by the fleas in the interval between the sixth and eighth day after the fleas themselves had acquired the infection; consequently the infection in the fleas could not have been more than eight days old.

Subsequent experiments performed by us have indicated a possible minimum of five days for the flea-cycle of the try-

panosome. In experiment 39 (see below, p. 630) it is proved that the rectum of the flea, injected into the rat, can produce an infection as early as the fifth day, and in such fleas the examination of films shows the presence of the small trypanosomes which are the final form of the development in the flea. In experiments 26 and 28, undertaken in order to ascertain whether a rat, in which the trypanosomes are still in the multiplication-period, is capable of infecting fleas (see below, p. 657), the results obtained indicated a short incubation-period in the fleas. Thus in experiment 26, 127 fleas, after being three days (from 8 : ii : '10 to 11 : ii : '10) on the infected rat were put on rat 187 for another three days (from 11 : ii to 14 : ii). Rat 187 showed trypanosomes in its blood after five days (19 : ii), and the multiplication-period ended five days later (24 : ii). Consequently the incubation-period in the fleas could not have been more than six days (8 : ii to 14 : ii). In experiment 28, 137 fleas were put first on the infected rat for four days (15 : ix : '10 to 19 : ix : '10), and then were put for one day (19 : ix to 20 : ix) on rat 209; after this they were put on rat 215 and left on it. Rat 209 had shown no infection when it died nine days later (29 : ix); rat 215 first showed trypanosomes on 30 : ix, and the multiplication was ended 3 : x. This result indicates that rat 215 was infected about 21 : ix, in which case the incubation-period in the flea could not have been more than six days (15 : ix to 21 : ix). Since rat 209 showed no trypanosomes for at least nine days after being exposed to infection it was probably not infected; so that the infection in the fleas was probably not ripe for at least five days (15 : ix to 20 : ix).

On the other hand we have instances, as already mentioned in our preliminary communication (1910), of an incubation-period in the flea apparently much longer than six days. Thus in experiment 19 a cage (J) was stocked with seventy-two fleas from the non-infected breeding-cage and an infected rat (No. 81, a wild black rat, naturally infected) was put with them for three days (20 : ix : '09 to 23 : ix : '09). Rat 81 was then removed and rat 82, a clean, tame rat, was put in its place (23 : ix) and left in the cage. Rat 82 first showed trypanosomes in its blood 29 : x; the multiplication-period was ended about 4 : xi, indi-

cating that the actual infection of rat 82 took place about 23 : x. In this case, therefore, the fleas did not produce an infection in the clean rat for at least a calendar month after their contact with the infected rat was interrupted. Such a result, however, permits of no conclusion whatever as to the length of the incubation-period in the flea; it merely demonstrates a point proved also by other experiments, namely that infective fleas often fail to infect. We have put forward already (1910) one possible explanation for this, that a rat, which is comparatively immune to begin with, may resist infection for a long time, but its resistance may be overcome at last. Another possible explanation may be given by the method in which infection of the rat by the flea is now known to take place, namely by the rat licking off the moist fæces of infective fleas that are deposited on its skin (see below, p. 648). It is evident that if the rat fails to lick off the fæces while still moist, or if the infective flea does not defæcate on the rat, no infection is brought about. A negative result of this kind is most likely to be attained when the number of infective fleas on the rat is very small, as seen in the large number of negative and small number of positive results in our series of experiments in which single fleas were used (see below, p. 661). That infective fleas in Cage J were rare is shown by the fact that between 23 : ix and 19 : x thirty fleas from this cage were dissected and examined without finding a single one infected. On the other hand, when fleas are sufficiently numerous and have been well infected, positive results are fairly certain (Experiment "C" of our preliminary report).

(v) Transmission is never effected until the Developmental Cycle is completed; that is to say, until at least Five Days have elapsed since the First Exposure of the Fleas to Infection.

We have found, as already stated, by direct observation, that the final form of the developmental cycle appears in the gut of the flea five days after the infective feed (see below, p. 630). We bring forward here a few instances to show that at least five days must elapse before the flea becomes infective, after having ingested trypanosomes from an infected rat.

(1) Experiment 20.—A cage colonised with forty-four fleas that had been exposed to infection from 4 : x : '09 to 8 : x : '09.

Rat 93 put into the cage from 8 : x to 12 : x, i. e. during a period in which the age of the infection in the fleas could not have been less

than two days old at the beginning nor more than eight days old at the end. Result negative.

(The next rat used in this experiment died, but subsequent rats used showed that the fleas had become infective.)

(2) Experiment 21.—A cage colonised with 157 fleas that had been exposed to infection from 11 : x : '09 to 15 : x : '09.

Rat 97 put in from 15 : x to 19 : x, i. e. during a period in which the age of the infection in the fleas could not have been less than a few hours at the beginning nor more than eight days at the end. Result negative.

(The next rat put in became infected, apparently about 27 : x; age of infection in the fleas then between twelve and sixteen days.)

(3) Experiment 22.—A cage colonised with 160 fleas exposed to infection from 24 : xi : '09 to 27 : xi : '09.

Rat 116 put in from 27 : xi to 30 : xi, i. e. during a period in which the age of the infection in the fleas could not have been less than a few hours at the beginning nor more than six days at the end. Result negative.

(The next rat put in became infected, apparently about 3 : xii; infection of the fleas then six to nine days old.)

(4) Experiment 23.—A cage colonised with 162 fleas exposed to infection from 7 : xii : '09 to 8 : xii : '09.

Rat 125 put in from 8 : xii to 11 : xii, i. e. during a period in which the age of the infection in the fleas could not have been less than a few hours at the beginning nor more than four days at the end. Result negative.

Rat 133 put in from 11 : xii to 13 : xii, i. e. during a period in which the age of the infection in the fleas could not have been less than four days at the beginning nor more than six days at the end. Result negative.

(The next rat put in became infected, apparently about 15 : xii; infection of the fleas then seven to eight days old.)

(5) Experiment 24.—A cage colonised with fifty fleas exposed to infection from 7 : xii : '09 to 8 : xii : '09.

Rat 126 put in from 8 : xii to 11 : xii, i. e. during a period in which the age of the infection in the fleas could not have been less than a few hours at the beginning nor more than four days at the end. Result negative.

(The next rat put in became infected, apparently about 3 : i : '10; age of the infection of the fleas then between twenty-six and twenty-seven days.)

(6) Experiment 25.—A cage colonised with seventy fleas exposed to infection from 31 : xii : '09 to 3 : i : '10.

Rat 152 put in from 3 : i to 6 : i, i. e. during a period in which the

age of the infection in the fleas was not less than a few hours at the beginning nor more than six days at the end. Result negative.

(The next rat put in became infected, apparently about 6 or 7 : i; the age of the infection at 7 : i was from four to seven days.)

(7) Experiment 45, Batch C (see below).—Bell-jar colonised with thirty fleas exposed to infection from 22 : vii : '13 to 23 : vii : '13.

No infection produced in rat 370 put in for a period of two days, during which the infection in the fleas could not have been less than five days old at the beginning nor more than seven days old at the end.

No infection produced in rat 370a, put in for a period of one day, during which the infection in the fleas could not have been less than eight days old at the beginning nor more than ten days old at the end.

(The next rat put in became infected.)

In the previous section it has also been pointed out that in Experiment 28, rat 209 escaped infection when exposed to infection by 138 fleas during a period of one day, at the beginning of which the age of infection in the fleas could not have been less than a few hours nor more than five days at the end. Rat 215 became infected by the fleas a day later, when the age of the infection in the fleas could not have been less than two or more than six days.

Putting together the results of this and the last section, it is seen that fleas in which there is a possibility, from the data of the experiment, of the infection being more than five days old, may fail to produce infection, although the subsequent history of those fleas shows them to have been infected effectively, but no infections have been obtained in any experiment of which the data are incompatible with the infection being at least six days old in the fleas that produced the infection.

(vi) The Infection of the Rat is brought about by the Small Trypanosome-form which is the Final Form of the Development.

This point is scarcely capable of direct proof, since it is impossible to be absolutely certain that when an infection has been produced no other forms of the developmental cycle in the flea were introduced into the rat except the trypanosome-forms. It can, however, be demonstrated in experiments planned for that purpose, that the trypanosome-forms

are present when an infection is produced. Nöller (1912) and Wenyon (1913), have shown that the trypanosome-forms were present in all cases in the fæces with which they infected rats per os.

The following experimental results indicate that the trypanosome-form is the effective agent in infection. In Experiment 35 B twelve fleas were taken at hazard from the infected breeding-cage and put on clean rat 243 for five days (23 : ii : '11 to 28 : ii : '11; Rat 243 was found to be infected on 3 : iii : '11). Ten of the fleas recovered were then dissected (the other two lost); of each flea the stomach was placed on one slide in a drop of salt-citrate solution, the rectum on another slide in another drop. Each stomach and each rectum were then teased up and examined microscopically in order to see if trypanosomes were present in any form; but since this examination had to be performed very rapidly and cursorily and without putting a coverslip over the drop, trypanosomes may have been often overlooked, when they were not present in abundance. Whether trypanosomes could be seen in the fresh specimen or not, each teased-up stomach was inoculated by means of a syringe into a separate clean rat; the rectum was only inoculated if trypanosomes were seen in it.[1] After the drop containing the teased-up stomach or rectum had been drawn up into the injecting syringe the film of moisture left on the slide was fixed with osmic vapour, stained with Giemsa's stain, and carefully searched for trypanosomes. The following are the results obtained with each flea.

Flea (1).—Nothing seen in the fresh stomach or rectum. Stomach inoculated into Rat 279. No infection produced. Nothing found in the preserved film.

Flea (2).—As last, stomach inoculated in rat 280, no infection, nothing found in the films.

[1] The reason for the differential treatment of the stomach and rectum was because we believed, at the time, that infection was brought about by regurgitation of infective trypanosomes through the proboscis from the stomach, and also because the presence of trypanosomes in the rectum is not so easily overlooked as in the stomach.

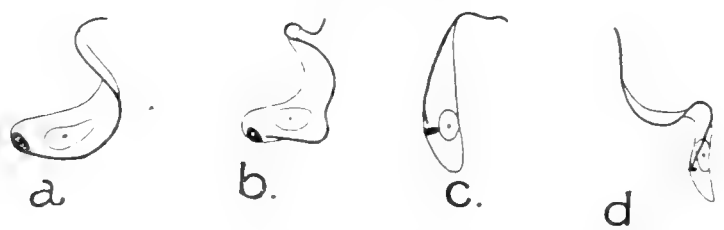
Flea (3).—As last, stomach inoculated into rat 281, no infection, nothing found in the films.

Flea (4).—One sluggish stumpy form, which may have been crithidial or trypaniform, was seen in the fresh teased-up stomach; nothing seen in the fresh rectum. Stomach inoculated into rat 283, result negative. Nothing found in the preserved film of the stomach.

Flea (5).—Nothing seen in the fresh stomach, numerous trypanosomes seen in the rectum. Stomach inoculated into rat 285, result positive. Rectum inoculated into rat 248, result negative. One trypanosome-form and one transitional form found in the preserved film of the stomach (Text-fig. 16, *b* and *c*). Nothing found in the preserved film of the rectum.

Flea (6).—Nothing seen in the fresh stomach; a few forms, some

TEXT-FIG. 16.



Small trypanosome-forms from the stomach-films of fleas 5 and 7 in Experiment 35 B, and flea 5 in Experiment 27 (see text). (× 2000.)

stout and of crithidial appearance and some slender, apparently trypaniform, seen in the rectum. Stomach inoculated into rat 286, result, negative; rectum not inoculated. No films preserved.

Flea (7).—Nothing seen in the fresh stomach or rectum. Stomach inoculated into rat 288, result positive. One trypanosome (Text-fig. 16, *a*) found in the preserved film of the stomach.

Fleas (8), (9), (10).—In each case nothing was seen in the fresh stomach or rectum. The stomachs were inoculated into rats 289, 262, 263 respectively, results in each case negative. Nothing found in the preserved films.

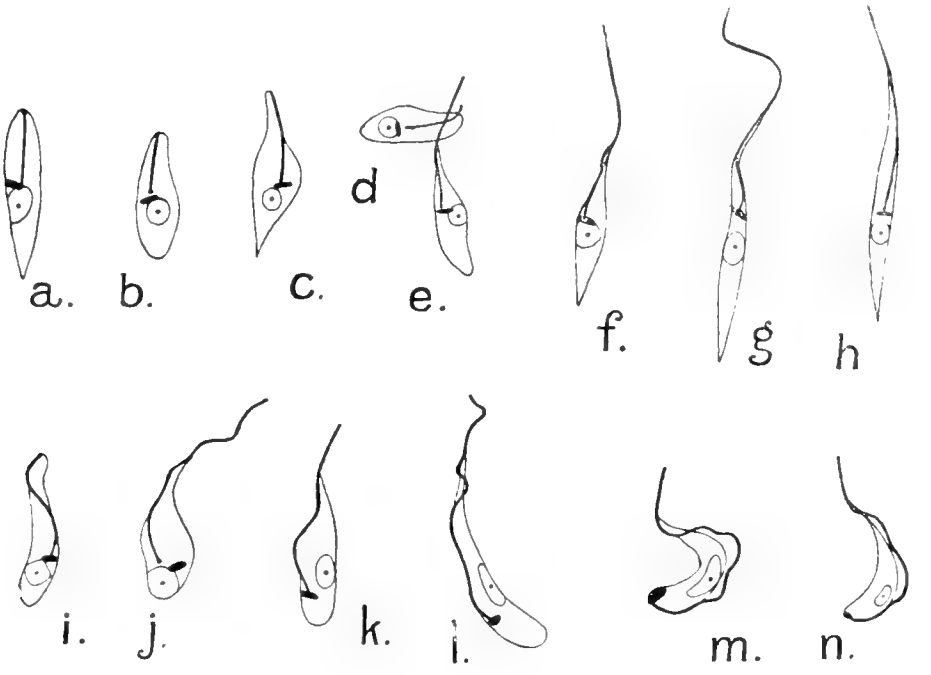
Summary.—In the case of two fleas out of the ten used, the stomachs, when inoculated into clean rats, produced an infection. The final trypanosome-stage was found in both the stomachs that produced infections, but in none of the remaining eight stomachs that produced no infection.

Experiments 27, 29 and 32 were conducted in a different

manner. Fleas taken from the infected breeding-cage were put each on a separate rat and left on it for three or four days. The flea was then recovered (if it could be found), dissected and examined.

Experiment 27.—Flea (5), placed on rat 207 for three days (2 : viii : '10 to 5 viii : '10) produced infection (see Table E). The flea dissected (5 : viii), and one large transitional form found in the slide of the stomach (Fig. 16, *d*).

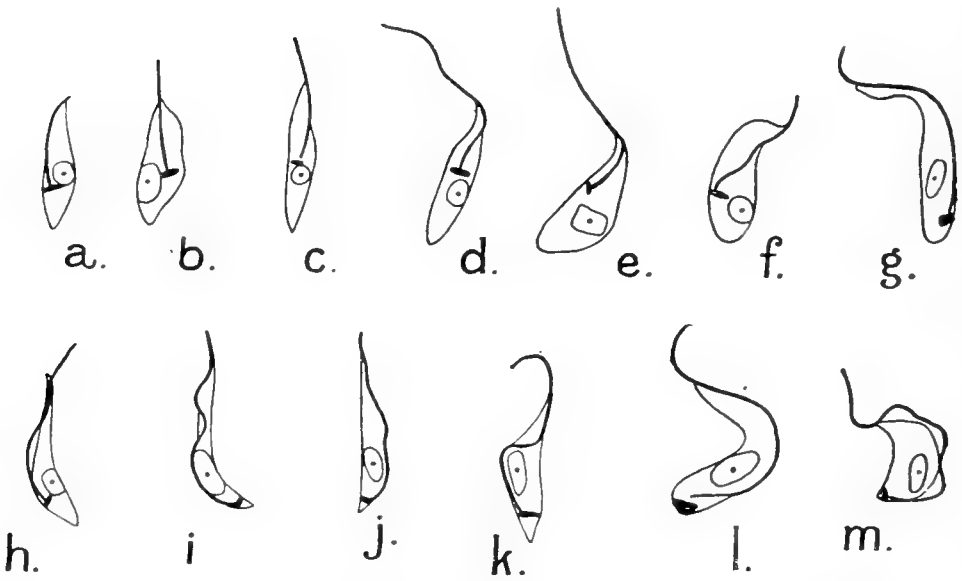
TEXT-FIG. 17.



Various forms (haptomonad, *a–d*, nectomonad, *e–h*, transitional, *i–l*, and trypaniform, *m* and *n*), from the stomach-film of flea 3, Experiment 29 (see text). (× 2000.)

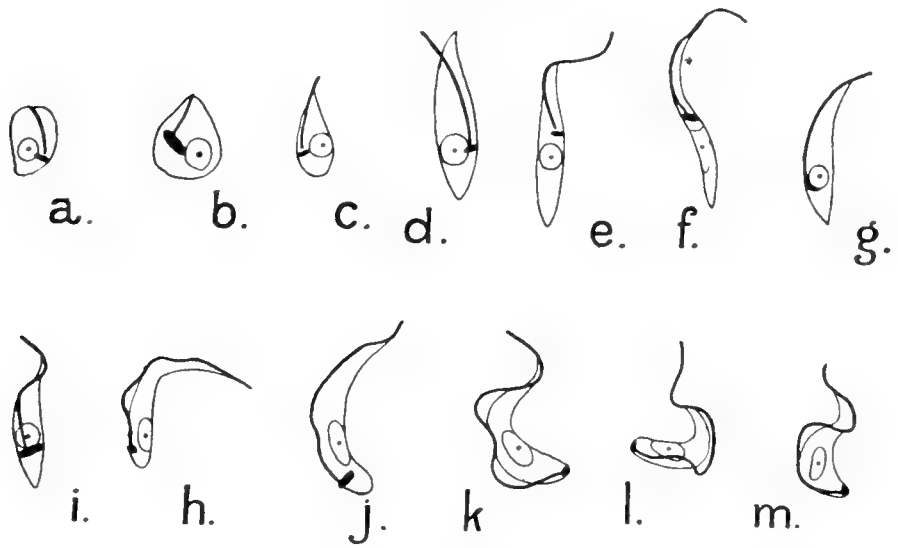
Experiment 29.—Flea (3) placed on rat 212 for three days, (19 : ix : '10 to 22 : ix : '10), recovered and dissected 22 : ix '10 (see Table E). Large clumps of attached forms were seen in the stomach and also free forms; nothing was seen in the intestine, rectum, salivary glands or proboscis. The preparations of the stomach showed crithidial, transitional and trypaniform types in abundance (Text-fig. 17). Rat 212 became infected and first showed trypanosomes in the blood on 28 : ix. Four other fleas in the same experiment failed to infect their rats; in two of these fleas nothing was found, in the third a small

TEXT-FIG. 18.



Various forms (haptomonad, *a*, *b*, nectomonad, *c*, *d*, transitional, *e–h*, and trypaniform, *i–m*), from the stomach-film of flea 3, Experiment 32 (g) (see text). (× 2000.)

TEXT-FIG 19.



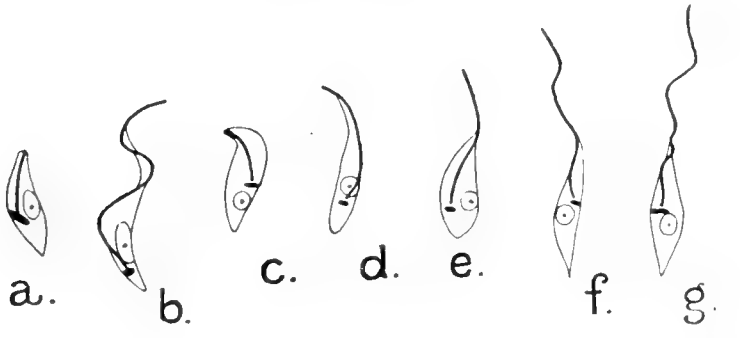
Various forms (haptomonads, *a–d*, nectomonad, *e*, transitional, *f–i*, and trypaniform, *j–m*) from the rectum-film of flea 3, Experiment 32 (h) (see text). (× 2000.)

attached clump was seen in the intestine, nothing in any other part of the flea. The remaining flea was not recovered.

Experiment 32 (g).—Flea (3) placed on rat 241 for four days (3 : xi : '10 to 7 : xi : '10) produced no infection. In the stomach of the flea, dissected 8 : xi, crithidial, transitional and trypaniform types were found (Fig. 18).

Experiment 32 (h).—Flea (3) placed on rat 244 for four days (11 : xi : '10 to 15 : xi '10) produced an infection (see Table E). The flea was dissected 15 : xi ; nothing was found in the stomach, intestine or salivary glands ; the rectum showed a typical swarming "pile-carpet" infection with all the usual types of form (Text-fig. 19).

TEXT-FIG. 20.



Flea 3, Experiment 32 (i). *a*, haptomonad, and *b*, trypanosome-form from the rectum-film; *c* and *d*, haptomonads, *e–g*, nectomonads, from the film of the intestine (see text). (× 2000.)

Experiment 32 (i).—Flea (3), placed on rat 247 for three days (12 : xi : '10 to 15 : xi : '10), produced no infection. The flea, dissected and examined (16 : xi), showed no trypanosomes in the stomach, but in the intestine were clumps attached behind the pylorus (Text-fig. 20, *c*), and the rectum contained a teeming infection (Text-fig. 20, *a* and *b*) of the usual types.

The cases cited show that when infections were produced the final trypanosome-form was found either in the stomach or rectum ; but it should also be mentioned that we have three instances in which the rat became infected under similar circumstances without our having been able to discover an infection of the flea, which must have been so scanty as to escape detection in our films. On the other hand the experiments also show that the infective form may be present in the

flea without any infection resulting when the flea is on the rat for not more than four days. The failure of the flea to infect in such cases must be correlated with the casual nature of the contaminative method of infection by the fleas, evidently not so sure a method as that of inoculation. It will be shown further (Experiment 39, below) that the period at which the flea becomes infective coincides with the first appearance of the small trypanosomes in the rectum.

(vii) The Final Infective Form of the Cycle is developed first in the Rectum on the Fifth Day of the Developmental Cycle, but may appear later in the Stomach.

In order to ascertain how soon the trypanosomes, ingested by the flea, attain to maturity in the different parts of the digestive tract of the flea an experiment (Experiment 39) was carried out in the following manner. A number of fleas (about one hundred) were collected from the non-infected breeding-cage, put into test-tubes, with clean sand, slightly damp, and kept there for four days (20 : iv : '11 to 24 : iv : '11), in order that they should be properly hungry and ready to feed. The fleas were then (24 : iv) put into a special flea-proof tin cage with a well-infected rat (No. 259).[1]

At regular intervals batches, each of ten fleas, were recovered from rat 259, kept in the test-tubes on sand, and dissected on the following day (to ensure that the fleas had not ingested blood containing trypanosomes for at least a day previous to being dissected). In the dissection of the fleas, the flea was first placed on a slide in a drop of salt-citrate solution and the proboscis removed by cutting through the head in the region of the eyes; the proboscis was then placed in a separate capsule in a small quantity of salt-citrate solution. Very often fæces were extruded when the

[1] Rat 259 was put in with a single flea on 22 : iii : '11; trypanosomes were first seen in the blood on 30 : iii; multiplication was ended on 4 : iv; see Table E, p. 617 above.

head was cut through. When this occurred the carcase of the flea was at once removed to another slide, and the extruded fæces examined microscopically. If trypanosomes were found in sufficient abundance the slide and coverslip were fixed and stained. The carcase of the flea was then opened in the hinder end of the abdomen, and the junction of stomach and intestine cut through behind the Malpighian tubes, after which the portion of the carcase containing the stomach was transferred to another slide. Then the stomach and Malpighian tubules, together with the proventriculus and œsophagus, were removed together and transferred to a second capsule, and the proctodæum (intestine with rectum) to a third. In making these dissections some of the contents of the stomach or rectum escaped on to the slide (the organs being purposely punctured to allow some contents to escape, when necessary). The escaped contents were examined microscopically, and if trypanosomes were found in them in sufficient numbers they were preserved.

After all the 10 fleas of each batch had been dissected in this way the 10 proboscides were inoculated into one clean rat, the 10 stomachs into another, and the 10 recta into a third. In each case the whole of the salt-solution in the capsule was injected also. The stomachs and recta were teased up as fine as possible, and the proboscides crushed up, before injecting them.

The following are the actual injections performed; in the results stated, 0 signifies that the rat inoculated acquired no infection, + that it became infected.

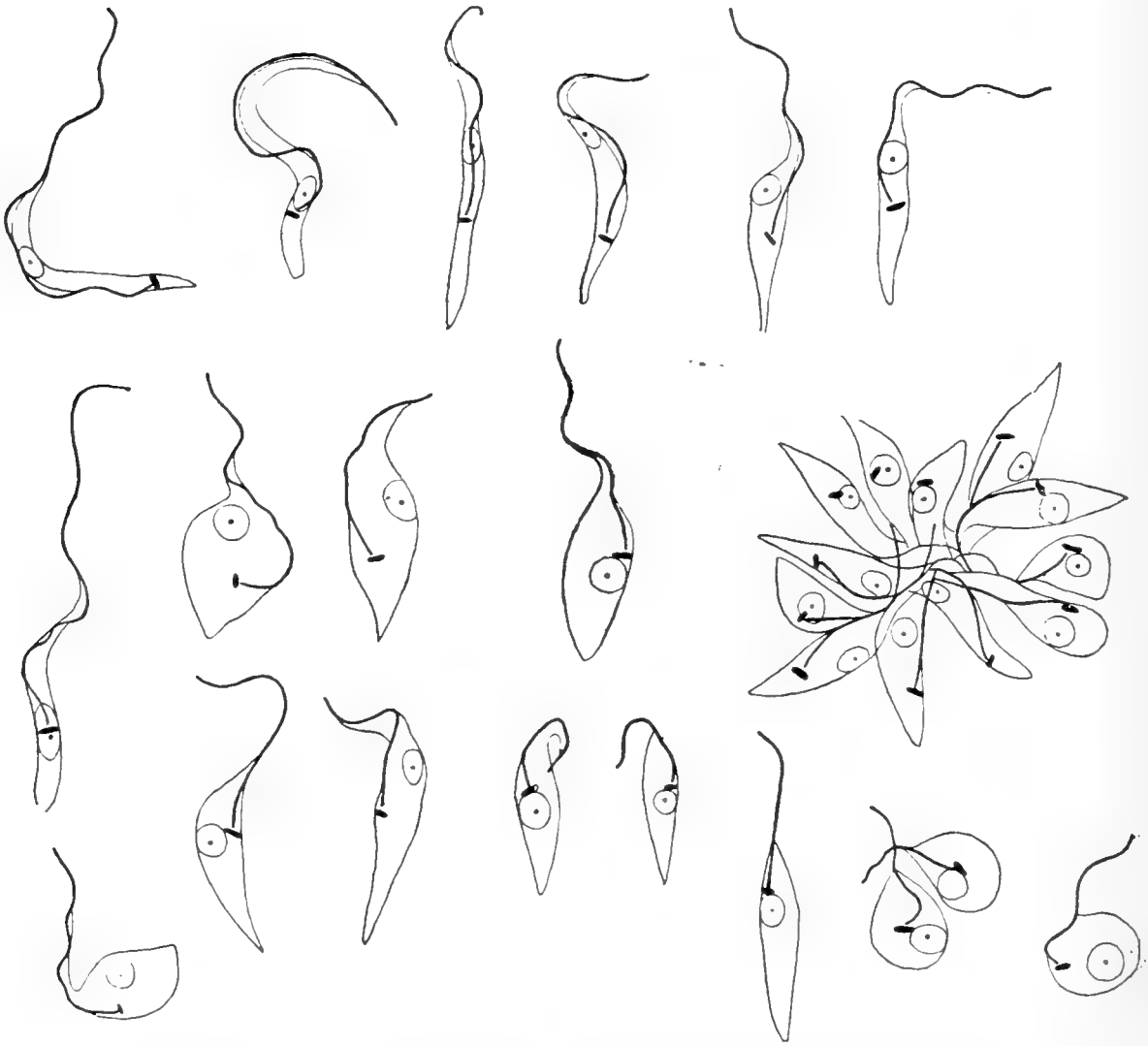
26 : iv : '11.—The 10 fleas recovered on the previous day were dissected; trypanosomes were seen in the stomach, rectum, and extruded fæces of several fleas. All trypanosomes seen appeared to be of quite ordinary type:

10 proboscides	injected	into	rat	289 :	result	0
10 stomachs	,,	,,	,,	299 :	,,	0
10 recta	,,	,,	,,	300 :	,,	0

27 : iv : '11.—The 10 fleas recovered on the previous day were dissected; trypanosomes were seen in the stomach of one, the stomach and extruded fæces of another, in the stomach and rectum of a third, and in

the rectum and extruded fæces, very abundantly, of a fourth; the rectal forms appeared pear-shaped or club-shaped in the living state (Text-fig. 21), but no post-crithidial trypanosome-forms were present:

TEXT-FIG. 21.



Various forms from the rectum and fæces of a flea of the batch of 27 : iv : '11. Note that no final trypanosome-forms are present (see text). (× 2000.)

10 proboscides	injected	into	rat	301:	result 0
10 stomachs	„	„	„	302:	„ 0
10 recta	„	„	„	303:	„ 0

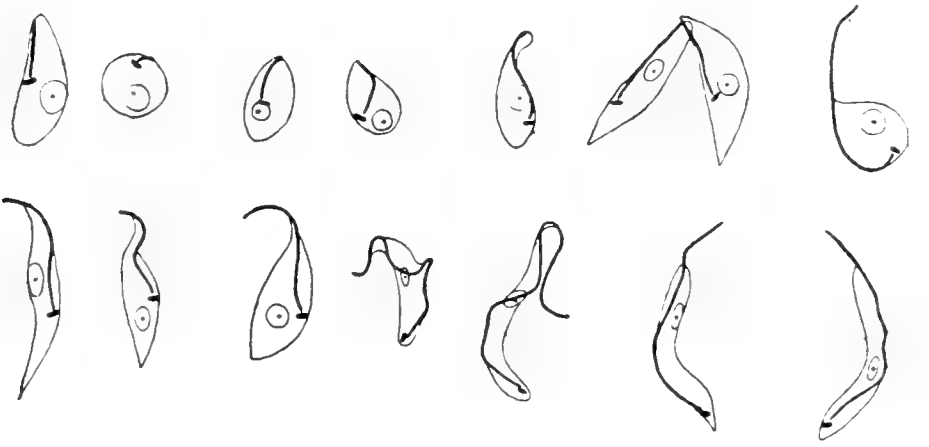
28 : iv : '11.—The 10 fleas recovered on the previous day were dissected; trypanosomes were seen in the stomachs of three, and in the stomachs and extruded fæces of two others. The fæces were preserved

in one case where they were seen, but no trypanosomes were found in the preparations:

10 proboscides injected into rat 304: result 0
10 stomachs „ „ „ 305: „ 0
10 recta „ „ „ 306: „ 0

29:iv:'11.—The 10 fleas recovered on the previous day were dissected; trypanosomes were seen in the extruded fæces of one, in the rectum and fæces of another (abundantly) and the rectum of a third (abundantly); of the last two, preparations were made of the rectal contents, and there were found pear-shaped crithidial, transitional, and post-crithidial trypaniform individuals (Text-fig. 22).

TEXT-FIG. 22.



Various forms from the rectum and fæces of two fleas of the batch of 29:iv:'11, Experiment 39 (see text). Note the trypanosome-forms (last 4 figs. to the right, second row). (× 2000.)

10 proboscides injected into rat 307: result 0
10 stomachs „ „ „ 308: „ 0
10 recta „ „ „ 309: „ +

1:v:'11.—The fleas recovered 29:iv were dissected; in one of them, a male, minute crithidial individuals were seen in the stomach contents, but not in the rectal contents; no preparation was made.

10 proboscides injected into rat 310: result 0
10 stomachs „ „ „ 311 „ 0
10 recta „ „ „ 312: „ +

4:v:'11.—The 10 fleas recovered on the previous day were dissected, trypanosomes were seen in the rectum of one, in the extruded fæces of another; no preparation made.

10 proboscides injected into rat 313: result 0
10 stomachs „ „ „ 314: „ +
10 recta „ „ „ 315: „ 0

Summary of Experiment 39.—None of the rats inoculated with organs of the fleas which had been exposed to infection two, three, or four days previously became infected. On the fifth and seventh days inoculation of the recta produced infections, while the inoculations of the stomachs were negative. On the tenth day, on the other hand, inoculation of the stomachs gave a positive, that of the recta a negative result. It is seen, therefore, (1) that the fleas first became infective on the fifth day, when also the post-crithidial trypanosomes were first found in preparations of the rectum; (2) that the developmental forms which produce infection were present in the rectum, but not in the stomach, on the fifth and seventh days; and in the stomach, but not in the rectum, on the tenth day.

(viii) The Developmental Forms of the Trypanosomes in the Flea are not infective when inoculated into the Rat during a period extending from a short time (half an hour?) after being taken up by the Flea until the Developmental Cycle is complete.

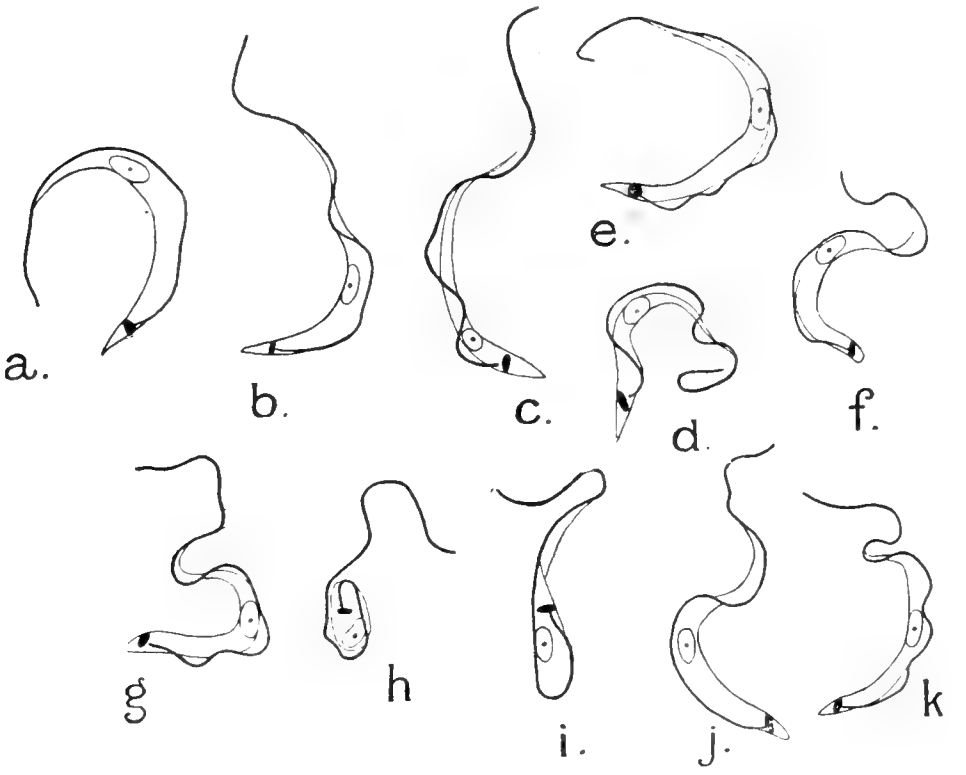
After we had shown, in Experiment 39 (see above), that the trypanosomes in the flea are not infective to the rat after they have been in the flea for two days, and that they do not acquire infectivity for five days after being ingested, we instituted a number of experiments with a view of discovering how soon the ingested trypanosomes lose their power of infecting.

In our first experiment a number of fleas collected from our non-infected breeding-cage and kept hungry for three days (7 : xi : '11 to 10 : xi : '11), were put on a well-infected rat at 6 a.m. (10 : xi : '11) and collected two hours later. They were then dissected in batches, the stomachs of each batch being placed together on the same slide, teased up in a drop of salt-solution, drawn up into an injection-syringe and inoculated into a clean rat. After the drop had been drawn up

into the syringe the film of moisture left on the slide was fixed with osmic vapour and stained with Giemsa's stain, in order to get an idea of the modification, if any, which the trypanosomes had undergone.

The following were the batches dissected and injected:

TEXT-FIG. 23.



Trypanosomes from the stomachs of fleas of the batches of 10 : xi : '11 (see text).

(1) Four fleas, injected into rat 323 at 11.35 a.m. The preserved film showed trypanosomes of the ordinary blood-type.

(2) Four fleas, injected into rat 324 at 12.35 p.m. The preserved film showed trypanosomes of the ordinary blood-type (Text-fig. 23, *a*, *b*).

(3) Four fleas, injected into rat 325 at 1.30 p.m. The preserved film showed trypanosomes for the most part unmodified, with *n* approximated to *N*, others dwarfed slightly as if beginning to degenerate (Text-fig. 23, *c*, *d*).

(4) Four fleas, injected at 2.35 p.m. into rat 326. The preserved film showed trypanosomes of the ordinary blood-type (Text-fig. 23, *e*, *f*).

(5) Four fleas, injected at 3.30 p.m. into rat 327. The preserved film

showed trypanosomes mostly modified, some rather lengthened out, others recurved (Text-fig. 23, *g*, *h*, *i*).

(6) Three fleas, injected at 4.35 p.m. into rat 327. The preserved film showed trypanosomes mostly unmodified, some rather long (Text-fig. 23, *j*, *k*).

All the results were negative, since none of the rats became infected. It is seen from the times of feeding and injecting the fleas that none of the trypanosomes had been in the fleas more than ten and half hours (6 a.m. to 4.35 p.m.), or less than three and half hours (8 a.m. to 11.30 a.m.).

After obtaining this result we made a number of other experiments, modifying slightly our method of procedure. Fleas taken from the non-infected breeding-cage were fed under observation on a well-infected rat and the time of feeding noted. The flea was recovered, dissected, and its stomach injected into a clean rat after being teased up in a drop of salt-citrate solution. As before, the film of moisture left on the slide was preserved in some cases.

In some cases also some blood from the infected rat was inoculated subcutaneously into a control clean rat. The controls were not always positive, however, since the subcutaneous method of injection is notoriously less efficient than the intra-peritoneal method, when a small quantity of blood is taken direct from the rat.

27 : xi : '11.—Fleas fed under observation on an infected rat after having been kept hungry for three days.

Fleas (1) and (2) fed at 11.35, injected into rat 329 at 12.10 (35 minutes). The preserved film showed trypanosomes quite unmodified.

Flea (3) fed at 11.45, injected into rat 329 at 12.20 (35 minutes). The preserved film showed trypanosomes quite unmodified.

Flea (4) fed at 11.30, injected into rat 330 at 12.30 (1 hour). Trypanosomes in preserved film quite unmodified.

Flea (5) fed at 11.50, injected into rat 330 at 12.50 (1 hour). Trypanosomes seen in the fresh stomach, but film not preserved.

Flea (6) fed at 11.50, injected into rat 330 at 12.50 (1 hour). Film badly preserved.

Flea (7) fed at 10.45, injected into rat 331 at 12.45 (2 hours). Trypanosomes in preserved film quite unmodified.

Summary.

Rat 329 was injected with 3 fleas 35 minutes after feeding.
„ 330 „ „ 3 „ 1 hour „ „
„ 331 „ „ 1 flea 2 hours after feeding.
The results in all three cases were negative. No control rat was injected.

8 : xii : '11.—Fleas fed under observation on an infected rat (322).

Fleas (1) and (2) fed at 11.40, injected into clean rat 324 at 12.26 (36 minutes). Active trypanosomes seen in the fresh stomachs.

Fleas (3) and (4) fed at 11·53, injected into rat 324 at 12.37 (44 minutes). Trypanosomes seen in the fresh stomachs.

Fleas (5), (6), and (7) fed at 12.4, injected into rat 324 at 12.42 (38 minutes). Trypanosomes seen in the fresh stomachs.

Fleas (8), (9), and (10) fed at 12.10, injected into rat 324 at 12.47 (37 minutes). Trypanosomes seen in the fresh stomachs.

Flea (11) fed at 12.20, injected into rat 324 at 12.55 (35 minutes).

Summary.—Rat 324 inoculated with the stomachs of eleven fleas fed on rat 322 between 35 and 44 minutes previously. Result negative.

Control rat 323 inoculated at the same time with a small drop of citrated blood from rat 322. Result positive (found to be infected on 15 : xii).

24 : i : '11.—Eight fleas, kept hungry for three days previously, were fed under observation on an infected rat (No. 392); the stomachs of six inoculated into clean rat 411.

Flea (1) fed 10 a.m, injected 11 a.m. (1 hour).
Flea (2) „ 10.10 a.m., injected 11.10 a.m. (1 hour).
Flea (3) „ 10.15 „ „ 11.14 „ (59 minutes).
Flea (4) „ 10.20 „ „ 11.20 „ (1 hour).
Flea (5) „ 10.25 „ „ 11.25 „ „
Flea (6) „ 10.29 „ „ 11.29 „ „

Fleas (7) and (8), fed 10.33 a.m. and 10.38 a.m. respectively, were dissected and fixed preparations made of them; numerous trypanosomes, quite unmodified in appearance, were found in them.

Summary.—Rat 411 inoculated with the stomachs of six fleas, each of which had fed on infected rat 392 an hour previously. Result negative.

Control rat 412 inoculated with a small drop of citrated blood from rat 392 at 12 noon. Result negative. (N.B.—Rat 412 put into the infected breeding-cage on 15 : ii : '13 contracted an infection in due course and was therefore not naturally immune.)

11: ii : '13.—Seven fleas fed on infected rat 402 under observation. The stomachs inoculated into clean rat 414.

Flea (1)	fed 10. 9 a.m.,	injected	11. 7 a.m.
Flea (2)	„ 10.12 „	„	11.12 „
Flea (3)	„ 10.17 „	„	11.17 „
Flea (4)	„ 10.25 „	„	11.25 „
Flea (5)	„ 10.28 „	„	11.28 „
Flea (6)	„ 10.37 „	„	11.38 „
Flea (7)	„ 10.58 „	„	11.58 „

(In all cases the time of feeding was reckoned from the moment the flea withdrew its proboscis.)

Some of the blood was allowed to escape from the stomach of flea (7) and permanent preparations made; numerous trypanosomes quite unmodified in appearance were found.

Summary.—Rat 414 acquired no infection; control rat 415, inoculated with one drop of blood from rat 402, became infected (trypanosomes first seen, 21 : ii : '13).

25 : ii : '13.—One flea was fed under observation at 10.45 a.m. on infected rat 415; the stomach was inoculated into clean rat 419 at 11.45. Results negative. (The other fleas refused to feed.)

27 : ii : '13.—Eight fleas fed under observation on infected rat 415; the stomachs inoculated into clean rat 419, in each case 59 minutes or an hour after feeding. Result negative.

Control rat 414 inoculated with a drop of blood from rat 415. Result negative (rat 414 was later infected by being put into the infected breeding-cage.)

It will be seen from the foregoing accounts that all experiments, in which infected blood ingested by a flea was inoculated into a clean rat from about half an hour onwards after ingestion by the flea, gave uniformly negative results. The controls were sometimes negative, sometimes positive.

(ix) The Flea, when once it has become infective, remains so for a considerable Length of Time.

This point was dealt with in our preliminary account (1910), when it was shown in two experiments ("C" and "D") that cages of fleas when once rendered infective continued to produce infections for some time without being re-infected. Experiment 22 ("C") was continued for some time after the publication of our paper, but on 28 : i : '10 an infected rat was unfortunately introduced into the cage, an oversight which vitiated all subsequent results, and the experiment was discontinued. During the period prior to this accident, however, the experiment was not open to objection, and produced a result which may be summarised as follows. A cage colonised with 160 clean fleas, into which an infected rat was introduced for three days (24 to 27 : xi : '09), and which produced the first infection between 30 : xi : '09 and 3 : xii : '09, continued to produce infections without being re-infected up to 24 : i : '10, a period of approximately 55 days. It is, therefore, a safe conclusion to affirm that the infectivity persisted in some fleas, or at least one flea, for that period of time.

Later a more exact experiment (Experiment 37) was carried out, in which single fleas were used. To begin with, 10 fleas were taken from the infected breeding-cage and put each on a clean rat for four days; at the end of that time the 10 fleas were recovered and each flea put by itself in a separate test-tube in damp sand for six days. Meanwhile the 10 rats were examined daily, and in due course two of them developed infection, the remaining eight being negative. The eight fleas that gave negative results were returned to the infected breeding-cage.

The two fleas that had been proved experimentally to be infective—henceforth known as Flea "A" and Flea "B"—were then placed singly on a succession of clean rats. That is to say, each flea was placed by itself on a clean rat for so many days, then was recovered and placed on another clean rat for so many days, and so on. The experiments with each

flea were continued until the flea disappeared: that is to say, until the flea could not be recovered when sought for.

The details and results of Experiment 37 have been summarised in tabular form in our preliminary communication (1911), but since we have frequently had occasion to refer to the experiment in the present memoir, we think it worth while to reproduce the table already published.

TABLE G.—Summary of Experiment 37.

Flea "A."

No. of rat.	Flea on the rat.	Result.	Trypanosomes.	
			First seen in blood.	Multiplication ended.
265	14–18 : ii : '11	+	24 : ii : '11	27 : ii : '11
249	24 : ii–1 : iii : '11	0		
260	1–6 : iii : '11	+	15 : iii : '11	(Rat died, 16 : iii : '11)
291	6–13 : iii : '11	0		
293	13–17 : iii : '11	0		
295	17–22 : iii : '11	+	27 : iii : '11	31 : iii : '11
249	23–27 : iii : '11	0		
291	27 : iii–1 : iv : '11	+	5 : iv : '11	9 : iv : '11
293	1–5 : iv : '11	0		
249	5–10 : iv : '11	0		
262	10–15 : iv : '11	0		
296	15–20 : iv : '11	+	22 : iv : '11	25 : iv : '11
263	20–25 : iv : '11	+	1 : v : '11	5 : v : '11
292	25–29 : iv : '11	+	1 : v : '11	6 : v : '11
293	29 : iv–4 : v : '11	0		
294	4–9 : v : '11	?	(Rat died, 9 : iv : '11)	
249	9–15 : v : '11	0		
	(15 : v : '11 flea not recovered.)			

Flea "B."

272	14–18 : ii : '11	+	24 : ii : '11	28 : ii : '11
259	24 : ii–1 : iii : '11	0		
253	1–6 : iii : '11	0		
292	6–13 : iii : '11	0		
294	13–17 : iii : '11	0		
296	17–22 : iii : '11	0		
259	23–27 : iii : '11	+	30 : iii : '11	4 : iv : '11
253	27 : iii–1 : iv : '11	+	5 : iv : '11	7 . iv : '11
292	1–5 : iv : '11	0		
294	5–10 : iv : '11	0		
	(10 : iv : '11 flea not recovered.)			

From the tabular summary it is seen that flea "A" remained infective from about 15 : ii : '11 to about 26 : iv : '11 —that is to say, for a period of 70 days—and flea "B" from about 16 : ii : '11 to about 27 : iii : '11—a period of 40 days. We have no data for determining the maximum period of time during which a flea can remain infective. It is, perhaps, not improbable that a flea, once rendered infective, may remain so as long as it lives, but we have no knowledge with regard to the average longevity of the flea. Flea "A" (♀) lived under our care from 14 : ii : '11 to 9 : v : '11—a period of 84 days; but we have no clue as to its age when it was first taken from the breeding-cage.

(x) The Trypanosome does not penetrate into the Salivary Glands of the Flea, but is confined, during its whole Development, to the Digestive Tract.

To prove this point we began by dissecting out salivary glands of fleas taken from the infected breeding-cage and examining the glands both in the fresh condition, by teasing them up or crushing them with a coverslip, in a drop of salt-citrate solution, and also by means of fixed permanent smears of the glands. In a large number of glands examined very carefully in this way many yeast-like organisms and other similar bodies were found, but never anything that appeared in the least like any possible stage of a trypanosome. Examination of fluid from the body-cavity gave negative results also.

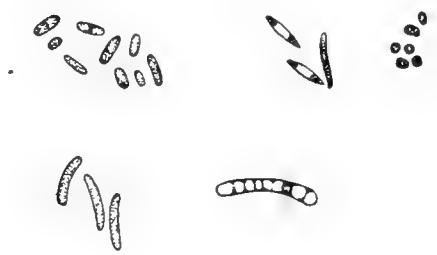
Thinking that we might have failed in these examinations to obtain an infective flea, we took fleas from the infected breeding-cage, put them singly on clean rats for three days or so, and then recovered and dissected them. The organs of the fleas were examined carefully in the fresh condition, and in some cases permanent preparations were also made of them and laid aside. If a rat became infected subsequently, and so proved the flea put on it to have been infective, the preparations of that flea were searched very carefully.

In this way we were able to examine the salivary glands and other organs of fleas which had been proved experimentally to be infective. The following are the details of the experiments and observations; the sign + signifies that trypanosomes were found in the organs mentioned, while 0 means that none were found:

(1) Flea (♀) put on rat 212 for three days (19 : ix : '10 to 22 : ix '10, Experiment 29). Rat 212 found to be infected 28 : ix; multiplication ended 30 : ix. Flea dissected 22 : ix; proboscis 0, salivary glands 0, stomach + (Fig. 17), rectum 0, intestines 0.

(2) Flea (♂) put on rat 223 for four days (10 : x : '10 to 14 : x : '10, Experiment 32a). Rat 223 found to be infected 17 : x; multiplication

Text-fig 24.



Yeast-like bodies of various kinds from the salivary glands of a flea. They are shown in groups, as they were found in the preparation. (× 2000.)

ended 22 : x. Flea dissected and examined 14 : x; no trypanosomes were seen in the proboscis, body-cavity, stomach, intestine, rectum or salivary glands; but unfortunately no permanent preparations were made.

(3) Flea (♀) put on rat 233 for five days (20 : x : '10 to 25 : x : '10, Experiment 32e). Rat 233 found to be infected 28 : x; multiplication ended 31 : x; scanty infection, with few trypanosomes. Flea dissected and examined 25 : x; no trypanosomes seen in proboscis, body-cavity, stomach, intestine, rectum or salivary glands; permanent preparations made of salivary glands, 0. (N.B.—Many yeast-like bodies in the salivary glands, see Text-fig. 24.)

(4) Flea (♂) put on rat 238 for three days (1 : xi : '10 to 4 : xi : '10. Experiment 32f). Rat 238 found to be infected 14 : xi; multiplication ended 16 : xi. Flea dissected and examined 4 : xi; no trypanosomes were seen in the proboscis, stomach, intestine, rectum or salivary glands; permanent preparations made of the stomach and salivary glands, both 0.

From the foregoing experiments it is seen that nothing which could be recognised as a trypanosome was found in the salivary glands of four fleas known to have been infective. In one of the four fleas the salivary glands were examined only in the fresh condition, but in the other three fleas the salivary glands were examined both in the fresh condition and in the permanent preparations.

It will be remarked, however, that no trypanosomes were found in any part of the digestive tract, when examined fresh, in three out of the four fleas, although there can scarcely be any doubt that trypanosomes must have been present. As a matter of fact, a scanty infection of the crithidial or final trypanosome-forms, small in size and sluggish in movement, is easily overlooked in the fresh preparations of the teased-up digestive tract, especially in an organ relatively so large as the stomach, which may be gorged with blood-débris greatly hindering and obscuring the examination. It has been our experience not infrequently that the smaller forms of the cycle have been found scantily in permanent preparations of stomachs in which nothing was seen in the fresh examination. This scarcely applies, however, to organs so minute as the salivary glands, the contents of which can be scanned comprehensively in one field of the microscope. We have, therefore, cited the second flea in spite of the fact that no permanent preparations of the salivary glands were examined.

After having made the negative observations recorded above, the idea occurred to us that any form of the trypanosome found in the salivary glands would probably be a final stage of the cycle, destined to be inoculated by the flea through the proboscis into the rat; and that consequently the examination of fleas which had produced an infection recently would be inconclusive, since in such fleas the salivary glands might be purged of their infection, temporarily at least.

We therefore carried out some experiments in which the object was to determine which organs of the flea contained the infective stages of the trypanosome, by dissecting fleas taken from the infected breeding-cage and injecting their

organs separately into clean rats. Thus a batch of fleas, at least five in number, was taken from the infected breeding-cage, and all the fleas in the batch were dissected at the same sitting. The stomachs of all the fleas were put together in one watch-glass and the salivary glands in another. Each flea has four salivary glands (two on each side of the body), but we did not succeed in every case in dissecting out all four of these minute organs; sometimes only two or three were obtained, or even one only of the four (on foggy mornings); but in every case at least one of the four glands was obtained. In the case of the recta (cut off behind the pylorus and therefore including the greater part of the intestine as well), each was examined microscopically and only kept for injection if seen to contain trypanosomes.

In our earlier experiment (Experiment 33) only the salivary glands were used for injection, with the following results:

TABLE H.—Experiment 33.

Date.	No. of fleas dissected.	No. of salivary glands injected.	Rat.	Result.
17 : xi : '13	11	34	248	0
5 : xii : '10	5	18	248	0

Hence fifty-two salivary glands, dissected out from sixteen fleas taken from the infected breeding-cage, failed to infect a clean rat when injected into it.

Our later experiment (Experiment 34) was carried out more elaborately. In the first place each batch of fleas, after having been collected from the infected breeding-cage, was first put on a clean rat, as a control. Then the fleas were recovered from the control rat and dissected; the salivary glands and stomachs were then injected into two clean rats respectively, and the recta, if anything was seen in them, into a third. The details of Experiment 34 are shown in the following table:

TABLE I.—Experiment 34.

No. of fleas.	Fleas put on control rat.	Fleas recovered from control rat.	Fleas dissected.	No. of salivary glands injected.	No. of rat (s. g.).	Result	No. of stomachs injected.	No. of rat (stom.).	Result.	No. of recta injected.	No. of rat (recta).	Result.	No. of control rat.	Result control.
6	22 : xi : '10	25 : xi : '10	25 : xi : '10	24	256	0	6	257	0	1	255	0	239	0
6	12 : xii : '10	15 : xii : '10	15 : xii : '10	22	256	0	6	257	+	—	—	—	239	+
5	5 : i : '11	9 : i : '11	12 : i : '11	19	243*	0	5	245	+	—	—	—	231*	0
6	6 : i : '11	10 : i : '11	13 : i : '11	24	246	0	6	247	+	2	259	0	241	+
6	19 : i : '11	25 : i : '11	26 : i : '11	24	253*	0	6	259*	0	1	258	0	250	+
6	19 : i : '11	25 : i : '11	26 : i : '11	22	260	0	6	261	+	—	—	—	251	+
5	19 : i : '11	25 : i : '11	27 : i : '11	15	262	0	5	263*	0	—		—	252	+

The rats marked * became infected in subsequent experiments, and were therefore not naturally immune.

From the details summarised in the above table it is seen that no infection was produced by 150 salivary glands taken from forty fleas, divided into seven batches. In three of these batches, comprising eighteen fleas, from which sixty-eight salivary glands were obtained, both the control rats and the rats infected with the stomachs[1] became infected. In a fourth batch, comprising five fleas from which nineteen salivary glands were obtained, the control rat was negative, the rat injected with the stomachs was positive. In a fifth batch, comprising five fleas from which fifteen salivary glands were obtained, the control rat became infected but the stomachs produced no infection; the infectivity of this batch must have been in the recta, which unfortunately were not injected. Thus, omitting the two batches that gave negative results throughout and reckoning only with five batches proved to contain infective fleas, it is seen that 102 salivary glands obtained from twenty-eight fleas produced no infection. These results convinced us finally that the salivary glands of the flea play no part whatever in the transmission or development of the trypanosome, and from this time we paid no further attention to them.

(xi) The Rat can become infected by eating infected Fleas, but not until the Developmental Cycle of the Trypanosome in the Flea is completed.

The fact that rats can become infected by eating infected fleas must now be considered as well established. It was first stated in print by Strickland (1911), but was then already known to us both from experiments performed by ourselves and from others carried out by Dr. Nicoll at the Lister Institute (see our preliminary report, 1911).

We carried out some further experiments to determine whether the rat could become infected in this way before the

[1] It should be noted that the stomachs here included the post-pyloric upper end of the intestine, which, as shown above, is often the site where the trypanosomes establish themselves.

developmental cycle of the trypanosome was completed in the flea. The experiments (Experiment 46) were carried on for a long period, each experiment occupying a week. The following sample is typical of the whole series, all being performed in the same manner and each corresponding stage of the experiment being carried out on the same day of the week.

Sunday, 20 : iv : '13.—A large batch of fleas (batch 6) collected from the non-infected breeding-cage the Friday previously were put on a well-infected rat.

Tuesday, 22 : iv : '13.—About fifty fleas were collected from the batch (6) and fed to the "two-day rat," clean rat 445. The method was to place the fleas, when collected, on the surface of water in a suitable vessel; then each flea was carefully picked off with a fine forceps, and either it was decapitated on a slide in a drop of water with a needle, or its head was crushed with the forceps. Several fleas so treated were stuck into a pellet of damp bread and given to the rat, previously kept hungry for a time. As a rule the rats when fed in this way ate both the bread and the fleas readily and even greedily.

Thursday, 26 : iv : '13.—About fifty more fleas of the infected batch (6) were collected and fed to the "four-day rat," clean rat No. 448, in the same manner.

Saturday, 24 : iv . '13.—About fifty more fleas of the infected batch (6) were collected and fed to the "six-day rat," clean rat 432, in the same manner.

On the Sunday following a fresh batch (batch 7) was exposed to infection in the same way. On the Tuesday following the "two-day rat" (No. 445), being found not to have become infected, was fed again with about fifty fleas of batch 7. On the Thursday following the four-day rat (No. 448), not having become infected, was fed again with about fifty fleas of batch 7. On the Saturday following (3 : v : '13) the six-day rat (No. 432), which then showed no trypanosomes in its blood, was fed with thirty-four fleas of batch 7. Rat 432 was found, however, to be showing trypanosomes in its blood when examined four days later (7 : v : '13); it must have been infected by the fleas of batch 6. For batch 8, in the following week, a fresh clean rat, No. 452, was appointed to be the new six-day rat; it later became infected by the six-day fleas of batch 12.

These experiments were continued in regular routine in the manner described, from 16 : ii : '13 to 22 : iii : '13, and from 20 : iv : '13 to 12 : vi : '13, in all thirteen weeks and

thirteen batches. The results may be summarized briefly: No rat fed either with fleas exposed to infection two days previously, or with fleas exposed to infection four days previously, became infected; on the other hand, two of the six-day rats fed with fleas exposed to infection six days previously became infected. It may be inferred, therefore, that rats cannot be infected by eating infected fleas until the infection in them is ripe, that is to say until the developmental cycle of the trypanosome in the flea is complete.

(It may be mentioned here that when any fleas remained over from any of the batches used in this experiment they were fed under observation on clean rats on the Monday, Tuesday or Wednesday following, that is to say, eight, nine or ten days after they had been first exposed to infection (see p. 654, below).)

(xii) Infection of the Rat is effected contaminatively, by way of the Rat's Mouth, by the Rat licking from off its Fur or Skin the Moist Fæces of Infective Fleas containing the Final Propagative Form of the Cycle.

This mechanism of infection was first demonstrated by Nöller (1912) and fully confirmed by Wenyon (1913) by means of experiments which put the matter beyond all reasonable doubt. Without repeating the experiments of these authors, we tested their results by another method, namely, by exposing rats, muzzled and pinioned, to infection by a large number of infected fleas.

For the purpose of our experiments, we made use in most cases of our infected breeding-cage, in which the fleas were swarming in great numbers, and in which an infected rat is kept habitually, so that the fleas ingest blood containing trypanosomes every time they feed. As a preparation for the experiment the infected rat was removed from the breeding-cage and kept apart, all fleas found on it being carefully cleaned off and returned to the breeding-cage. The breed-

ing-cage was then left without a rat in it for a certain time, sometimes merely from morning to evening, in other cases a day or two, in order to induce hunger in the fleas. Then a clean rat was introduced into the cage for a single night or from morning to evening, after having been muzzled in the following manner: The muzzle was a conical cap of fine wire gauze, with meshes too fine for a flea to pass through. The cap was large enough to cover the whole head, including the ears; the opening of the cone had a broad rim or sleeve of soft cloth, and a draw-tape was run through the free edge of the sleeve, so that by pulling the two ends of the tape the sleeve could be drawn up as tight as required. When fixing the muzzle, it was slipped over the head and then the sleeve was tightened round the neck behind the ears; the two free ends of the tape were then passed downwards and forwards over the chest and backwards under each axilla; each end of the tape was given a single turn round the upper joint of the fore-leg of its side and then passed backwards and upwards to be tied to the other end of the tape from the other side of the body over the back of the rat. In this way, not only was the head of the rat muzzled so that it could not lick itself or eat fleas, but owing to the fore-legs being secured firmly it could not use them to tear off its muzzle, which the rat always makes violent efforts to do, and which, in spite of all precautions, it sometimes succeeds in doing.

The clean rat, having been muzzled in the manner described, and exposed to the attentions of the fleas for a certain time, was removed from the breeding-cage, and before being unmuzzled it was subjected to a cleansing process which consisted of removing all fleas from it and of washing its fur all over thoroughly with a disinfectant (lysol, about 2 per cent.). The liquid used for washing usually became coloured reddish-brown from the fæces deposited on the fur by the fleas. After the rat had been cleansed thoroughly, its fur was dried by holding it close to an ordinary electric lamp and then its muzzle was removed and it was allowed to lick itself as much as it wished, being kept apart from all fleas and its blood examined at regular intervals.

As a variation of the above procedure the muzzled rat was not put into the infected breeding-cage in some instances, but into a bell-jar with a certain number of fleas taken from the infected breeding-cage and previously kept hungry.

At first the experiments were controlled by putting unmuzzled rats into the infected breeding-cage, but it was found superfluous to do this, since in many of our actual experiments the muzzled rats succeeded in tearing off their muzzles and thus furnished improvised but very efficient controls.

The following is a brief statement of the experiments (controls marked *) :

(1) Rat 447, muzzled and put into the infected breeding-cage from 4.30 p.m., 23 : iv : '13, to 10 a.m., 24 : iv : '13. Examined from 28 : iv to 30 : v, not infected ; inoculated later from wild rat and acquired infection in due course.

(2) Rat 449, muzzled, put into infected breeding-cage for night of 25–26 : iv : '13. Examined up to 30 : v, no infection ; put into infected breeding-cage, 30 : v, became infected in due course.

(3) * Rat 450, muzzled, put into infected breeding-cage evening of 30 : iv : '13 : found next morning with its muzzle off ; it became infected in due course.

After the above-mentioned experiments had been performed in the manner described, namely, by cleaning and disinfecting the rat before unmuzzling it, a change of procedure was adopted. The rat that had been muzzled and put in the infected breeding-cage was not disinfected when taken out, but, after having been freed from fleas, its fur was merely dried thoroughly by holding it near an electric lamp before unmuzzling the rat and allowing it to lick its fur. This was in order to see whether dried fæces would produce an infection when licked off.

(4) Rat 467, muzzled, put into infected breeding-cage, 8 a.m. to 4 p.m., 16 : ii : '14 (the infected rat having been removed from the cage two days previously). Examined up to 5 : iii, no infection.

(5) Rat 468, muzzled, put into infected breeding-cage 10 a.m. to 2 p.m., 19 : ii : '14. Procedure otherwise same as in last, no infection.

(6) *Rat 469, muzzled, put into infected breeding-cage, 7 p.m., 20 : ii : '14; found next morning (8 a.m.) with muzzle off; became infected in due course.

(7) Rat 470, muzzled, put into infected breeding-cage 11.30 p.m., 23 : ii : '14, treated as 467, etc.; no infection. Muzzled and put into bell-jar with 200 hungry fleas taken from infected breeding-cage 9 a.m. to 2.30 p.m., 3 : iii : '14; no infection.

(8) *Rat 472, muzzled, put into bell-jar with 200 hungry fleas from the infected breeding-cage, 9 a.m., 26 : ii : '14; found at 2.30 p.m. with its muzzle off; became infected in due course.

From the data quoted, it is seen that when the experiments were carried out successfully, that is to say, when the muzzle kept on, the rat did not become infected, alike whether its fur was cleaned and disinfected, or merely dried, before it was unmuzzled. But in those cases in which the rat succeeded in ridding itself of its muzzle by its own efforts, it became infected in due course.

It is evident that the sole effect of the muzzle is to exclude infection of the rat by way of its mouth. The way is still open for the rat to become infected through the skin, either (1) by the trypanosomes passing in through the puncture made by the proboscis of the flea, a possibility suggested by Nöller; or (2) by the fæces being rubbed into wounds or abrasions on the skin; or (3) by the small trypanosomes in the fæces penetrating by their own efforts through the skin. But since all the results with rats muzzled efficiently were negative, it is evident that no infection *per cutem* by any of these possible ways occurred in these experiments and it becomes highly probable that it does not take place naturally in any of these ways. On the other hand, since the muzzle excludes only infection *per os*, it becomes also probable that the rats that succeeded in tearing off their muzzles were infected in this manner.

It may be mentioned finally that an experiment (Experiment 43) was carried out in which the fæces, collected overnight in a moist glass capsule, of fleas from the infected breeding-cage were injected under the skin of a clean rat, but the result was negative.

(xiii) Can the Flea infect the Rat by inoculating the Trypanosomes into it through the Proboscis?

The first to throw doubt upon the occurrence of this mode of transmission were Strickland and Swellengrebel (1910, '12), who made a number of attempts to infect rats by feeding infected fleas on them through gauze. Every such experiment gave negative results. We repeated these experiments, and always with the same negative results.

We also carried out a large number of experiments in which fleas taken from the infected breeding-cage were fed on clean rats under observation. Our course of procedure, in its latest and most highly elaborate form, was as follows :[1]

A certain number of fleas, collected from the infected breeding-cage, or fed on an infected rat more than seven days previously, or known experimentally to be infective, were kept in a flask containing some damp sand for three or more days, to make them hungry. When the experiment was about to take place the fleas that it was proposed to use were put each into a separate test-tube. A clean rat was prepared for the experiment by shaving a small region of its skin, usually on the belly, sometimes on the inside of the thigh (a favourite spot for fleas to feed). The rat was then held still by an assistant, with its tonsure upwards. The test-tube was inverted on to the tonsure and at first kept pressed upon it; the flea was thus emptied out on to the shaved area of the skin. At first the flea runs round and round inside the circle formed by the rim of the test-tube, but usually comes to rest very soon, and inserts its proboscis into the skin. In some cases, however, the flea refuses to feed, and either continues to run about or remains perfectly still in one place, presenting a deceptive appearance

[1] We did not adopt Nöller's method of tethering the fleas, since it seemed to us better that the flea should be free and unhampered in its movements, and would then be more likely to feed in a natural way. Our colleagues, Dr. Martin and Mr. Bacot, who were doing experiments at the same time on transmission of plague, also found it unnecessary to tether the fleas.

of being engaged in feeding, but when recaptured and examined it is found to have been merely thinking.

In fleas carefully watched it is not difficult to observe with a hand-lens the penetration of the proboscis into the skin, the rush of blood into the stomach of the flea, and the withdrawal of the proboscis when the flea is replete. From a number of fleas that were timed carefully it was found that the male flea took about $1\frac{1}{4}$ minutes to fill its stomach, the female about $2\frac{1}{4}$ minutes. In all the fleas we have fed under observation we have never once observed the flea to defæcate while feeding, though particular attention was directed to this point. Only in one case, when the flea after feeding succeeded in making good its escape into the fur, it was found to have defæcated there.[1]

While the flea is feeding an assistant holds over it a paint-brush dipped in a thick syrupy solution of sugar and water. As soon as the flea has filled its stomach it withdraws its proboscis and makes a rush for the fur of the rat, but as soon as it does so the assistant dabs the paint-brush down on it and catches it. From the paint-brush, to which it sticks, the flea is put in water, which cleans off the sugar-syrup; it can then be dissected or put back in the cage, none the worse for its adventure. If it succeeds in getting into the fur of the rat it must be recaptured, and any fæces it may have deposited must be washed off with a disinfectant.

Fleas that refuse to feed can either be put back in their test-tubes and given another chance on the following day, or dissected and examined as controls.

In the majority of cases the fleas fed under observation were either fleas taken at random from the infected breeding-cage or fleas which had been fed on an infected rat at a

[1] Our experience of the feeding habits of Ceratophyllus fasciatus does not agree in the least with the account given by Nöller for Ctenocephalus canis. The habits of the two species are evidently quite different. The former lives in the burrows of the rat, and only goes on to the rat in order to feed, while the latter lives more or less permanently in the fur of the dog.

definite time, and of which the age of the infection was known exactly. In a few cases we used fleas which had been put singly on clean rats and had produced an infection, and were therefore known to be infective. Thus rat 367a had a "known infective" flea fed on it on 26 : vii : '12 and again on 29 : vii : '12, and another such flea was fed on the same rat on 3 : viii : '12, but the rat did not become infected (Experiment 44a).

We have in our notebooks records of 150 fleas fed on rats under observation in this way. Since the results were uniformly negative it is quite unnecessary to refer to them in further detail, but it may be of some interest to mention some cases in which the fleas were dissected and examined immediately after the experiment. These were fleas left over from the batches used in Experiment 46 (see above, p. 648), and were therefore, all of them, fleas in which the age of the infection was known.

11 : iii : '13.—Six fleas left over from batch 3; infection of the fleas nine days old. Five fleas fed, in three of which no trypanosomes were seen, in a fourth the stomach contained an infection of crithidial forms, and in a fifth both stomach and rectum contained crithidial forms. (In a sixth flea, which had not fed, both stomach and rectum also contained crithidial forms.)

12 : iii : '13.—Four fleas of the same batch as yesterday; infection ten days old. All fed. In two nothing was seen, the other two had crithidial forms in the rectum.

13 : iii : '13.—Two fleas of the same batch as last; infection eleven days old. One, which fed, had a few crithidial forms in the rectum. (In the other, not fed, nothing was found.)

17 : iii : '13.—Four fleas of batch 4; infection eight days old. One which fed, had a swarming infection of the rectum and a few crithidial forms in the stomach. (Of the three which did not feed all had crithidial forms in the rectum, one in the stomach also.)

18 : iii : '13.—Three fleas of the same batch as last; infection nine days old. One, which fed, had crithidial forms in the stomach. (Of the two which did not feed, in one nothing was found, the other had scanty crithidial forms both in stomach and rectum.)

19 : iii : '13.—Six fleas of the same batch as last; infection ten days old. Four fed. In one nothing was found; in two there were crithidial forms in the rectum only; in the fourth there were crithidial forms in

the stomach only. (Two did not feed. In one of them nothing was found, in the other there were crithidial forms in the rectum only.)

5 : v : '13.—Ten fleas of batch 8; infection eight days old. Five fed. Of these three showed crithidias in the rectum only, one in the rectum and stomach and one was quite negative. (Five did not feed. Of these one had crithidial forms in the stomach only, one in the rectum only, one both in the stomach and rectum, and two were quite negative.)

13 : v '13.—Six fleas of batch 8, infection nine days old. One fed, five did not; the examination of all the six was negative in result.

14 : v : '13.—Eight fleas of the same batch as last; infection ten days old. Five fed, three did not; the examination in all cases was negative in result.

19 : v : '13.—Six fleas of batch 9; infection eight days old. Only one flea fed; all the six negative.

20 : v : '13.—Six fleas of the same as last; infection nine days old. Four fed, two did not; all six negative.

26 : v : '13.—Six fleas of batch 10; infection seven and a half days old. One fed which was negative. (Of the five which did not feed, one was quite negative, three had crithidial forms in the rectum only, and one both in the stomach and rectum.)

27 : v : '13.—Six fleas of the same batch as last; infection eight and a half days old. Of four that fed, two were quite negative, two had crithidial forms in the rectum. (The two that did not feed had crithidial forms, both in stomach and rectum.)

2 : vi : '13.—Six fleas of batch 11; infection eight days old. Three that fed in two cases crithidial forms in the rectum only, the third was quite negative. (In the three that did not feed, two were quite negative, one had crithidial forms in the rectum only.)

3 : vi : '13.—Six fleas of same batch as last; infection nine days old. Two that fed were both negative. (In the four that did not feed, two had crithidial forms in the rectum only, one in the stomach only, one in both rectum and stomach.)

9 : vi : '13.—Seven fleas of batch 12; infection eight days old. Three fed, two of which had crithidial forms in the rectum only; one was quite negative. (All the four that did not feed had crithidial forms in the rectum, and one of them in the stomach also.)

It is seen from the data that in many cases the fleas that fed on the rats contained copious infections in the rectum, the stomach or both; but not in a single case was any infection produced in the rats fed upon by the fleas. At the present time, therefore, the answer to the question posed at the head of this section must be a very decided negative.

(xiv) Hereditary Transmission of the Trypanosome from Flea to Flea does not, in our Experience, take place.

In order to obtain, if possible, fleas infected hereditarily, a clean, freshly-prepared breeding-cage was colonised with 648 flea-larvæ taken from the infected breeding-cage at various dates between 23 : ix : '10 and 26 : x : '10. Adult fleas were first seen in the cage on the latter date, subsequently to which 100 more larvæ were added (3 : xi : '10).

Thinking, however, that the larvæ in the infected breeding-cage might possibly infect themselves directly from the fæces of adult fleas in the cage, we also colonised another clean breeding-cage with larvæ newly-hatched from eggs laid by fleas taken from the infected breeding-cage. The method was to take a certain number of fleas from the infected breeding-cage and keep them overnight in a glass capsule containing a glass coverslip at the bottom. In the morning a certain number of eggs were usually found, some on the glass of the capsule, some on the coverslip. The fleas having been returned to the infected breeding-cage, the eggs were carefully removed by means of a soft camel's-hair paint-brush, slightly moistened, from the glass of the capsule and each egg was placed on a small piece of black paper, to which it adhered. At first the eggs, attached either to the coverslip or to the black paper, were put in the new breeding-cage and allowed to hatch there; between 1 : x : '10 and 11 : x : '10 there were sixty eggs introduced in this way, but since they did not all hatch, another method was adopted. The eggs laid were kept in a glass capsule till they hatched, and then the newly-hatched larvæ were put into the breeding-cage. In this way 159 larvæ were introduced into the breeding-cage between 18 : x : '10 and 22 : xi : '10. The time taken by the eggs to hatch varied between six to eight days in October and ten to twelve days towards the end of November (laboratory-temperature).

In the two cages colonised in this way clean rats were kept

for a long time, but no infection was produced in either case.

(xv) The Trypanosomes in the Blood of the Rat can render Fleas infective very soon after they make their First Appearance in the Blood, before their Multiplication-Period is over.

In their paper on the life-history of Trypanosoma lewisi in the rat-louse, Breinl and Hindle (1909) state that they were unable to produce an infection of the louse when it was fed on the rat during the multiplication-period; they state that "during the first stages of infection, so long as dividing and segmenting forms were present in the blood, the trypanosomes taken up by the louse only degenerated." Wishing to find if this was true for the flea also, we did two experiments (Experiments 26 and 28) in which a number of fleas were first fed on an infected rat during the multiplication-period of the trypanosomes. The fleas were then recovered and used to colonise a freshly-prepared flea-cage, into which a clean rat was put. In both cases the result was positive, showing that fleas can become infective after having fed on infected rats in which the trypanosomes are undergoing multiplication.

We then planned and carried out a more elaborate experiment (Experiment 40) to determine how soon a rat infected by fleas can infect fleas again. For this purpose rat 317 was placed for one day (15–16 : v : '11) in the infected breeding-cage after the cage had been kept without a rat in it for three days, to make the fleas hungry. Rat 317 first showed trypanosomes in its blood on 21 : v; the multiplication-period was ended 25 : v or the following day.

Meanwhile, a succession of eight flea-cages, numbered A to H, were colonised with clean fleas, taken from the non-infected breeding-cage, and rat 317 was put in each cage successively for one day, thus :

Cage	A,	colonised	with	70	fleas,	12 : v ;	rat 317 put in	16–17 : v
,,	B	,,	,,	70	,,	13 : v	,, ,,	17–18 : v
,,	C	,,	,,	50	,,	15 : v	,, ,,	18–19 : v
,,	D	,,	,,	50	,,	16 : v	,, ,,	19–20 : v
,,	E	,,	,,	50	,,	17 : v	,, ,,	20–22 : v
,,	F	,,	,,	50	,,	18 : v	,, ,,	22–23 : v
,,	G	,,	,,	50	,,	19 : v	,, ,,	23–24 : v
,,	H	,,	,,	50	,,	20 : v	,, ,,	24–25 : v

After rat 317 had been taken out of each of the cages, a clean rat was put into the cage in its place and left in, with the following result :

Rat 218	put in	Cage	A,	17 : v ;	result	0	
,, 264	,,	,,	B	18 : v	,,	0	
,, 266	,,	,,	C	19 : v	,,	0	
,, 267	,,	,,	D	20 : v	,,	0	
,, 268	,,	,,	E	22 : v	,,	0	
,, 269	,,	,,	F	23 : v	,,	0	
,, 270	,,	,,	G	24 : v	,,	+	Found to be infected. 14 : vi.
,, 271	,,	,,	H	25 : v	,,	0	

From the above data it is seen that rat 317, exposed to infection 15–16 : v, did not render any fleas infective before the batch that fed on it in cage G, 23–24 : v, seven to nine days after it had been infected and two days after trypanosomes were first seen in its blood, at a time when the multiplication of the trypanosomes was proceeding actively. The late appearance of the infection in rat 270 (the record of which indicates that infection took place about 6 : vi, twelve days after it had been in contact with the fleas and thirteen days after the fleas themselves had been exposed to infection), indicates that only a small percentage of the fleas in cage G became infective.

From these experiments we deduce that infection of the flea is not dependent on the presence of special propagative forms of the trypanosome produced late in the rat's blood.

(3) Problems of Special Nature.

(xvi) The Trypanosomes succeed in establishing themselves in the Flea and rendering it infective to the Rat in only a Small Proportion of the Fleas (Ceratophyllus fasciatus) that ingest them.

It has already been pointed out above, in the description of the developmental cycle of the trypanosome, that in the greater number of the fleas fed on infected rats the trypanosomes degenerate and die out completely, and that they succeed in establishing themselves in but a small percentage of the fleas. We have also tested this question experimentally by the method of taking fleas from the infective breeding-cage and putting these fleas on clean rats. At first we carried out such experiments by taking small batches, each of five or six fleas, from the infected breeding-cage and putting each batch separately on a clean rat. Eleven such experiments were performed with the results summarised in Table J, from which it is seen that three batches, each of five fleas, produced one infection, and that eight batches, each of six fleas, produced four infections. In each case, as in many of the subsequent experiments now to be recorded, the fleas were left on the rat three or four days on the supposition, which is probably correct, that a flea will become sufficiently hungry to feed in the course of three days, and on the further supposition, which has now proved to be incorrect, that the infection passes into the rat through the proboscis of the flea.

The results of the experiments summarized in Table J are inconclusive, and could only permit of deductions approximately exact if carried out in great number. When a batch gives a positive result there is no clue as to the number of infective fleas contained in it. Further, it has been brought home to us by subsequent experience that an infective flea often fails to produce an infection. Thus, comparing Table J with Table I, it is seen that the batch of 5 : i : '11 failed to infect rat 231 (a rat infected subsequently in another ex-

TABLE J.—Experiments to determine the Percentage of Infective Fleas by taking batches of Fleas from the Infected Breeding-cage and putting each batch on a Clean Rat.

Experiment No.	Date.	No. of fleas in batch.	Fleas left on rat.	Rat No.	Result.	Tryps. first seen.	Multipl. ended.
27 bis	14 : ii : '10	5	4 days	186	0	—	—
,,	,,	5	4 ,,	187	+	21 : ii : '10	26 : ii : '10
,,	,,	5	4 ,,	188	0	—	—
34	22 : xi ; '10	6	3 ,,	239	0	—	—
,,	12 : xii : '10	6	3 ,,	239	+	19 : xii : '10	22 : xii : '10
,,	5 : i : '11	6	4 ,,	231	0	—	—
,,	6 : i : '11	6	4 ,,	241	+	16 : i : '11	19 : i : '11
,,	9 : i : '11	6	4 ,,	242	0	—	—
,,	19 : i : '11	6	4 ,,	250	0	—	—
,,	,,	6	4 ,,	251	+	26 : i : '11	30 : i : '11
,,	,,	6	4 ,,	252	+	25 : i : '11	30 : i : '11

Compare also Table I above (p. 645).

periment), but, nevertheless, the stomachs of this batch, injected into rat 245, produced an infection.

In order to obtain results from which more exact conclusions could be drawn, we experimented in a different manner, acting under the advice of Dr. M. Greenwood, Statistician of the Lister Institute. We took batches of fleas from the infected breeding-cage and put them on clean rats, one flea on each rat. A large number of such experiments were carried out with the results summarized in Table K, from which it is seen that 115 fleas placed each on a clean rat produced but eleven infections, a percentage of 9·56 approximately.

It is evident, however, that the bare numerical results of the experiments summarized in Table K cannot be taken as final, for the following reasons :

(1) The number of days given in the table as the time during which the flea was left on the rat is reckoned from the date the flea was put on the rat to the date on which the flea was sought for. But in many cases the flea when sought

TABLE K.—Infections produced by Fleas taken at Random from the Infected Breeding-cage and put singly on Clean Rats.

Fleas left on the rat.	Date put on.	Negative results.	Positive results.	Total negative.	Total positive.	Percentage of positive.
One day	6 : xii : '10	1	—	—	—	—
	12 : xii : '10	3	—	—	—	—
	18 : xii : '10	2	—	—	—	—
Total .	—	—	—	6	—	0
Three days	2 : viii : '10	4	1	—	—	—
	19 : ix : '10	2	1	—	—	—
	10 : x : '10	3	—	—	—	—
	1 : xi : '10	2	1	—	—	—
	18 : xi : '10	3	—	—	—	—
	19 : xi : '10	3	—	—	—	—
Total .	—	—	—	17	3	15
Four days	19 : ix : '10	2	—	—	—	—
	10 : x : '10	2	1	—	—	—
	13 : x : '10	3	—	—	—	—
	14 : x : '10	3	—	—	—	—
	3 : xi : '10	3	—	—	—	—
	11 : xi : '10	2	1	—	—	—
	12 : xi : '10	3	—	—	—	—
	29 : xi : '10	1	—	—	—	—
	14 : ii : '11	8	2	—	—	—
	28 : ii : '11	9	—	—	—	—
Total .	—	—	—	36	4	10
Five days	19 : x : '10	3	—	—	—	—
	20 : x . '10	2	1	—	—	—
Total .	—	—	—	5	1	16·6
Six days	27 : i : '11	10	—	—	—	—
Total .	—	—	—	10	—	0
Ten days	29 : vii : '11	7	1	—	—	—
Total .	—	—	—	7	1	12·5
Sixteen days	9 : i : '12	9	1	—	—	—
	13 : i : '13	7	1	—	—	—
Total .	—	—	—	16	2	11·1
Eighteen days	3 : iv : '13	5	—	—	—	—
	21 : iv : '13	2	—	—	—	—
Total .	—	—	—	7	—	0
Grand total .	—	—	—	104	11	9·56

for was not found, and it must be supposed that the flea died. If it died a natural death before going on to the rat the result would of course be negative whether the flea was infective or not. If eaten by the rat, an infective flea would probably produce a positive result.

(2) The fact, now established, that the flea does not infect by the puncture of the proboscis, but contaminatively, renders the infection a very casual affair, especially when only one flea is on the rat, and the probability of the infection taking place is relatively low. This is clearly shown by Experiment 37, tabulated above (Table G, p. 640), in which one infective flea, put on seventeen rats successively, over a period of about three months, infected seven of them, and another flea, put on ten rats over a period of about two months, infected only three of them. An analysis of these results is given in Table L, from which it is seen that twenty-seven exposures of rats to infection by two fleas known to be infective produced ten infections, equivalent to 37 per cent. of positive results.

TABLE L.—Infections produced by Two Fleas known experimentally to be Infective and placed singly on Clean Rats.

Flea left on the rat.	Negative results.	Positive results.	Total negative.	Total positive.
Four days . . .	4	4		
Five days . . .	10	6		
Six days . . .	1	—		
Seven days . . .	2	—		
			17	10

A side-light on the problem of the percentage of infective fleas might be obtained from the dissection and examination of fleas six days or more after they have been fed on an infected rat. From Table B it is seen that, of 118 such fleas examined, fifty-three were found to contain stages of the trypanosome; of these twenty-eight had a scanty, twenty-five

an abundant, infection. The value of these data, however, is somewhat uncertain, as an aid to the solution of the problem under consideration.

From the foregoing summary of the data, it is evident that the computation of the percentage of fleas that become infective, of those exposed to infection, is a somewhat complicated statistical problem. We have submitted the data to Dr. Greenwood, who has kindly supplied us with the report appended below, from which it is seen that the percentage of infected fleas lies probably between 5·9 per cent. and 45·7 per cent., the mean being 25·8 per cent.

Report of Dr. M. Greenwood.

The problem it is desired to solve is the following:

Within what limits does the true proportion of infective fleas in the population of which those enumerated in Table K are a sample probably lie?

Of 115 fleas left not more than eighteen days on clean rats, eleven produced infections. But all infective fleas do not produce infections, and, according to Table L, in twenty-seven trials with unquestionably infective fleas only ten produced infections in clean rats. Accordingly, it follows that the proportion of fleas in the first experiment which actually produced infection must be divided by the proportion found in the other experiment to arrive at the ratio of potentially infective fleas, which is the quantity sought. This is—

$$\frac{\frac{11}{115}}{\frac{10}{27}} = \cdot 2583 \text{ or } 25{\cdot}8 \text{ per cent.}$$

But the two ratios from which this result is derived are each subject to errors of random sampling, and it is necessary to compute the "probable error" of sampling to which the final proportion is subject. Since the two proportions are entirely independent one of another, the square of the standard deviation of their ratio is $\frac{s_A^2.B^2 + s_B^2.A^2}{B^4}$ where A

is the proportion observed among the 115 fleas, and s_A its standard deviation and B and s_B similar quantities in the case of the twenty-seven trials of the other experiments.

Using this formula we reach 9·84 per cent. as the value of the standard deviation or ·67449 times this = 6·64 per cent. for the "probable error." Taking the usual margin, three times the "probable error," the conclusion may be drawn that the real proportion of infective fleas is very unlikely to be beyond the limits 5·9 per cent. and 45·7 per cent.

This is the conclusion which might, I think, legitimately be drawn from the two experiments, but two cautions must be had in mind. The first is that the number of trials in the second Experiment, 27, is rather small, and consequently the application of the customary theory of sampling errors must be made with hesitation. The second caution is that we are using twenty-seven trials with two fleas, not twenty-seven separate fleas, consequently, the two experiments are not strictly in pari materia. The two fleas used gave very different proportions of successes, and it might happen that were a larger number of definitely infective fleas used, the factor for division would be substantially modified. The above calculation can naturally give us no information on this point since it proceeds in terms of trials, twenty-seven trials with two fleas being assumed to be the same as twenty-seven trials with twenty-seven fleas, and that the differences do not depend upon the idiosyncrasies of the fleas, but upon the fluctuations of chance, the fleas being used simply as dice or counters.

(xvii) Can the First Phase of the Development of the Trypanosomes, namely, the Intra-Cellular Multiplication in the Stomach of the Flea continue beyond the Second Feed of the Flea (counting as the First Feed that by which it became infected)?

With regard to this point, it should first be made quite clear that our observations on fleas examined during early

periods of the development show conclusively that the trypanosomes may have disappeared from the stomach, and the rectal-phase may be well started, even so early as 18, 24, or 36 hours after the first feed.[1] A very clear case of this is the stomach mentioned above (p. 555), in which the infection was thirty-six hours old, and which was examined after being cut into a series of sections; no trypanosomes of any kind were found in the stomach, but immediately behind the pylorus were two attached clumps of quite normal crithidial type. On the other hand, in fleas not fed again after the infective feed we have found normal forms of the stomach-phase as late as three, four, or even five days after the first feed. From such observations it is evident that the stomach-phase is of very variable duration, for some reason, and that in some cases it is ended[2] long before the time when the flea would, under natural conditions, feed again, while in other cases it persists at least up to this time.

The question, therefore, is not, "Does the stomach-phase continue beyond the second feed?" (since it is certain that it is very often ended before the second feed), but "Can it do so?" and with regard to the question so posed it must be pointed out that a single clear instance of the stomach-phase persisting beyond the second flea would suffice to give an answer in the affirmative with certainty; but so long as the

[1] We do not refer to those cases in which the trypanosomes had disappeared from the stomach by degeneration, and in which the rectum was either empty or contained only degenerative forms; but only to those cases in which the presence of true crithidial forms in the intestine or rectum showed that the development of the trypanosome was following its normal course.

[2] Assuming, that is, that the intracellular multiplication is an essential part of, and takes place invariably in, the normal development of T. lewisi; we believe this to be the case, but we are unable to assert that it is so. It is at least within the bounds of possibility that the development may take occasionally a short cut, that is to say, that the trypanosomes may pass on to the rectum, and there establish the normal crithidial phase without undergoing intracellular multiplication in the stomach.

TABLE M.—Experiments to Test the Influence of a Second Feed on the Persistence of the Stomach-phase of the Trypanosomes in the Flea.

A.—Fleas not Fed a Second Time.

	Date of first (infective) feed.	Date of dissection of flea.	Age of infection in the flea.	Contents of stomach.	Contents of rectum.
1.	24 : vi : '12	27 : vi : '12	3 days	A few active trypanosomes of developmental type	Nothing.
2.	,,	,,	,,	Many active trypanosomes and intracellular stages	A few forms, apparently degenerative
3.	,,	,,	,,	A few active trypanosomes and many intracellular stages.	Two seen, apparently degenerative forms.
4.	,,	,,	,,	Trypanosomes free and intracellular	Nothing.
5.	,,	,,	,,	Nothing	Small forms, apparently degenerative.
6.	,,	,,	,,	A few sluggish trypanosomes, apparently degenerative	Small forms, apparently developmental.
7.	,,	,,	,,	Trypanosomes free and intracellular	Developmental crithidial forms.
8.	,,	,,	,,	Nothing	Nothing.
9.	,,	,,	,,	A few free trypanosomes . .	A few small forms and one "tadpole."
10.	,,	,,	,,	Nothing	A few small forms, apparently developmental
11.	,,	28 : vi : '12	4 days	,, ...	Developmental crithidias.
12.	,,	,,	,,	,, ...	A few small forms of crithidial appearance.
13.	,,	,,	,,	,, ...	Nothing.
14.	,,	,,	,,	Trypanosomes free and intracellular	A few crithidial (?) forms.
15.	,,	,,	,,	Nothing	Nothing.
16.	,,	,,	,,	,, ...	Crithidias fairly numerous.
17.	,,	,,	,,	A few trypanosomes free and intracellular	Crithidias fairly numerous.

18.	1 : vii : '12	3 : vii : '12	2 days	Nothing	Degenerative forms.
19.	,,	,,	,,	,, ...	Nothing.
20.	,,	,,	,,	,, ...	Degenerative forms.
21.	,,	,,	,,	,, ...	Nothing.
22.	,,	,,	,,	,, ...	Developmental crithidias.
23.	,,	4 : vii : '12	3 days	,, ...	Degenerative (?) forms.
24.	,,	,,	,,	A few free and intracellular trypanosomes	Degenerative (?) forms.
25.	,,	,,	,,	Nothing	Nothing.
26.	,,	,,	,,	,, ...	Degenerative (?) forms.
27.	,,	,,	,,	A few free trypanosomes . .	Nothing.
28.	,,	,,	,,	A few small forms	Swarms of crithidias.
29.	,,	5 : vii : '12	4 days	Nothing	Nothing.
30.	,,	,,	,,	,, ...	,,
31.	,,	,,	,,	One long trypanosome seen . .	Crithidial (?) forms.
32.	,,	,,	,,	Active free trypanosomes . .	Two degenerative (?) forms.
33.	,,	,,	,,	Nothing	Nothing.
34.	,,	,,	,,	A few long trypanosomes . .	Two active slender forms.
35.	8 : vii : '12 (night)	11 : vii : '12	2½ days	A few trypanosomes . . .	Nothing.
36.	8 : vii : '12	,,	,,	Nothing	A few degenerative forms.
37.	,,	,,	,,	Long free trypanosomes and attached clumps	A few attached pointed forms.
38.	,,	,,	,,	Nothing	Crithidial forms.
39.	,,	,,	,,	A few trypanosomes . . .	Developmental (?) crithidias.
40.	,,	,,	,,	Nothing	Crithidial forms.
41.	,,	,,	,,	,, ...	Crithidial (?) forms.
42.	,,	,,	,,	,, ...	Clump of pointed forms.
43.	,,	12 : vii : '12	3½ days	,, ...	Crithidias swarming.
44.	,,	,,	,,	,, ...	Crithidias.
45.	,,	,,	,,	Trypanosomes free and intracellular	Nothing.
46.	,,	,,	,,	Nothing	,,
47.	,,	,,	,,	,, ...	One developmental form.
48.	,,	,,	,,	,, ...	Nothing.
49.	,,	,,	,,	Free trypanosomes	,,

TABLE M—(*continued*).

B.—Fleas Fed a Second Time.

	Date of first (infective) feed.	Date of second feed.	Date of dissection of flea.	Age of infection in the flea.	Contents of stomach.	Contents of rectum.
1.	1 : vii : '12	3 : vii : '12	4 : vii : '12	3 days	Nothing	Nothing.
2.	,,	,,	,,	,,	,, ...	,,
3.	,,	,,	,,	,,	,, ...	,,
4.	,,	,,	,,	,,	,, ...	,,
5.	,,	,,	,,	,,	,, ...	Crithidial forms.
6.	,,	,,	,,	,,	,, ...	Nothing.
7.	,,	,,	5 : vii : '12	4 days	,, ...	,,
8.	,,	,,	,,	,,	,, ...	,,
9.	,,	,,	,,	,,	,, ...	,,
10.	,,	,,	,,	,,	,, ...	,,
11.	,,	,,	,,	,,	,, ...	,,
12.	,,	,,	,,	,,	,, ...	,,
13.	8 : vii : '12 (night)	11 : vii : '12 (9–10 a.m.)	11 : vii : '12	$2\frac{1}{2}$ days	,, ...	Degenerative (?) forms.
14.	8 : vii : '12	11 : vii : '12	,,	,,	One or two small pear-shaped forms (post-pyloric (?))	A few crithidial forms.
15.	,,	,,	,,	,,	Nothing	Nothing.
16.	,,	,,	,,	,,	,, ...	Crithidial forms.
17.	,,	,,	,,	,,	,, ...	Nothing.
18.	,,	,,	,,	,,	,, ...	Degenerative (?) forms.
19.	,,	,,	12 : vii : '12	$3\frac{1}{2}$ days	,, ...	Crithidial forms.
20.	,,		,,	,,	,, ...	Nothing.
21.	,,	,,	,,	,,	,, ...	A few crithidias.
22.	,,	,,	,,	,,	,, ...	A few degenerative (?) forms.
23.	,,	,,	,,	,,	,, ...	Crithidias.
24.	,,	,,	,,	,,	,, ...	A few pointed forms.
25.	,,	,,	,,	,,	,, ...	Pointed forms.

26.	8 : vii : '12	11 : vii : '12	12 : vii : '12	3½ days	Nothing	A few pointed forms.
27.	,,	,,	,,	,,	,, ...	Nothing.
28.	24 : vi : '12	27 : vi : '12	28 : vi : '12	4 days	,, ...	,,
29.	,,	,,	,,	,,	,, ...	,,
30.	,,	,,	,,	,,	,, ...	Numerous crithidias.
31.	,,	,,	,,	,,	,, ...	Nothing.
32.	,,	,,	,,	,,	,, ...	A few crithidial (?) forms.

N. B.—When a query (?) is affixed to the statement concerning the contents of the rectum, it signifies that the determination of the nature of the forms seen in the fresh state was not quite certain and that it was not confirmed by the examination of permanent preparations.

ANALYSIS OF THE RESULTS.

Series A.

No trypanosomes seen in 11 fleas.

Trypanosomes seen only in the rectum in 18 fleas; in 4 of the cases doubtfully, in 3 certainly, of degenerative type; in 3 of the cases doubtfully, in 8 certainly, of developmental type.

Trypanosomes seen only in the stomach in 6 fleas.

Trypanosomes seen both in the stomach and rectum in 14 fleas; the rectum contained in 5 of these fleas forms doubtfully of degenerative type; it contained in 4 of them forms doubtfully, in 5 of them forms certainly, of developmental type.

Series B.

No trypanosomes seen in 18 fleas.

Trypanosomes seen only in the rectum in 13 fleas; in 6 of these fleas doubtfully of degenerative type; in 1 doubtfully, in 6 certainly, of developmental type.

Trypanosomes seen both in stomach and rectum in 1 flea; in this case all the forms seen were crithidial in character, and those seen in the teased-up stomach may have been post-pyloric in position.

Summary.

A.—In 49 fleas not fed a second time, trypanosomes were found in the stomach in 20 cases, and were not found in 29 cases, in 11 of which they appeared to have disappeared altogether in the flea.

B.—Counting only those cases in which the rectum certainly contained developmental forms—namely, 7 fleas—the typical stomach-forms were absent in all.

answer is in the negative, it cannot be regarded as certain, but only as possessing a greater or less degree of probability.

In order to test this point we fed batches of fleas on infected rats and then divided each such batch usually into two batches, A and B. Batch A in each such case was kept starved until it was examined; batch B was fed again before being examined. In cases where fleas of batch B were found on examination not to have availed themselves of the chance of feeding, they were reckoned in batch A. Sometimes the original batch was not divided, but treated as a whole either as an "A" (not re-fed) or "B" (re-fed) batch.

The results of these experiments are tabulated in Table M, which seems at first sight decidedly in favour of the conclusion that the trypanosomes cannot persist beyond the second feed of the flea. It is seen that in forty-nine fleas not fed again after the infective feed, trypanosomes were present in twenty cases; in thirteen of the cases the trypanosomes were of the long stomach type and in eight cases intracellular forms were seen. On the other hand, in thirty-two fleas examined after having been fed a second time, the typical multiplicative stomach-phase was not present in a single instance. Unfortunately, the force of these figures is rather weakened by the fact that, of the fleas fed a second time it can only be asserted positively in seven cases that the rectum contained true developmental crithidial forms and that the developmental cycle was in these cases a "going concern," so to speak. While the figures make it probable, to a certain degree, that the stomach-phase, if it persists up to the time of the second feed, must come to an end then, this conclusion cannot be considered established and must remain a point for further investigation.

If it be true that the stomach-phase cannot persist beyond the second feed, we may enquire how such a result is brought about. It is intelligible that a fresh meal of blood might sweep on all free, extracellular trypanosomes from the stomach towards the rectum, but this would not account for the disappearance of the intracellular forms. It has been

mentioned above that in some insects the epithelium of the mid-gut is regenerated completely after each meal, and we stated further that we were not in a position either to affirm or to deny that, in the case of the flea, the regeneration of the stomach-epithelium takes place in regular correlation with the feeding. If it were so, however, it would become quite intelligible why a second feed should put an end to the intracellular multiplication in the stomach.

(xviii) Starvation of the Flea during the Incubation Period of the Cycle does not inhibit, nor does it necessarily retard, the Developmental Cycle of the Trypanosome in the Flea.

Experiment 45.—A batch of about 250 clean fleas, having been collected from the non-infected breeding-cage and kept hungry for three days, were put (22 : vii : '13) on a well-infected rat, for about twenty-four hours. The next day 150 of the fleas were recovered and kept in a flask containing some damp sand.

Two days later (25 : vii), thirty of these 150 fleas in the flask were put into a freshly-prepared bell-jar A with a clean rat 368. The next day (26 : vii) rat 368 was removed from bell-jar A, all the fleas on it being cleaned off carefully and put back into the bell-jar. Rat 368, examined regularly up to 26 : viii, did not become infected.

Two days later (28 : vii), clean rat 368a was put into bell-jar A, containing the fleas that had been in contact with rat 368. On the same day, thirty more fleas from the flask were put into bell-jar B with clean rat 369; another thirty in bell-jar C with clean rat 370; and another thirty in bell-jar D with clean rat 371. It will be remembered that the fleas in the flask had been exposed to infection for one day (22–23 : vii), and kept without food since then; consequently, bell-jars B, C, and D were colonised each with thirty fleas that had been exposed to infection between five and six days previously and starved since then.

The next day (29 : vii), rat 368a was removed from bell-jar A and all fleas recovered from it put back into the bell-jar. Rat 368a did not acquire infection. The same day rats 369, 370, and 371 were removed from bell-jars B, C, and D, all fleas being recovered from them and put back into the respective bell-jars. Rats 369 and 370 did not become infected; rat 371, on the other hand, became infected in due course (see Table C, p. 616).

Two days later (31 : vii), rat 368b was put into bell-jar A, rat 369a into bell-jar B, rat 370a into bell-jar C, and rat 371a into bell-jar D.

The next day (1 : viii), rats 369a, 370a and 371a were removed from the bell-jars B, C, and D, and the fleas on them carefully recovered and put back into their respective bell-jars. Rat 368b was left in bell-jar A. In the sequel rats 368b, 369a, and 370a did not become infected; rat 371a showed infection in due course.

Two days later (3 : viii), clean rats 369b, 370b and 371b were placed in bell-jars B, C, and D, and left in till they should become infected. Rat 369b did not become infected; rats 370b and 371b became infected in due course.

The results of the experiment may be summarised in the following manner. We start with four batches (A, B, C, D), each of thirty fleas, which had been exposed to infection on the same rat for one day (22 : vii to 23 : vii).

(1) Batch A (bell-jar A) :

Put on rat 368 from 25 : vii to 26 : vii (about three days after exposure to infection); result negative.

Put on rat 368a from 28 : vii to 29 : vii (about six days after exposure to infection); result negative.

Put on rat 368b, 31 : vii (about nine days after exposure to infection), and left on the rat; result negative.

This batch therefore did not become infective at all.

(2) Batch B (bell-jar B) :

Starved for five days (23 : vii to 28 : vii), then put on rat 369 from 28 : vii to 29 : vii (about six days after exposure to infection); result negative.

Put on rat 369a from 31 : vii to 1 : viii (about nine days after exposure to infection); result negative.

Left in with rat 369b on 3 : viii; result negative.

This batch therefore did not become infective at all.

(3) Batch C (bell-jar C) :

Starved for five days, then put on rat 370 from 28 : vii to 29 : vii (about six days after exposure to infection); result negative.

Put on rat 370a from 31 : vii to 1 : viii (about nine days after exposure to infection); result negative.

Left in with rat 370b on 3 : viii; result positive (the examinations of the rat indicate that infection took place between 4 : viii and 7 : viii).

This batch therefore became infective.

(4) Batch D (bell-jar D) :

Starved for five days, then put on rat 371 from 28 : vii to 29 : vii (about six days after exposure to infection) ; result positive.

Put on rat 371a from 31 : vii to 1 : viii (about nine days after exposure to infection) ; result positive.

Left in with rat 371a on 3 : viii ; result positive.

This batch evidently became strongly infective.

Remarks.—From the above summary it is seen that batch A (not starved) and batch B (starved) failed to become infective, while batches C and D (both starved) became infective.

Batch C did not produce its first infection before 3 : viii ; that is to say not until eleven or twelve days, at least, after exposure of the fleas to infection. It is not legitimate, however, to conclude from this that the developmental cycle of the trypanosome was retarded, since it has been shown above that infective fleas often fail to infect. The fleas may very well have been infective when placed in contact with rats 370 and 370a, but they were in contact with these rats for only about twenty-four hours. The most probable explanation for the two failures to infect is that only a small number of fleas in this batch were infective.

Batch D produced its first infection between 28 : vii and 29 : vii, and since it was exposed to infection between 22 : vii and 23 : vii it follows from these figures that the incubation-period—that is to say, the length of time taken by the developmental cycle of the trypanosomes—must have been between five and seven days. We are justified therefore in concluding that the trypanosomes in this batch went through a cycle of perfectly normal duration. The further fact that this batch never failed to produce infection during the time the experiment was carried on, indicates that the trypanosomes went through their cycle and established themselves successfully in a relatively large number of the fleas.

To conclude : Batches C and D show that starvation of the

fleas during the incubation-period does not inhibit the development of the trypanosomes; and batch D shows further that the development is not necessarily retarded by starvation.

(xix) Starvation of the Flea following immediately on an Infective Feed favours the Establishment of the Haptomonad Phase in the Rectum, while Starvation begun after the Incubation-Period in the Flea is over favours Migration to the Post-Pyloric End of the Intestine and the Establishment of the Haptomonad Phase there.

Experiments 49 and 50 were carried out with the object of ascertaining what effect, if any, varying food-conditions might have on the incidence and location of the established haptomonad phase in the flea's gut.

Experiment 49.—21 : iii : '14.—A number of fleas collected from the non-infected breeding-cage two days previously were put into a bell-jar with a well-infected rat at eight a.m., and were recovered again at twelve noon. They were then divided into two batches. Batch A, consisting of fifteen fleas, was put into a flask with moist sand at the bottom. Batch B (about forty fleas) was put into a bell-jar with a clean rat (rat 477).

26 : iii : '14.—Five fleas of batch A and four of batch B were dissected and examined.

Of batch A four were positive, one was negative. Of the four positive three showed developing forms of the trypanosomes in both the stomach and the rectum. Two of the three showed large numbers in both stomach and rectum, and in one of the stomachs intracellular forms were found. The fourth positive showed a haptomonad infection in the rectum.

Of batch B only one of the four dissected showed trypanosomes, and these were found free in the stomach-slide.

27 : iii : '14.—Eight fleas of batch A and eight of batch B were dissected and examined.

Of batch A seven were positive; one was negative. Of the seven positive one showed long active forms in the stomach and haptomonads in the rectum, while six showed developing forms in the rectum only—three scanty and three in fair numbers attached mostly round the

rectal surface of the projecting intestine, but in other parts as well. Of batch B only one was found infected, and it showed haptomonads attached about the middle region of the rectum.

The remaining fleas of batch B were now divided into two batches—batch A1 and batch B1. Batch A1, consisting of fifteen fleas, was put into a flask with moist sand at the bottom, and batch B1 was left in the bell-jar with rat 477.

4 : iv : '14.—Rat 477 was removed from the bell-jar and clean rat 478 was put in its place with batch B1.

Four fleas of batch A1 and four of batch B1 were dissected and examined.

Of batch A1 three were positive and one was negative. Of the three positive two showed trypanosomes in abundance in the post-pyloric region of the intestine and nowhere else. In these the trypanosomes were long and slender, and some were club-shaped; while in a third, which showed one or two in the rectum also, there was a swarming infection of haptomonads, as well as long, slender and club-shaped forms in the post-pyloric region. The fourth flea was negative.

Of batch B1 all were negative.

The remaining fleas of batch A1 were now allowed to feed on a clean rat for a short time.

9 : iv : '14.—Five fleas of batch A1 and four of batch B1 were dissected and examined.

Of batch A1 only one flea was positive, and it showed a fair number of slender trypanosomes in the post-pyloric region and nowhere else. Of batch B1 all were negative.

11 : iv : '14.—The remaining fleas of batch A1 were allowed to feed on a clean rat for a short time.

16 : iv : '14.—Five fleas of batch A1 and five of batch B1 were dissected and examined.

Of batch A1 two were positive and three were negative. The two positives showed large numbers of trypanosomes, some free, long and active, some club-shaped, and others were small, round and pear-shaped, in clumps and attached so as to form a lining to the wall of the gut. All were in the post-pyloric region and nowhere else. Of batch B1 all five were negative.

Rat 477 became infected in due course. Rat 478 never became infected. This agrees with results of the examinations.

Experiment 50.—20 : v : '14.—A number of fleas collected from the non-infected breeding-cage two days previously were put into a bell-jar with a well-infected rat late in the evening, were left overnight, and were recovered next morning. They were then divided into two batches. Batch A was kept in a flask with moist sand at the bottom.

Batch B was put into a freshly-prepared bell-jar with a clean rat (rat 496).

26 : v : '14.—Five fleas of batch A and five of batch B were dissected and examined.

Of batch A three were positive and two were negative. Of the three positive two showed haptomonads in the rectum, one being a swarming infection, and the third showed many long, active forms in the post-pyloric region only.

Of batch B all were negative.

28 : v : '14.—Seven fleas of batch A and seven of batch B were dissected and examined.

Of batch A five were positive and two were negative. Of the five positive four showed developing forms in the rectum only, and of these two were swarming infections. The fifth showed haptomonad infection of the rectum, and also free active forms in the stomach.

Of batch B only one of the seven was positive, and it showed a scanty infection of the rectum only.

The remaining fleas of batch B were now divided into two batches—batch A1 and batch B1. Batch A1 was put into a flask with moist sand in the bottom. Batch B1 was put into a bell-jar with a clean rat (rat 497).

2 : vi : '14.—Fourteen fleas of batch A1 and fourteen of batch B1 were dissected and examined.

Of batch A1 four were positive and ten were negative. Of the four positives one showed haptomonads on the rectal surface of the projecting intestine and three showed trypanosomes (one swarming) in the post-pyloric region and nowhere else.

Of batch B1 five were positive and nine were negative. Of the five positives one which had its stomach full of red blood showed a scanty infection in the rectum only. The other four showed infection in the post-pyloric regions only. Of these two (females) had small ova and their stomachs were empty. The remaining two had a fair quantity of brownish-coloured blood-débris in their stomachs. Rats 496 and 497 became infected in due course.

Summary.

A.—Fleas starved from immediately after the infective feed, dissected and examined five and six days after the infective feed.

No. of experiment.	Number of fleas examined.	Number infected.	Site of infection.	Remarks.
49	13	11	Stomach and rectum, 4; rectum only, 7	Intracellular forms in one stomach 3 scanty, 4 swarming —infections mostly in upper part of rectum
50	12	8	Stomach and rectum, 1; rectum only, 6; post-pyloric only, 1	3 scanty and 3 swarming

B.—Fleas that were put into bell-jar immediately after the infective feed, along with clean rat, on which they could feed at any time. Dissected and examined five and six days after the infective feed.

No. of experiment.	Number of fleas examined.	Number infected.	Site of infection.	Remarks.
49	12	2	Stomach only, 1	—
			Rectum only, 1	Haptomonad infection in middle region.
50	12	1	Rectum only, 1	—

A1.—Fleas in which starvation was begun six days after the infective feed. During the six days the fleas had been fed on clean rats. Those of Experiment 49 were dissected and examined fourteen, nineteen, and twenty-six days after the infective feed. Those of Experiment 50 were dissected and examined thirteen days after the infective feed.

No. of experiment.	Number of fleas examined.	Number infected.	Site of infection.	Remarks.
49	14	6	Post-pyloric only, 5; post-pyloric and rectum, 1	Swarming haptomonad infection in 2.
50	14	4	Rectum only, 1; post-pyloric only, 3	Pile carpet infection upper part; swarming haptomonad infection in 1.

B1.—Fleas kept with clean rats in bell-jar ever since the infective feed. Dissected and examined as in A1.

No. of experiment.	Number of fleas examined.	Number infected.	Site of infection.	Remarks.
49	13	0	—	—
50	14	5	Rectum only, 1; post-pyloric only, 4	Stomach full of red blood; [1]stomach empty and ova small in 2; stomach contained fair quantity of blood-débris in 2.

These results seem to throw light on the important function that the nectomonad forms described as occurring in the established rectal-phase may have in maintaining the infection in the flea. The optimum food-conditions for the establishment of the haptomonad stage seem to lie somewhere between abundance and poverty, and between partial and complete digestion of the blood-supply. More extended

[1] Although the fleas of batches B and B1 in both experiments had the chance of feeding on clean rats at any time from immediately after the infective feed onwards, all may not have equally availed themselves of the opportunity. It is certain, in fact, from the condition of the ova and of the stomachs of two females of batch B1 that showed post-pyloric infection, that they, for some reason not ascertained, had starved in the midst of plenty; and these should really be transferred to batch A1. The remaining two fleas of batch B1 that showed post-pyloric infection had evidently fed, but not quite recently. The other infected flea of batch B1 had its stomach distended with red blood, and in it the infection was in the rectum only.

In order to test the infectivity of batches A1 and B1 in Experiment 50, twelve fleas of each batch were put on clean rats, two fleas to each clean rat. The result was that two of the six rats belonging to batch A1 became infected, while none of the six belonging to batch B1 became infected. This may indicate that a period of starvation heightens the infectivity of infected fleas, perhaps by inducing increased production of the final propagative forms of the cycle; but further experiments would be required to justify such a deduction.

and varied observations are required, but so far as these experiments go they show that the incidence, location, and continued existence of the haptomonad stage in the flea's gut depend to a large extent on the food-supply. When, under conditions of partial starvation, a sufficient supply of nourishment cannot be obtained in the rectum, the haptomonad stage, if established there, would die out, and the flea would lose its infection were it not that the nectomonads produced in the rectum migrate forwards and re-establish this stage nearer to the food-supply. In like manner it may be assumed that when the food-supply in the post-pyloric end of the intestine becomes continuously too rich and abundant, the nectomonads produced there migrate backwards to the rectum and so the balance is maintained and the infection in the flea is kept up.

Lister Institute,
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DESCRIPTION OF PLATES 36 TO 45,

Illustrating Mr. E. A. Minchin and Dr. J. D. Thomson's paper on "The Rat-Trypanosome, Trypanosoma lewisi, in its Relation to the Rat-Flea, Ceratophyllus fasciatus."

(In all cases where the age of a trypanosome is stated, it is to be understood as reckoned from the first infective feed of the flea, or, in cases where the fleas were left on the infected rat for some days, from the time when the fleas were first given the chance of feeding on it.)

PLATE 36.

[Various stages of the stomach-phase in film-preparations fixed with osmic vapour and stained with Giemsa's stain; drawn with the camera lucida at a magnification of 3000.]

Figs. 1–12.—Free (extracellular) trypanosomes.

Figs. 1 and 2.—Six hours; fig. 1, practically unmodified; fig. 2, slightly modified in structure.

Figs. 3 and 4.—Twelve hours.

Figs. 5 and 6.—Eighteen hours.

Figs. 7–10.—Twenty-four hours.

Figs. 11 and 12.—Forms of crithidial structure from an abnormal flea (see p. 519); fig. 11, a free specimen; fig. 12, a couple adhering together.

Figs. 13–17.—Recurved forms.

Figs. 13, 14.—Twelve hours; both from the same preparation, but 14 s flattened out by having dried up before fixation, while 13 is normal.

Figs. 15–17.—Twenty-four hours.

Figs. 18–20.—Forms in which the multiplication of the nuclei have begun without the typanosomes having assumed the recurved form (or perhaps forms which have straightened themselves out again secondarily); twenty-four hours, all from the same preparation. In fig. 19 the trypanosome is enclosed in the remains of the host-cell.

Figs. 21–23.—Rolled-up forms with $2\,nn$ and $2\,NN$ each; from the same preparation as the preceding.

Figs. 24–29.—Rolled-up forms with *n* and *N* still single, in 27 beginning to divide. Figs. 24 and 25 are from the same preparation as the preceding; figs. 26–29 are also twenty-four hours; the specimen in fig. 26 is evidently enlarged artificially by having dried before fixation.

Figs. 30–37.—Multiplication of nuclei to 2 or 3 *nn* and 2 *NN*.

Fig. 38.—Small sphere with 4 *nn* and 4 *NN*.

Fig. 39.—Stage with 3 *nn* and 2 *NN*. N.B.—Figs. 35–39 are all from the same preparation, showing the intense staining and consequent opacity of the body characteristic of the intracellular stages; only the principal flagella can be made out clearly, the daughter-flagella being scarcely, or not at all, visible. Twenty-four hours.

Fig. 40.—Sphere with 3 *nn* and 3 *NN*, from the same preparation as fig. 26; evidently enlarged by flattening due to drying. Twenty-four hours.

Fig. 41.—A large sphere containing 13 *nn* and 13 *NN*, and two others; the stain is over-extracted and the flagella are not seen. These specimens are from the same stomach as figs. 88–94 on pl. 37, in all of which flagella are plainly seen. Thirty-six hours.

Fig. 42.—Two very large tailed spheres from the same preparation as figs. 18–25, 30, 33, 34; the flagella cannot be made out. Twenty-four hours.

Figs. 43–46.—Small rolled-up forms, possibly in process of degeneration, from the same preparation as the last.

PLATE 37.

[Various stages of the stomach-phase from films preserved in sublimate mixtures—sublimate-acetic, Schaudinn's fluid, or Maier's fluid—and stained with iron-hæmatoxylin, drawn with the camera lucida at a magnification of 3000.]

Figs. 47–68.—Free (extracellular) trypanosomes, except 58 and 59.

Figs. 47, 48.—Six hours; fig. 47, quite unmodified; fig. 47, slightly modified.

Fig. 49.—Eighteen hours.

Figs. 50–55.—Twenty-four hours.

Figs. 56–59.—Three days, all from the same preparation. Figs. 58 and 59 are evidently early stages of the intracellular multiplication.

Fig. 60.—Four days.

Figs. 61–68.—From an abnormal flea taken from the infected breeding cage (see p. 519). Every possible grade of transition occurs in the preparation from quite ordinary forms, such as fig. 61, to large crithidial

forms, such as fig. 66, and of the latter some occur in couples adhering together, as in figs. 67 and 68.

Figs. 69–73.—Recurved forms, twenty-four hours; in the specimen shown in fig. 72 the flagellum appears to have become torn away from the body.

Fig. 74.—First division of *n* in a rolled-up form.

Fig. 75.—Recurved form with 2 *nn* and 2 *NN*; forty-eight hours.

Figs. 76.—A rolled-up form in which the flagellum has become untwisted from the body; from the same preparation as the last.

Figs. 77, 78.—Two rolled-up forms from the same preparation, twenty-four hours.

Figs. 79–81.—Twenty-four hours, all from the same preparation; fig. 79, *n* divided, daughter-flagellum arising, *N* beginning to divide; figs. 80 and 81, similar stages.

Figs. 82–86.—Twenty-four hours, all from the same preparation; figs. 82, 83, each 2 *nn*, 1 *N*; fig. 85, 2 *nn*, 2 *NN*; fig. 84, 3 *nn*, 2 *NN*; fig. 86, tailed sphere with 8 *nn*, 8 *NN*.

Fig. 87.—Recurved form, twenty-four hours.

Figs. 88–94.—Various stages, all from the same preparation and from the same flea as fig. 41 on Pl. 36. Thirty-six hours.

Fig. 95.—Small sphere with 3 *nn* and 3 *NN*; twenty-four hours.

Fig. 96.—Large, nearly ripe sphere containing about ten trypanosomes, shown also in the photograph on Pl. 44, fig. 318; forty-eight hours.

PLATE 38.

[Epithelial cells of the stomach and stages of the stomach-phase from sections of the flea's stomach, stained with Giemsa's stain (with the exception of figs. 260 a, 261 a, 262 a, 263 a, and 264, for which see description of Pl. 42). Figs. 99–103 were fixed with Flemming's fluid, but all the rest were fixed with Maier's fluid. Drawn with the camera lucida at a magnification of 2000.]

Figs. 97, 98.—Epithelial cells; fig. 97 shows a cell in the columnar form; fig. 98 a flattened cell with, just beneath the border, a layer of granules staining quite differently from the blood-débris externally.

Figs. 99–103.—Various details of the cells after Flemming-fixation; fig. 99, red-staining grains in the upper end of the cell; fig. 100, red-staining grains mixed with osmic-blackened (fatty) grains; fig. 101, cell comparatively free from granules; fig. 102, pseudosphere in the upper end of a cell; fig. 103, two "yellow bodies" in the upper end of a cell.

Fig. 104.—Portion of the epithelium close to a crypt of regeneration, showing a recurved trypanosome attached to an epithelial cell and a trypanosome of ordinary type free in the débris close by. Twenty-four hours.

Fig. 105.—Section shaving the side of an epithelial cell obliquely, showing a trypanosome of ordinary appearance attached to it. Twenty-four hours.

Fig. 106.—Stout form of trypanosome free in the débris. Twenty-four hours.

Fig. 107.—Recurved trypanosome within a cell. Twenty-four hours.

Fig. 108.—Tailed sphere within a cell, also two smaller spheres and a portion of a trypanosome cut across; the nucleus of the cell does not come into the section. Twenty-four hours.

Fig. 109.—Portion of the epithelium showing a very large tailed sphere, three smaller ones and a nucleus (*N*); the cells are much exhausted. Twenty-four hours.

Fig. 110.—Section of a cell (not passing through the nucleus) showing a large tailed sphere and fragments of three smaller ones. Twenty-four hours.

Fig. 111.—A large tailed sphere and a slice of a smaller one, in a cell near the nucleus (*N*). Twenty-four hours.

Fig. 112.—Numerous multiplication-stages in a partly broken-down cell of flattened type, and a cell nucleus (*N*). Twenty-four hours.

Fig. 113.—Six spheres in a broken-down cell cast off into the blood-débris. Twenty-four hours.

Figs. 114–116.—Three figures drawn from the same slide. Fig. 114, exhausted cell filled with trypanosomes; fig. 115, a large sphere ripe for breaking up into a mass of trypanosomes as in the last; fig. 116, large sphere. The preparation was over-extracted and does not show the flagella. Twenty-four hours.

PLATE 39.

[Stages of the stomach-phase, all from the same series of sections through nine stomachs preserved thirty-six hours after the infective feed in Flemming's fluid and stained with iron-hæmatoxylin and Licht-grün-picric. Drawn with the camera lucida at a magnification of 2000.]

Figs. 117–129.—Extracellular trypanosomes.

Fig. 117.—Slightly club-shaped form, near the epithelium but not attached.

Figs. 118, 119.—Recurved forms, not attached.

Figs. 120–123.—Attached recurved forms,

Fig. 124.—A small bunch of trypanosomes attached in the interspace between two cells. Structure of the trypanosome difficult to make out clearly.

Fig. 125.—Trypanosomes in or attached to the débris of a necrosed cell. Two of them are recurved forms, the third possibly crithidial in type.

Fig. 126.—A slightly hypertrophied epithelial cell containing three spheres of different sizes, with portions of the cells on either side of it. *N*, the nuclei of the cells; *l.m.*, longitudinal muscles of the stomach in transverse section; *c.m.*, one of the circular muscles of the stomach.

Fig. 127.—From the next section of the series, showing the same cell in which appears one of the same three spheres lodged in a distinct vacuole.

Fig. 128.—Large hypertrophied cell with five nuclei in process of being thrown off from the epithelium; it contains two spheres (*sph.*) and numerous yellow bodies. The same cell is shown in the photograph in Pl. I, fig. 313 *c*.

Fig. 129.—Flattened cell showing a sphere lodged in a vacuole beside the nucleus (*N*).

Fig. 130.—Cell containing four spheres close to the nucleus (*N*).

Fig. 131.—Young epithelial cell close to a crypt containing an early multiplication-stage just external to the nucleus (*N*).

Fig. 132.—Degenerated and cast-off cell, containing a sphere, the flagellum of which is sticking out from the remains of the cell.

Fig. 133.— Recurved trypanosome within a cell.

Fig. 134.—Normal columnar epithelial cell containing spheres both above and below the nucleus (*N*).

Fig. 135.—Degenerated and cast-off epithelial cell, full of fatty deposits and yellow bodies, containing two spheres.

PLATE 40.

[Epithelial cells of the flea from sections of stomachs, all, except fig. 147, fixed with Flemming's fluid and stained with iron-hæmatoxylin followed by Lichtgrün-picric. Figs. 136–140 are drawn at a magnification of 1000; all the rest at 2000.]

Fig. 136.—Four young cells showing the partially developed border and the different positions of the nucleus in the cell.

Fig. 137.—A cell showing the more granular condition.

Figs. 138, 139.—Two stages of the fatty degeneration of the cell. In 138 the nucleus, though obscured, is still faintly visible; in 139 the cell has become an opaque black mass.

Fig. 140.—Two contiguous cells, of which the one to the left shows the beginning of the "yellow necrosis."

Figs. 141, 142.—The upper ends of cells containing "yellow bodies"; in fig. 141 several such bodies, in fig. 142 one large one.

Figs. 143, 144.—To show the deposition of osmic-blackened fatty grains in cells beginning to degenerate; fig. 143 in a flattened cell, fig. 144 in a columnar cell.

Figs. 145, 146.—Cells containing pseudospheres (*ps.*) in their upper portions.

Fig. 147.—To show the deposition of deeply-staining grains below the border of the epithelial cell. External to the cell are seen the coarse black grains of the blood-débris. Maier's fluid, iron-hæmatoxylin, Lichtgrün-picric.

Fig. 148.—Outer end of a cell containing a sphere (*sph.*) which is seen to contain a darkly-staining mass (chromatin? or fatty deposit?).

PLATE 41.

[Various stages of the rectal-phase. Figs. 201, 202 are from sections fixed with Flemming's fluid, stained with Giemsa's stain and drawn at a magnification of 2000; all the other figures are from smear-preparations fixed with osmic vapour, stained with Giemsa's stain, and drawn at a magnification of 3000.]

Figs. 149–152.—Early "tadpole" forms from the rectum, twenty-four hours; fig. 152 is perhaps an early stage of division.

Figs. 153, 154.—Early stages from the rectum, twenty-four hours.

Fig. 155.—Rectum, thirty-six hours.

Fig. 156.—Rectum, twenty-four hours.

Figs. 157, 158.—Rectum, thirty-six hours.

Figs. 159–161.—From the rectum of another flea, thirty-six hours.

Fig. 162.—Rectum, sixty hours.

Figs. 163, 164.—rectum, three days.

Figs. 165–170.—From a swarming rectal infection, three days.

Figs. 171–176.—From a swarming rectal infection, about three and a half days old.

Figs. 177, 178.—From the rectum, forty-eight hours.

Figs. 179–182.—From the rectum about three and a half days.

Figs. 183–187.—Early stages from the rectum, four days. In the clump shown in fig. 183 two trypanosomes of quite ordinary type are seen; these are probably forms which have come in with a later feed and have attached themselves secondarily to the clump.

Figs. 188–190.—From the rectum, five days.

Fig. 191.—From the rectum of another flea, five days.

Figs. 192–200.—From the recta of fleas taken from the infective breeding-cage, age uncertain. Fig. 192, early form from the rectum figs. 193–196, from stomach-preparations (post-pyloric?); fig. 197, rectum; fig. 198, rectum; fig. 199, stomach (post-pyloric); fig. 200, rectum.

Fig. 201.—Free clump from a section of a rectum.

Fig. 202.—From a section of the intestine close behind the pylorus, showing a number of haptomonads attached to the cuticle, and between them, projecting up above the level of the haptomonads and distinguished from them by their lighter stain and terminal *nn* are numerous examples of the final trypanosome-forms (*T*).

PLATE 42.

[Various stages of the rectal-phase. Figs. 273–277 are from sections of recta fixed with Flemming's fluid, stained with iron-hæmatoxylin, and drawn at a magnification of 2000; all the other figures are from smear-preparations fixed with sublimate mixtures, stained with iron-hæmatoxylin and drawn at a magnification of 3000.]

Figs. 203, 204.—Forms from the intestine in process of migration to the rectum, twenty-four hours (see p. 568).

Figs. 205–212.—Early "tadpole" forms from the rectum, twenty-four hours.

Figs. 213, 214.—Early division-forms, rectum, forty-eight hours.

Figs. 215, 216.—Early forms, one dividing, rectum, three days.

Fig. 216 a.—Couple produced by an early division?

Figs. 217–220.—From a well-infected rectum, about three and a half days.

Figs. 221–240.—Various forms from a swarming rectal infection of a flea that had been on an infected rat for five days; figs. 221–225, haptomonads; figs. 226–230, division-forms; figs. 231–234, growth of flagellum figs. 235, 236, nectomonads; figs. 237, 238, transitional forms; figs. 239, 240, slender and stout trypanosome-forms.

Figs. 241, 259.—Various forms from a swarming rectal infection, eight days; figs. 241–245, rounded haptomonads without free flagella; figs. 246, 250, 251, flagella growing out from rounded haptomonads; figs. 247, 248, 249, pear-shaped haptomonads without free flagella; fig. 252, clump of pear-shaped haptomonads, some with well-developed

flagella; fig. 253, division-stage; fig. 254, nectomonad; figs. 255–258 transitional forms; figs. 259, final trypanosome-form.

Figs. 260–264.—Various forms from a swarming rectal infection, taken from the infected breeding-cage, age unknown; figs. 260 a–263 a, the same specimens as 260–263, restained in Twort's stain; fig. 264, a nectomonad stained with Twort's stain. (N.B.—Figs. 260 a–263 a and 264 have been transferred to Pl. 38.)

Figs. 265–272.—Various forms from a swarming rectal infection, age unknown; fig. 265, early form? fig. 266, division-form; fig. 267, rounded haptomonad, without flagellum, beginning to divide; figs. 268, 269, rounded haptomonads with free flagellum beginning to grow out; fig. 270, transitional form; figs. 271, 272, final trypanosome-forms.

Fig. 273.—Clump of crithidial forms attached in the intestine just behind the pylorus, in sections of a stomach of a flea preserved thirty-six hours after the infective feed. (× 2000.)

Figs. 274–276.—From sections through the rectum of a flea preserved eight days after the infective feed. Fig. 274, free clump; fig. 275, clump attached to the cuticle of the rectum; fig. 276, forms attached singly to the cuticle. (× 2000.)

Fig. 277.—Haptomonads attached to the cuticle of the rectum, from sections through the rectum of a flea, preserved eleven days after the infective feed. (× 2000.) (This drawing is from the same section as that photographed in Pl. 44, fig. 317; the patch drawn lies just to the right hand of the middle of the three pointers that start from *c* in fig. 317.)

Figs. 278–288.—Leptomonas pattoni, various forms from the rectum of the flea. Figs. 278–284 a are from preparations fixed with Maier's fluid and stained with iron-hæmatoxylin; figs. 285–288 are from preparations fixed with osmic vapour and stained with Giemsa. In the specimens drawn in figs. 281, 282, note small bodies marked *x* which appear to be endogenous buds ("infective granules"); in fig. 283 a similar body is shown free, and figs. 284, 284 a appear to be stages in the development of the bud into the leptomonad. Fig. 285 is evidently a nectomonad form, and fig. 286 a stage in the development of such a form; they differ from the nectomonads of T. lewisi in the great prolongation of the body behind *N*.

PLATE 43.

[Degenerative forms, fixed with osmic vapour and stained with Giemsa, drawn with the camera lucida to a magnification of 3000.]

Figs. 289, 290.—Six hours, rectum.

Figs. 291–296.—Twelve hours, rectum.

Figs. 297, 298.—Recurved forms from the rectum, twelve hours.

Figs. 299–301.—From the rectum of another flea, twelve hours.

Figs. 302–304.—Rectum, thirty-six hours.

Fig. 305.—Stomach, forty-eight hours.

Fig. 306.—Rectum, twenty-four hours.

Fig. 307.—Rectum, sixty hours.

Fig. 308.—Clump of degenerative forms, rectum, eighteen hours.

Figs. 309, 310.—Agglomerating trypanosomes from a flea fed for the second time on an infected rat the day previously.

PLATE 44.

Fig 311.—Clump of degenerative forms (compare fig. 308). Photo. (× 1500.)

Fig. 312.—Clump of developmental crithidial forms; at *T* is seen a final trypanosome-form attached to the clump. From the same preparation as figs. 241–259. Photo. (× 2000.)

Fig. 313.—Infected patch of stomach-epithelium in which the cells are breaking down and being thrown off. The large multinucleate cell marked *C* is the cell, part of which is drawn in Pl. 39, fig. 128. Photo. (× 600.)

Fig. 314.—Section through an epithelial crypt of regeneration. Close beside it is seen a black, degenerated epithelial cell. Photo. (× 800.)

Fig. 315.—Section of an infected patch of the stomach-epithelium showing the cells being thrown off; the cells contain coarse black (fatty) granules and stages of intracellular multiplication, which are not clearly seen. Photo. (× 600.)

Fig. 316.—Section of the stomach-epithelium showing an epithelial crypt towards the middle; to the left of the crypt the epithelium is old and degenerate and full of blackened fat; to the right of the crypt is new, clear epithelium budded off from it. Compare Text-fig. 2. Photo. × 300.

Fig. 317.—Section of the wall of a rectum showing a swarming crithidial infection of the "pile-carpet" type. Fig. 277 is drawn from this section, from the part between the middle and the right-hand pointers which start from *c* to indicate the serried ranks of the crithidial forms. Photo. (× 500.)

Figs. 318.—A sphere (the same that is drawn in fig. 96) and near it one of the long free trypanosomes of the stomach-type, from a smear. Photo. (× 1000.)

Fig. 319.—Section passing through the opening of the pylorus into the intestine. Photo. (× 300.)

PLATE 45.

[General diagram of the entire life-cycle of Trypanosoma lewisi in the flea, combined from the observations and experiments set forth in this memoir in order to give a summarised idea of the complete course of events. The stomach-phase is represented on the upper side of the diagram, the rectal phase on the lower side and to the left; in the right-hand lower corner is shown the secondary infection of the pylorus. Magnification 2000.]

1. Trypanosome as taken up by the flea from the rat.

2. Trypanosome slightly modified after a few hours in the flea's stomach.

3–12. Cells of the epithelium of the stomach containing the various phases of the intracellular multiplication.

3. Cell with two trypanosomes attached to it, one of ordinary, the other of recurved, type.

4. Penetration of an epithelial cell by a trypanosome, drawn from Nöller's description of the process.

5. Recurved form in the cell.

6. Rolled-up form in the cell.

7. Early multiplication-form.

8. Later multiplication; 8 *nn*, 8 *NN*.

9. Large tailed sphere.

10. Large ripe sphere without tail.

11. Daughter-trypanosomes free in the exhausted cell after bursting of the sphere.

12. Daughter-trypanosomes escaping from the cell. After being set free, the trypanosomes may do one of two things as shown by the arrows. They may each penetrate another epithelial cell and repeat the process of multiplication by which they were produced. Or they may pass through the pylorus and down the intestine to the rectum to give rise there to the rectal (crithidial) phase.

13–18. To show the two possible ways in which the established rectal phase may arise from the stomach-trypanosomes:

(*a*) 13, 14, 15, 16. Four successive stages of the contraction of a stomach-trypanosome into a pear-shaped form which at 17 divides by

equal or subequal binary fission to produce two equipotential daughter-products, similar to 18, which by further-repeated binary fission produce the ordinary crithidial phase.

(*b*) 14 a, 14 b. Two successive stages of the contraction of a stomach-trypanosome into a club-shaped form, which at 17a divides by unequal binary fission to produce two inequipotential products, the larger parent-form which does not develop further and the smaller daughter-form, similar to 18, which by repeated binary fission produces the ordinary crithidial phase.

19. Established rectal infection, showing the various forms of the rectal phase. *h.h.* Haptomonads, some of them dividing, some of them transitional to the other types. *n.n.* Nectomonads. *tr. tr.* Forms transitional from the crithidial type to *T.T.*, the final trypanosome-form.

The final trypanosome-forms, as shown by the arrows, pass finally out of the flea with the fæces.

The nectomonads can, under certain conditions of food-supply, migrate back along the intestine and fix themselves close behind the pylorus in order to give rise to—

20. Secondary infection of the pyloric region of the intestine: the letters have the same significance as before. Final trypanosome-forms (*T.T.*) arise and pass down the intestine into the rectum and so out with the fæces; nectomonads (*nn*) also arise and may migrate back again to the rectum and re-establish the crithidial phase there; these migrations are indicated by the arrows.

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NEW SERIES.

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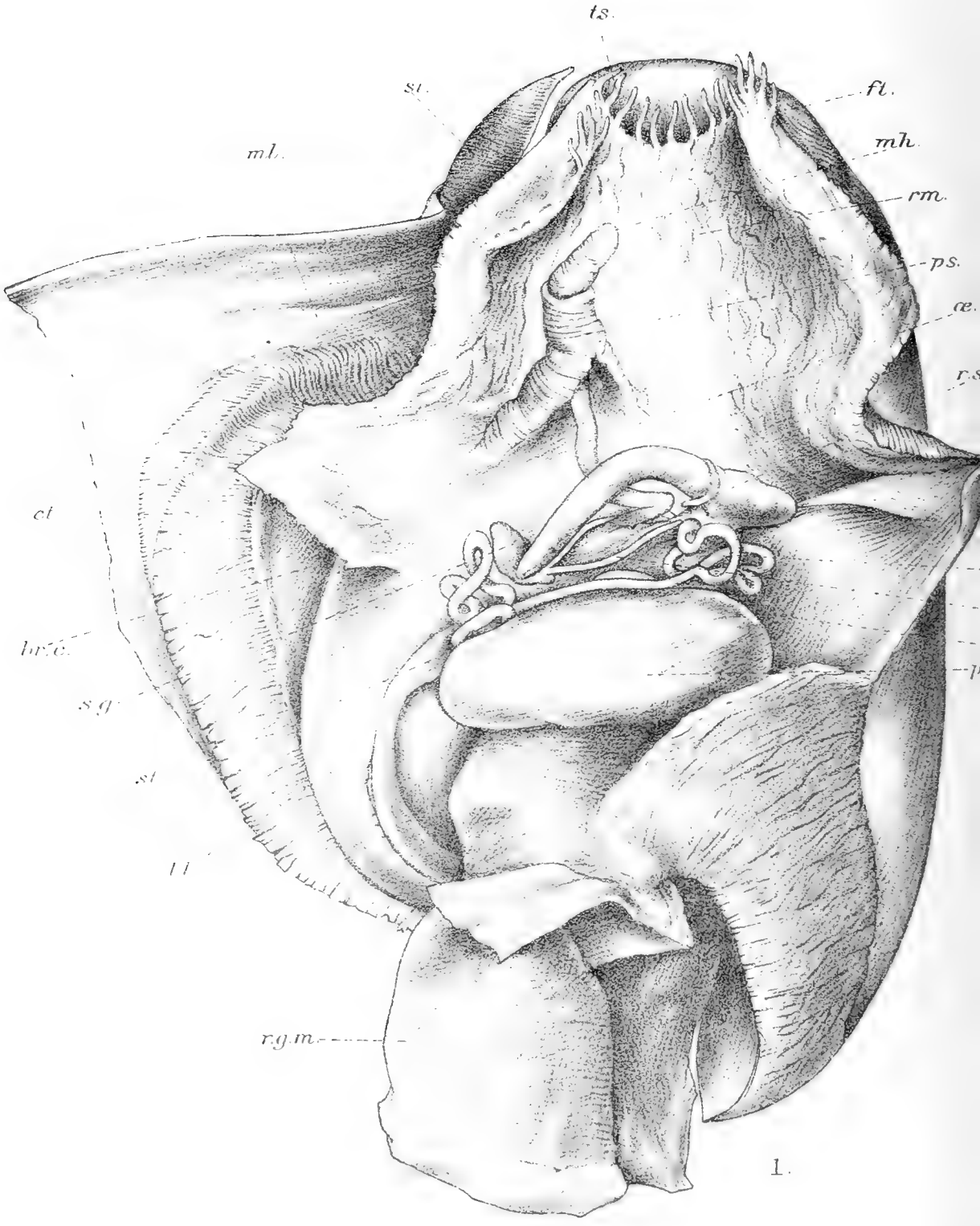
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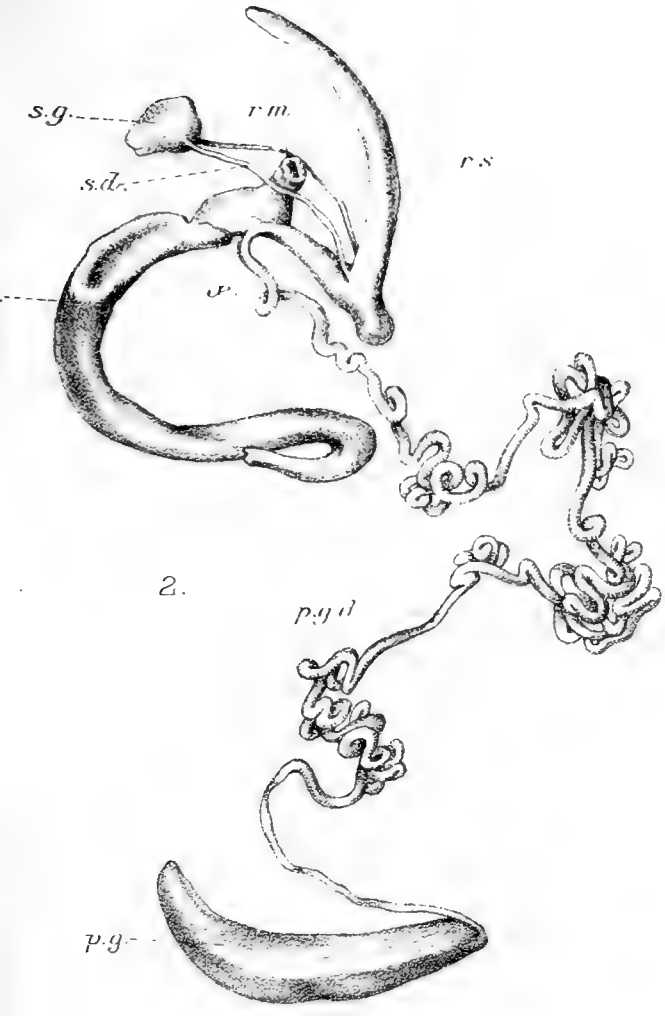

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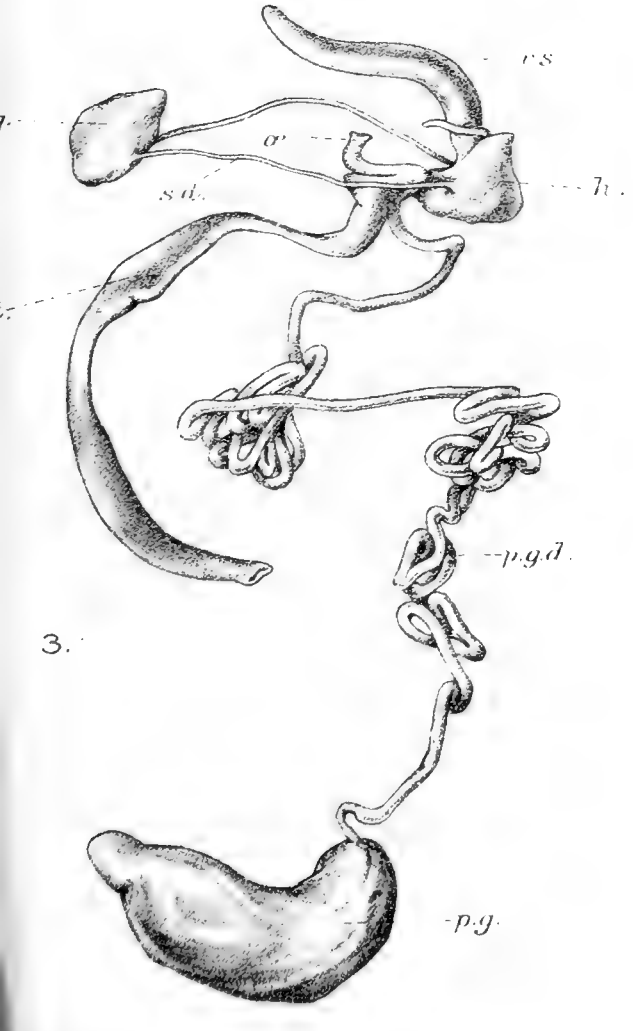

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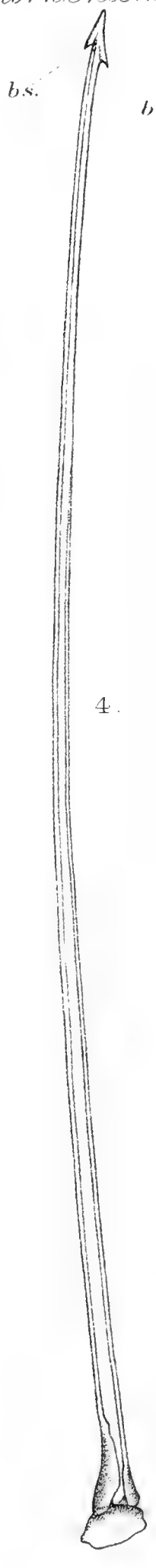

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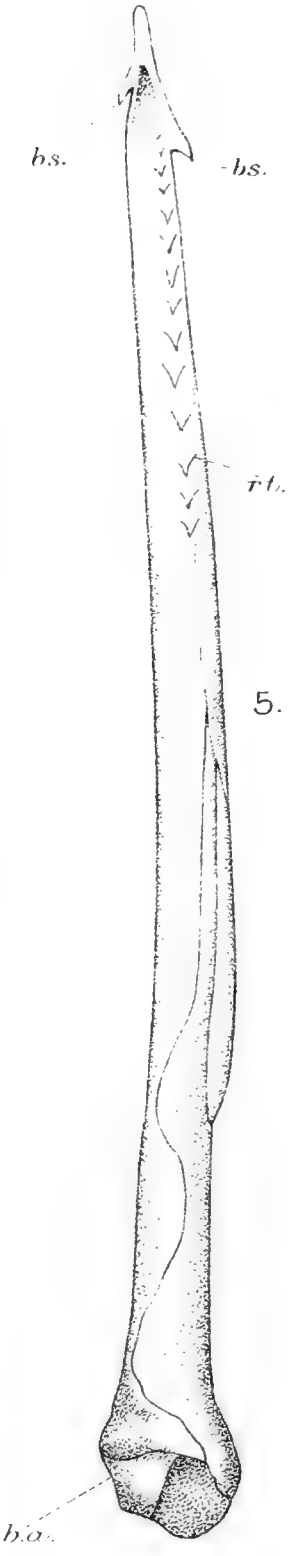

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18.

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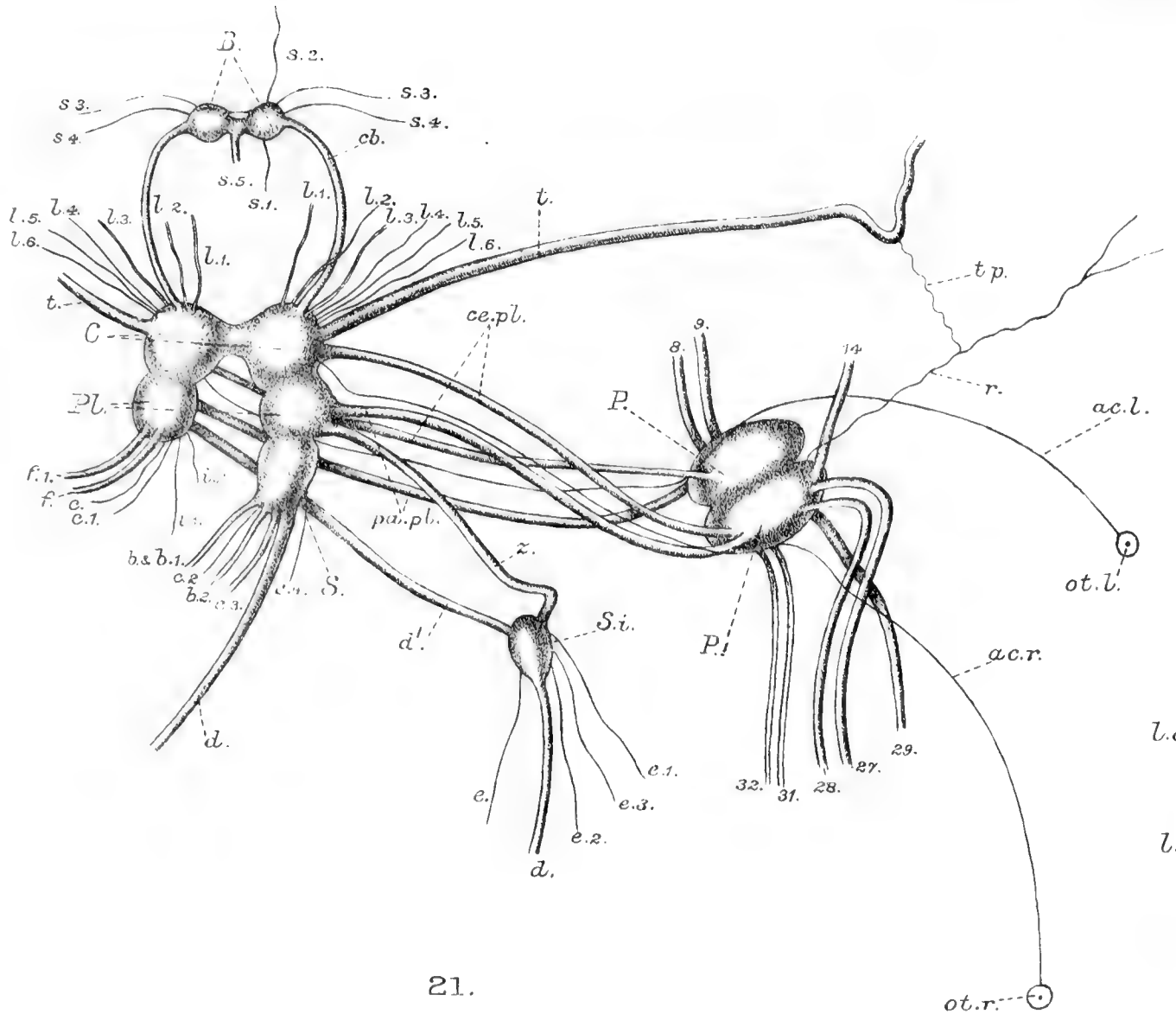

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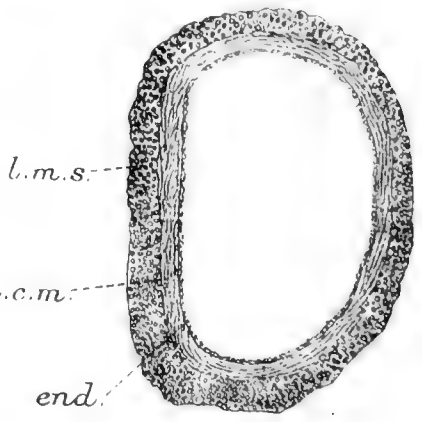

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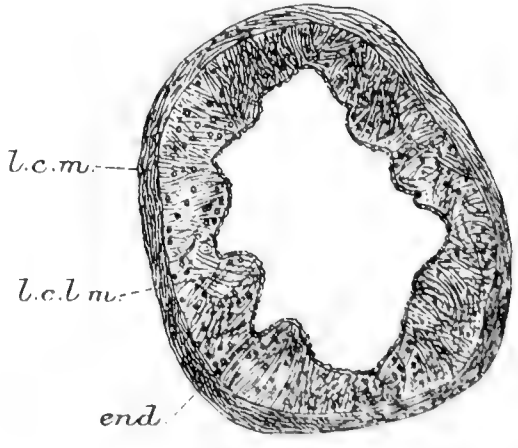

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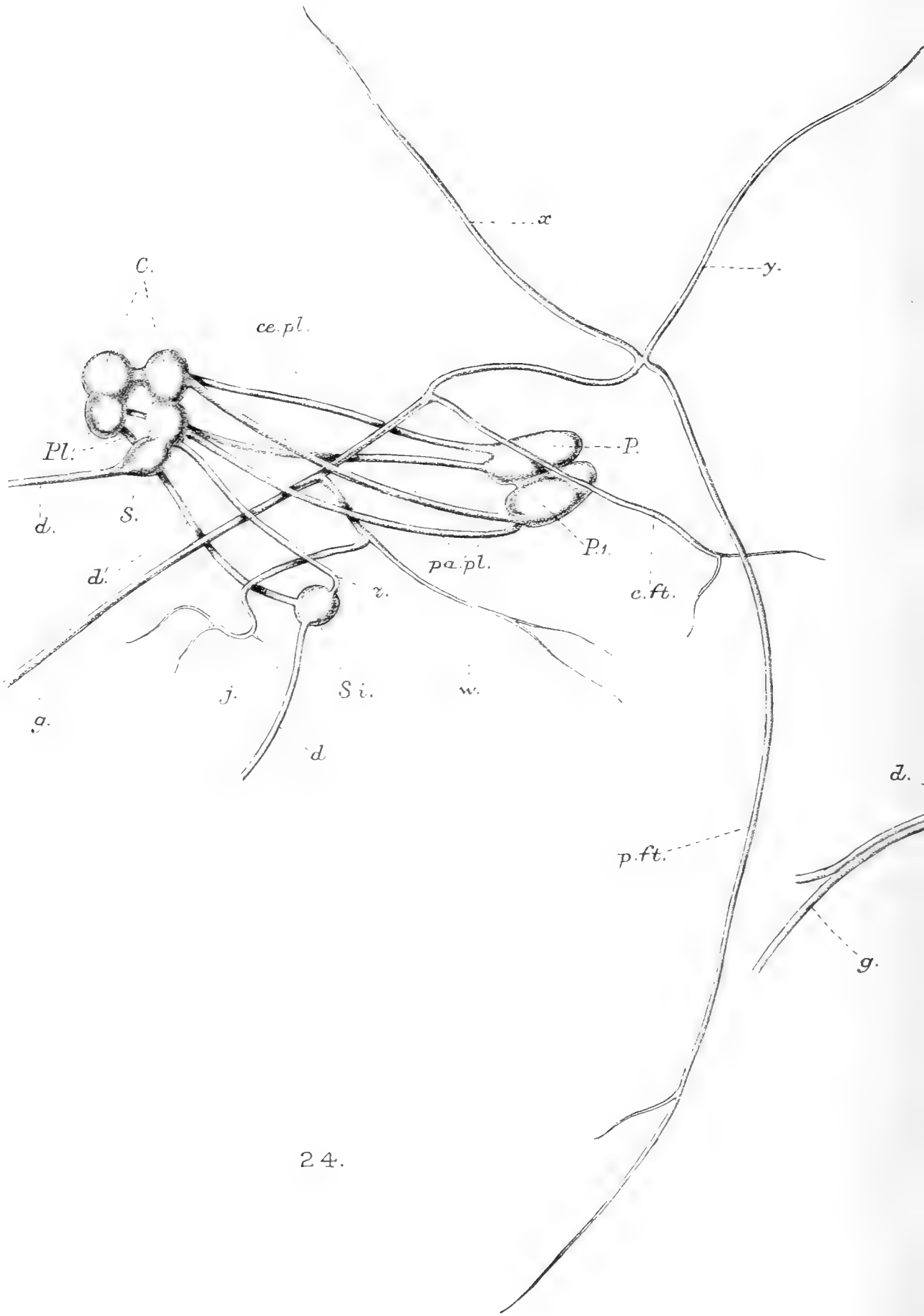

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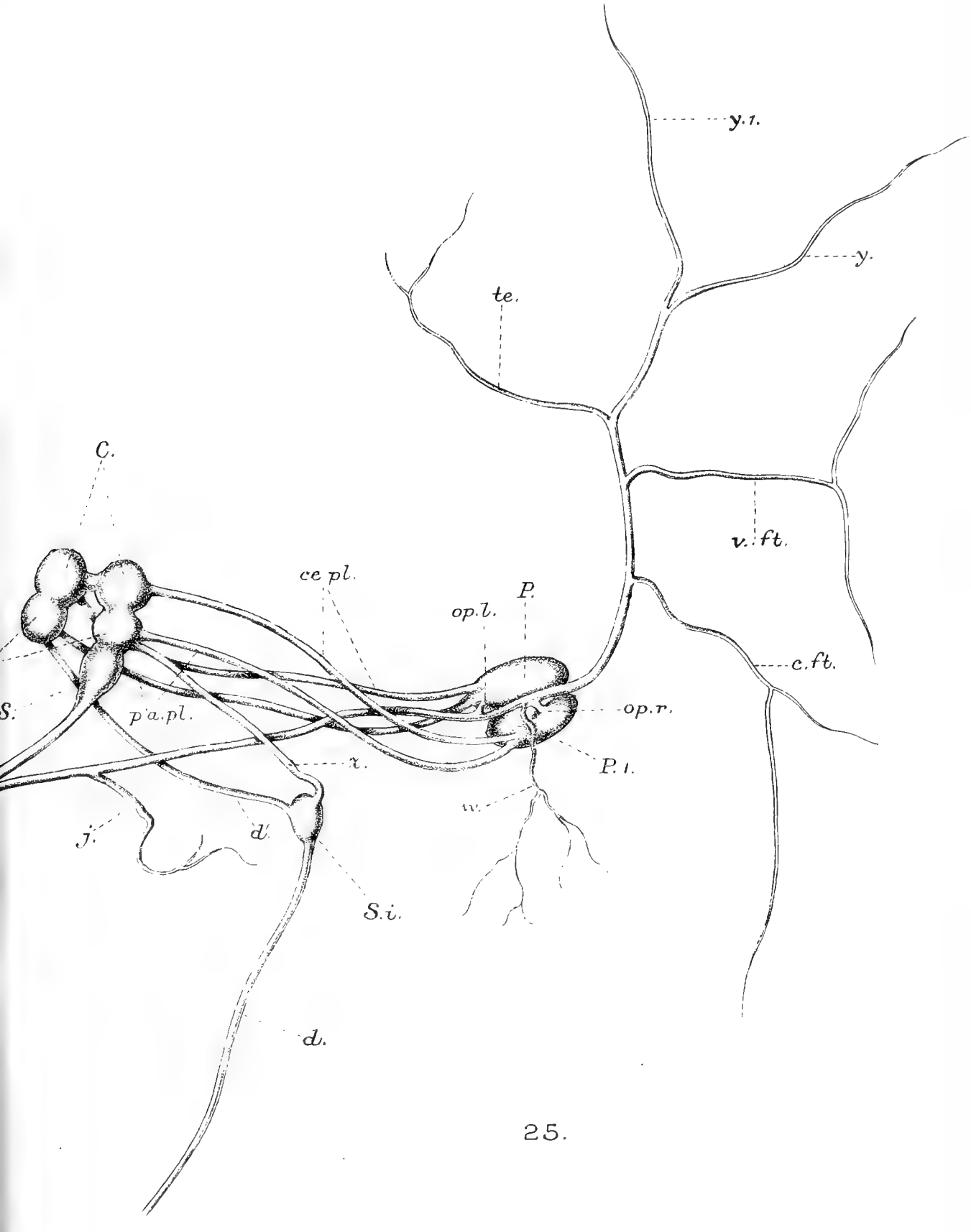

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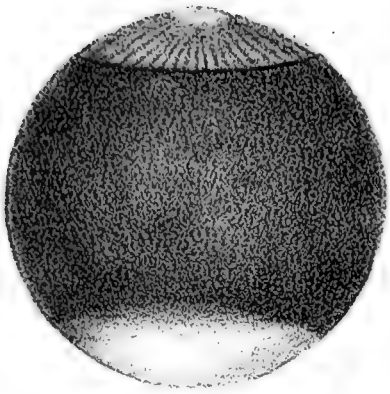

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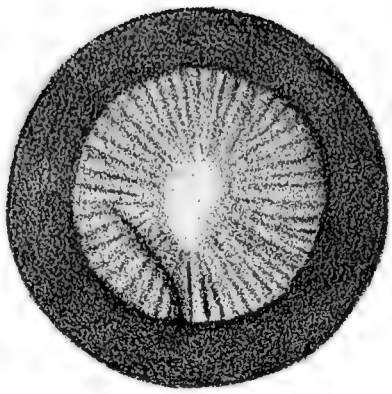

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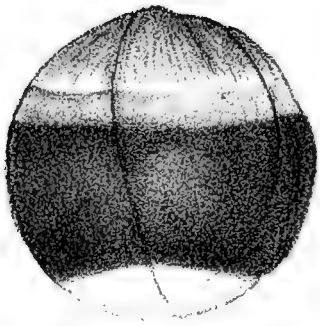

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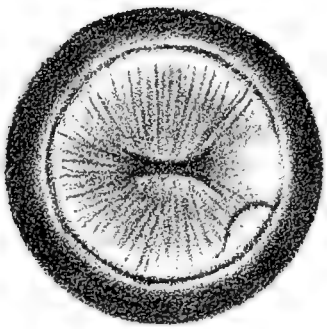

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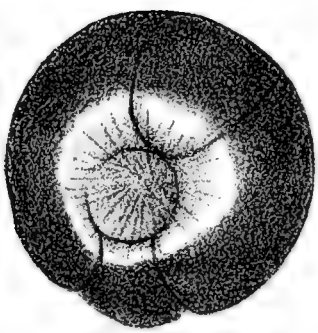

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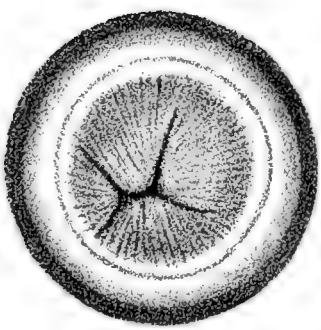

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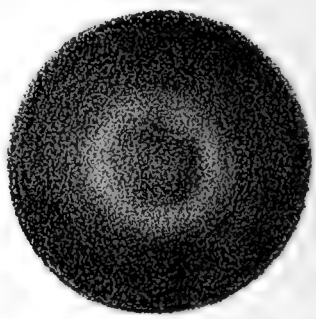

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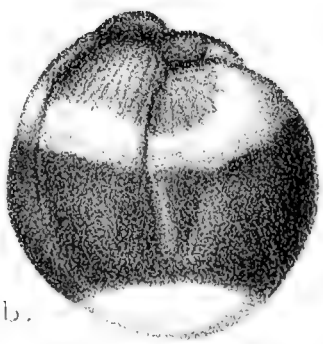

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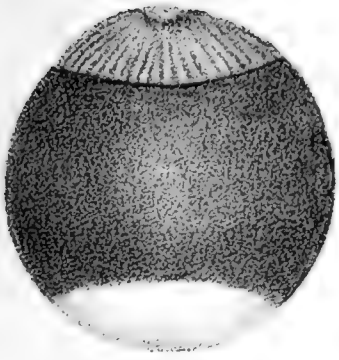

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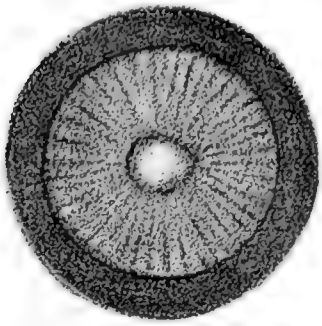

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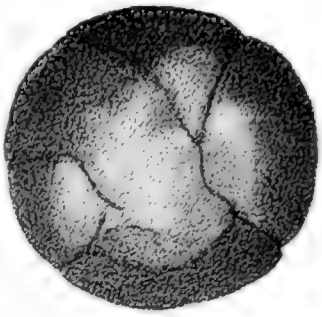

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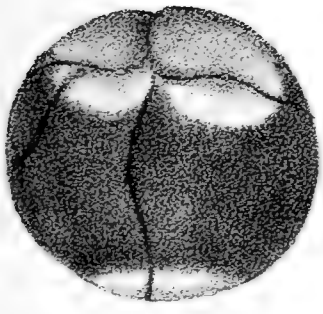

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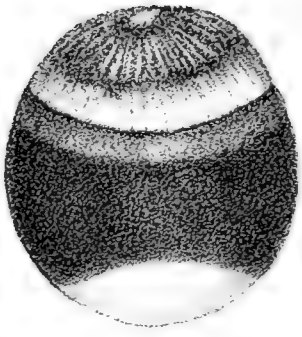

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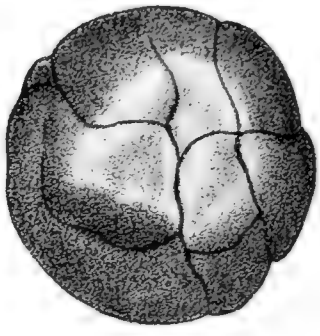

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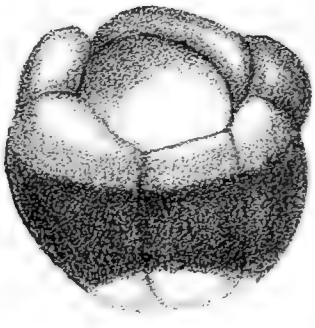

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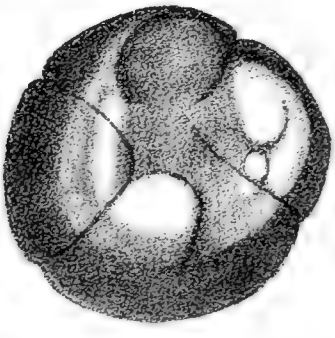

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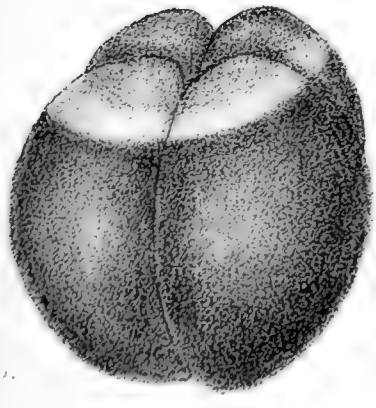

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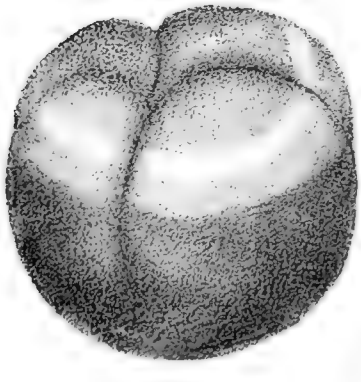

3 b

Hutchings Lith. London

UGED FROGS EGG.


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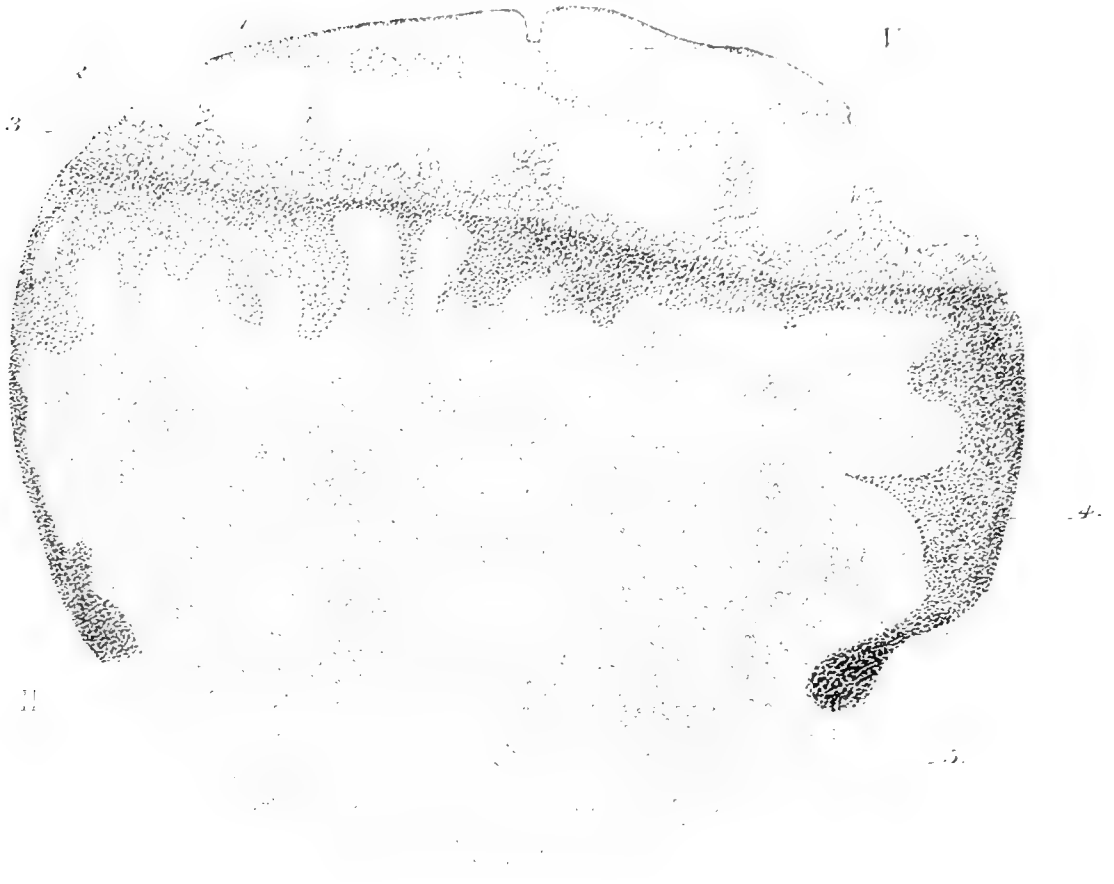
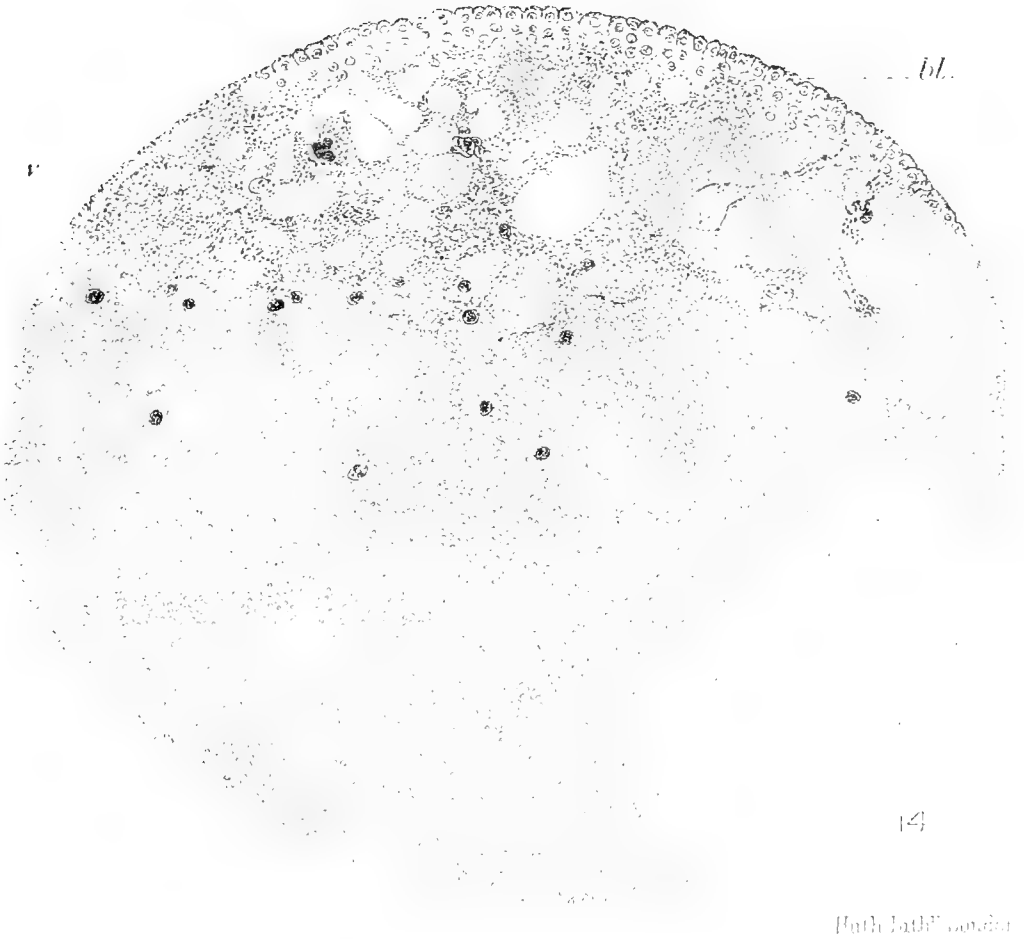


Hanhart Lith. London

RIFUGED FROGS EGG.



Huth Lith. London

JENKINSON—CENTRIFUGED FROGS EGG.

15

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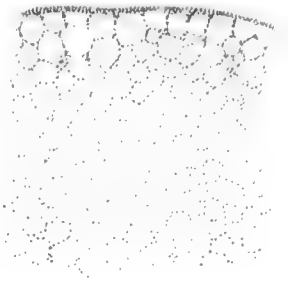

a

16

b

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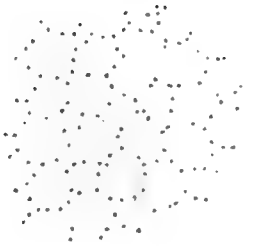

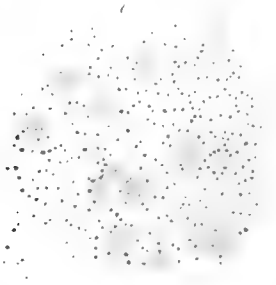

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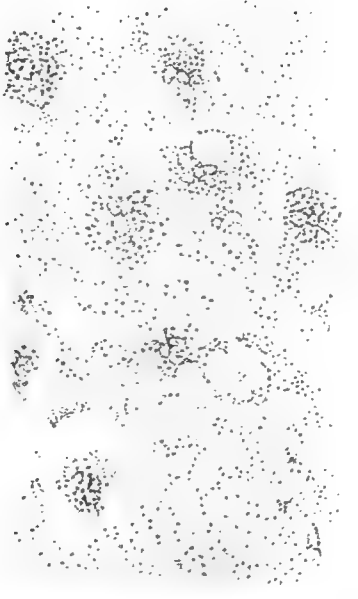

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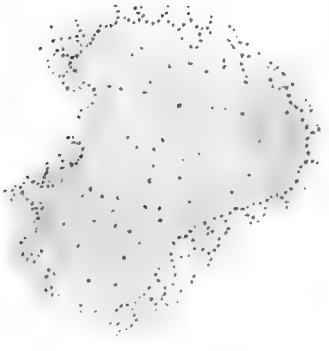

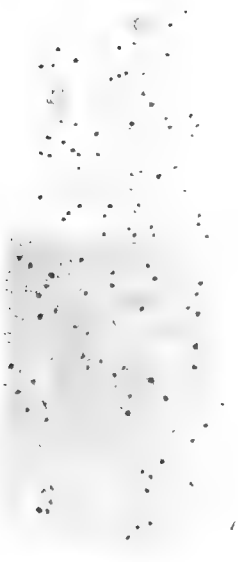

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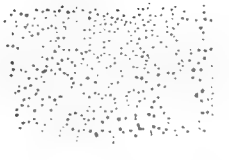

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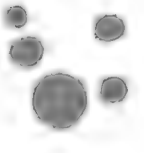
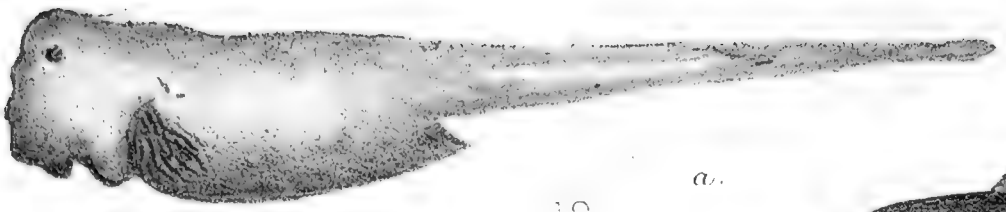


Huth, Lith.r London

JENKINSON—CENTRIFUGED FROGS EGG.



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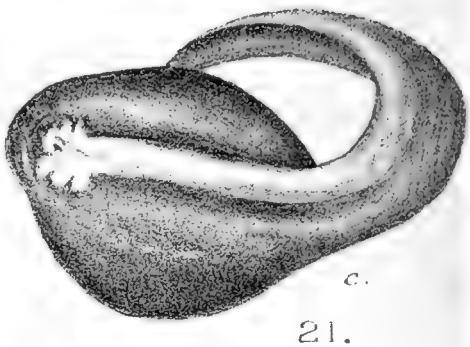

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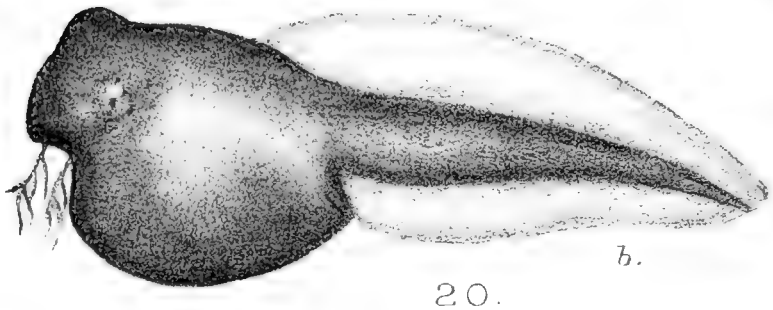

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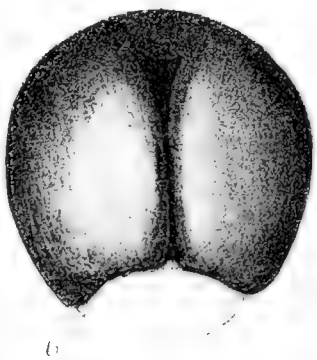

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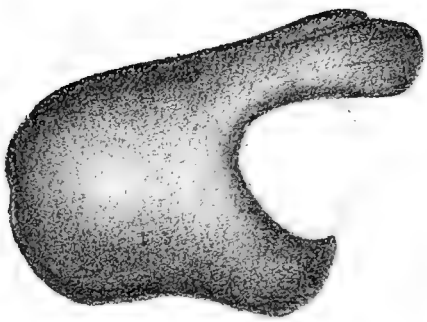

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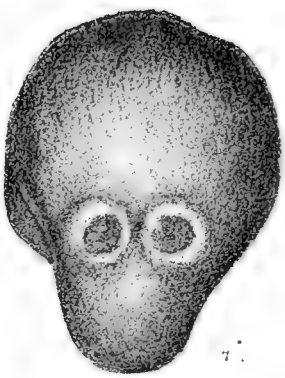

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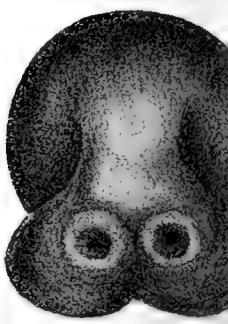

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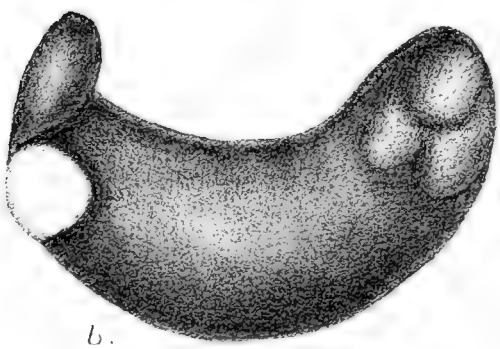

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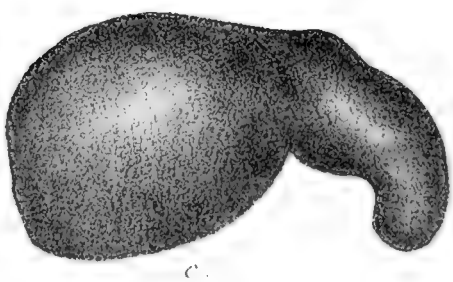

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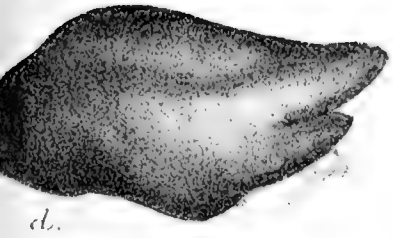

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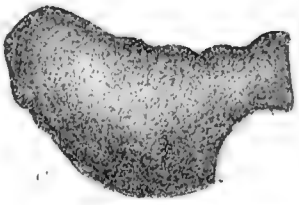

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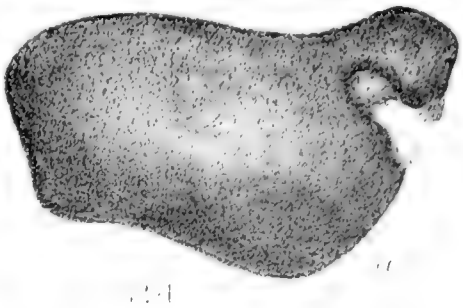

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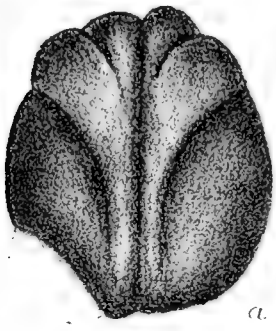

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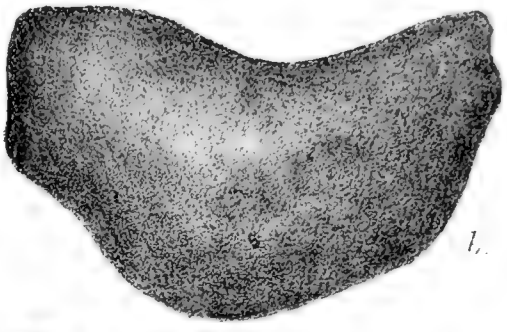

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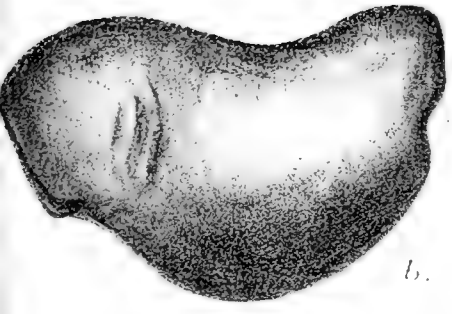

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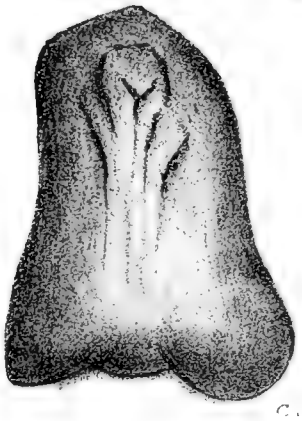

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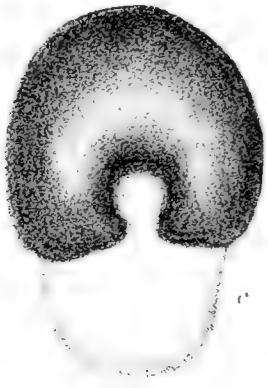

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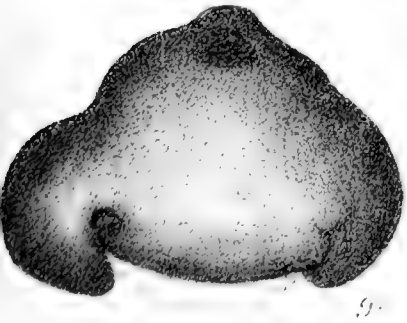

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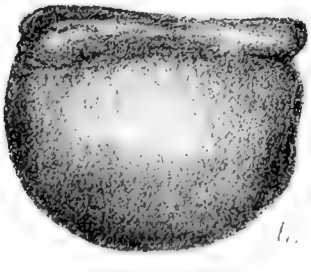

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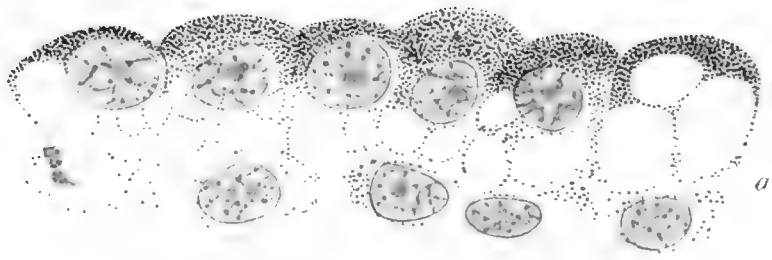

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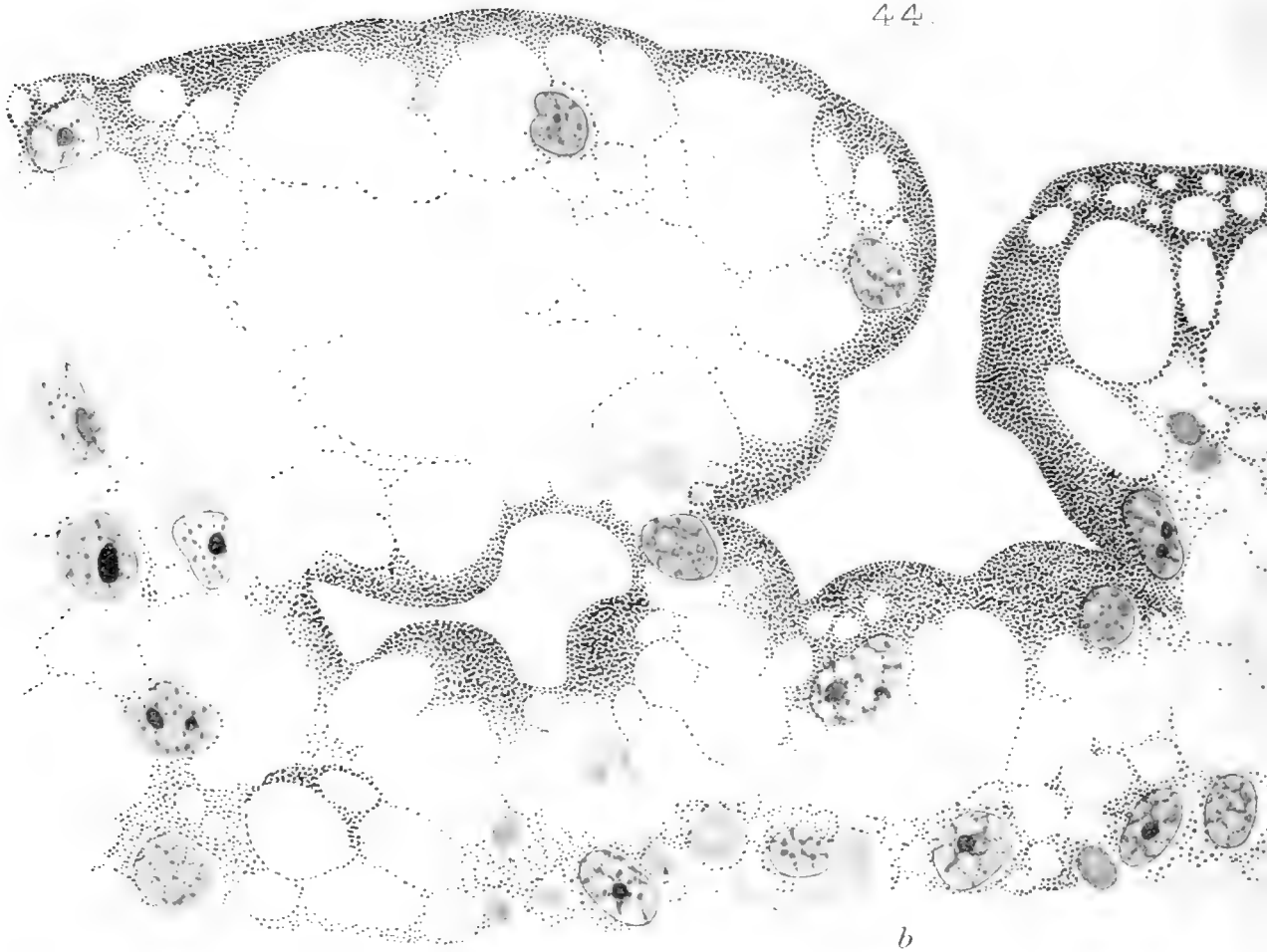

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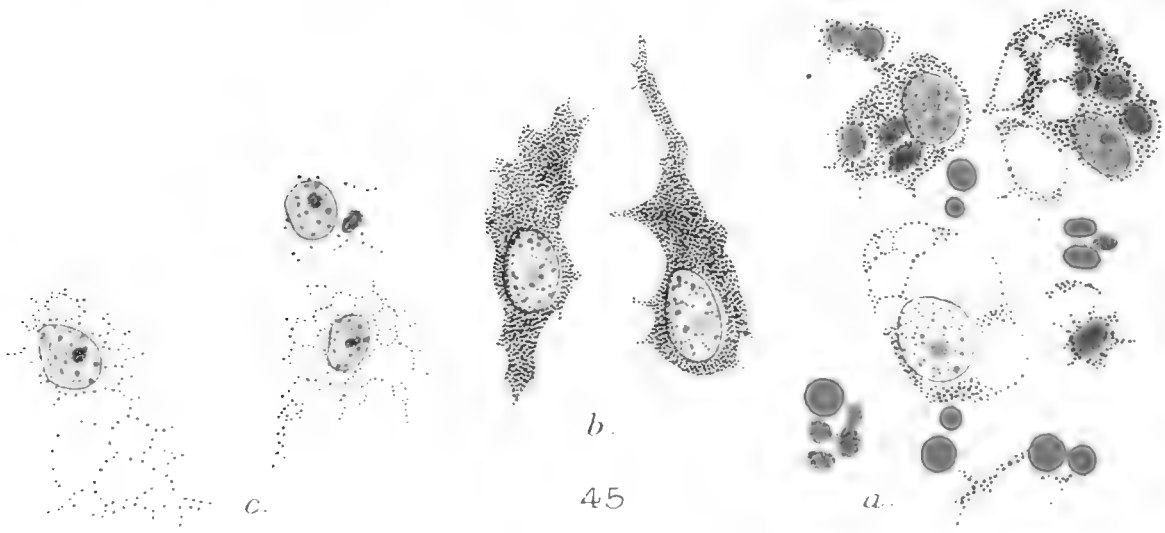

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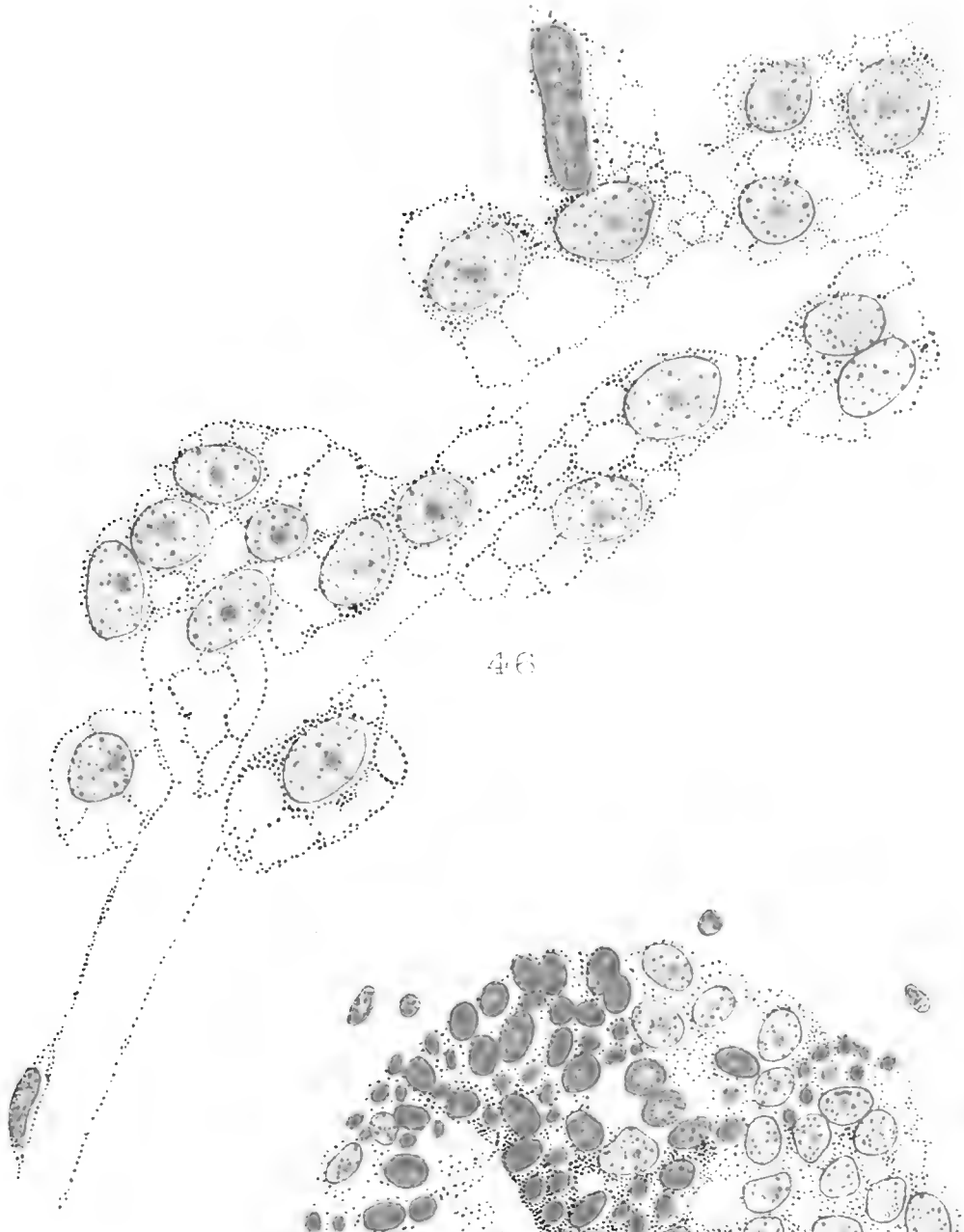

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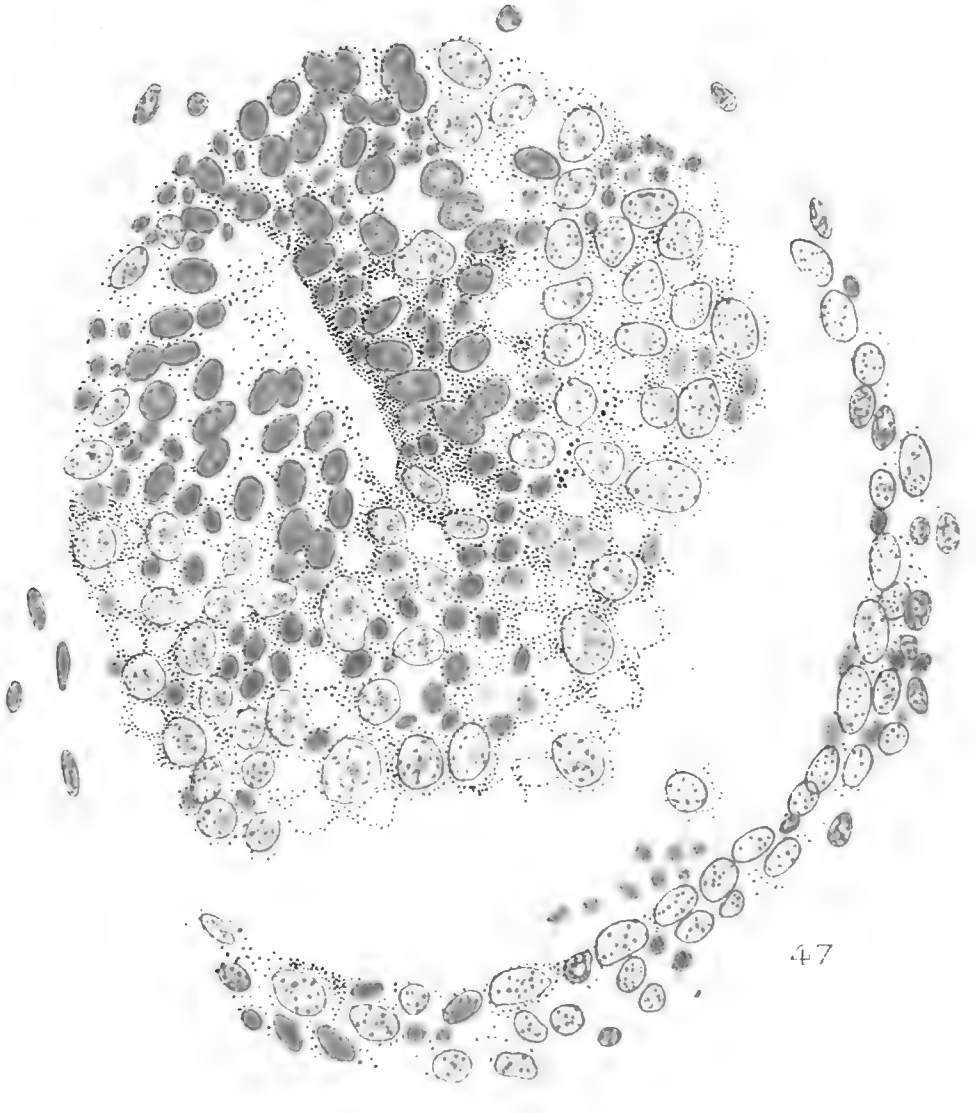

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Huth, Lith. London.

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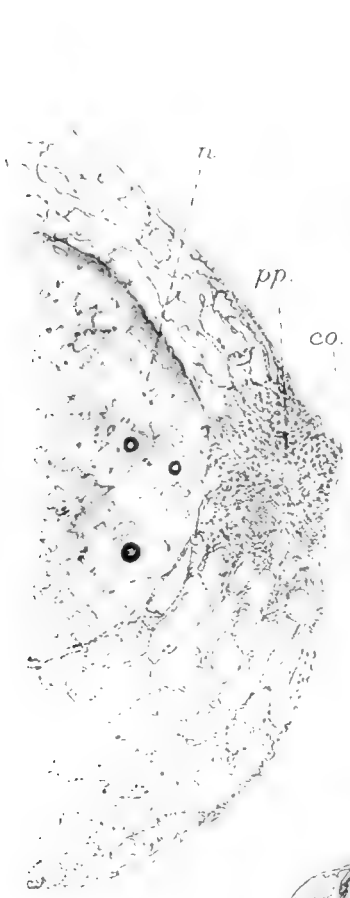

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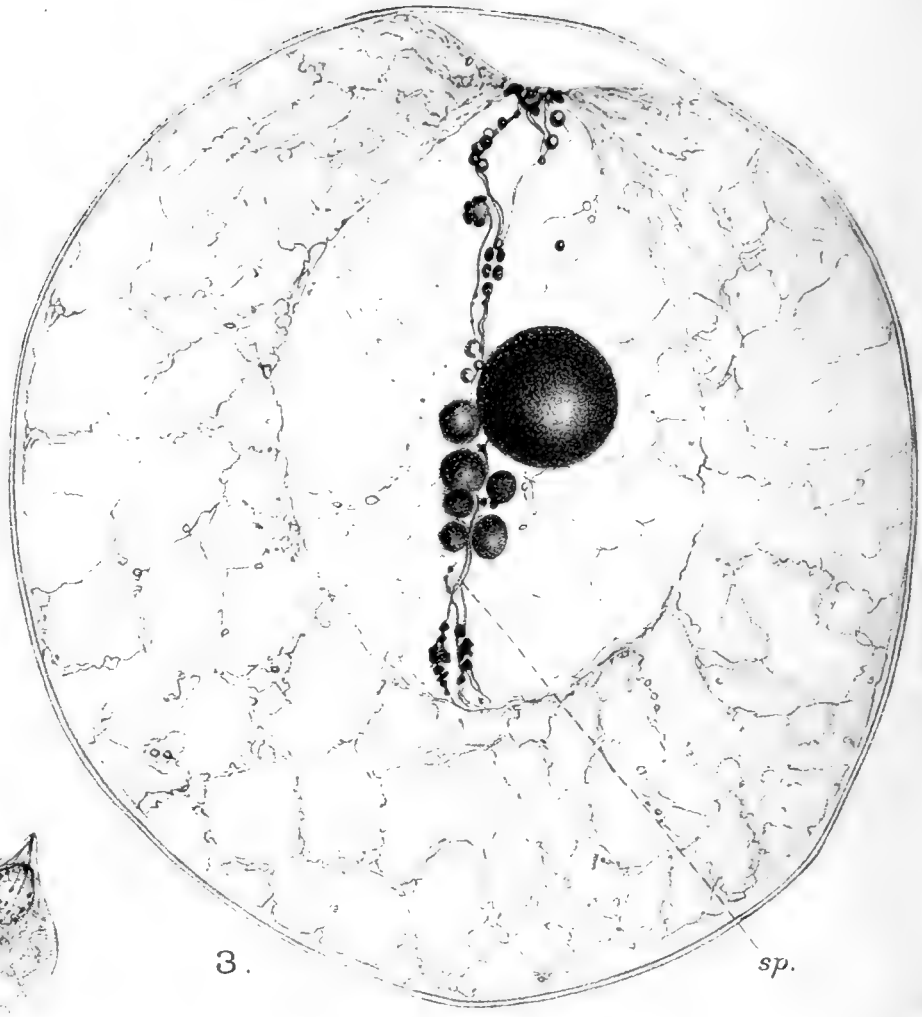

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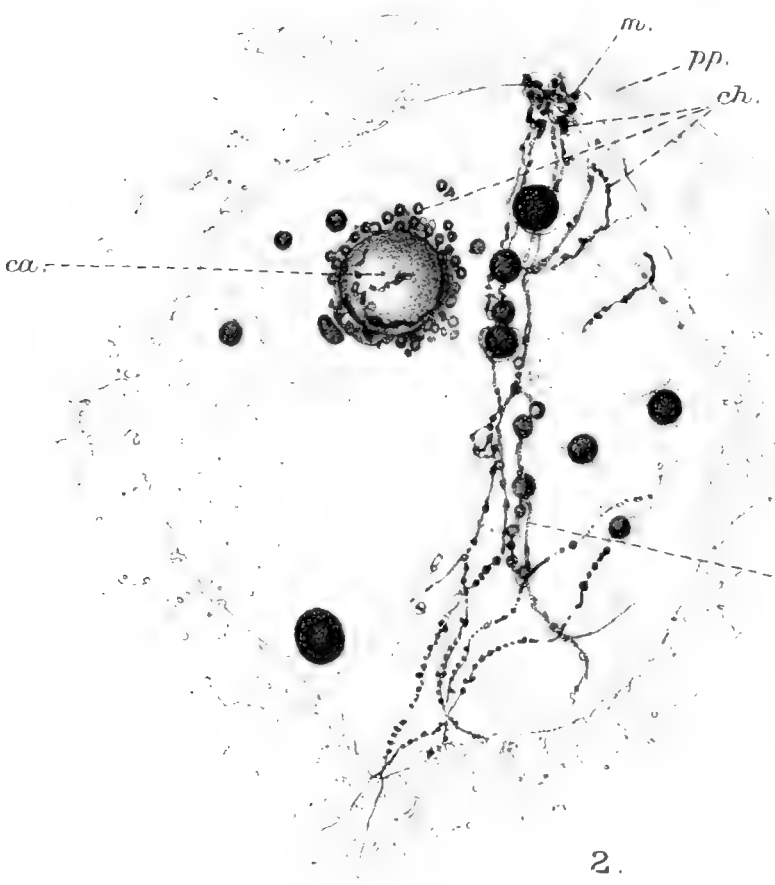

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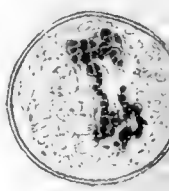

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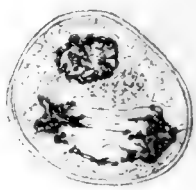

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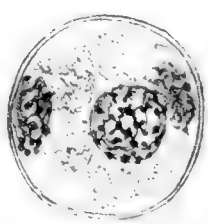

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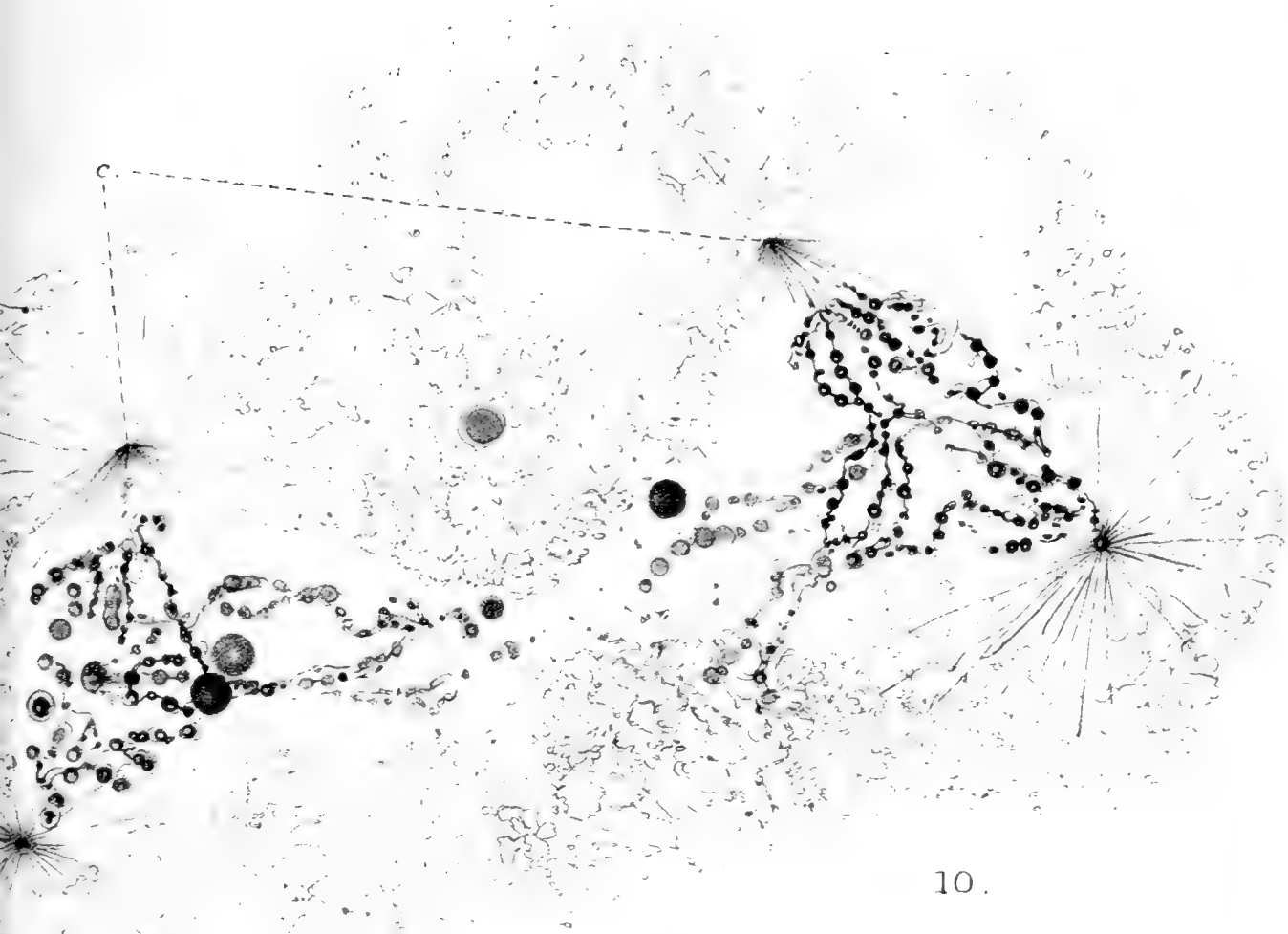

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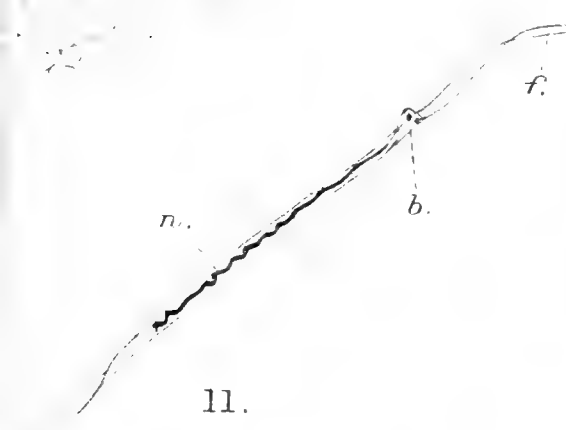

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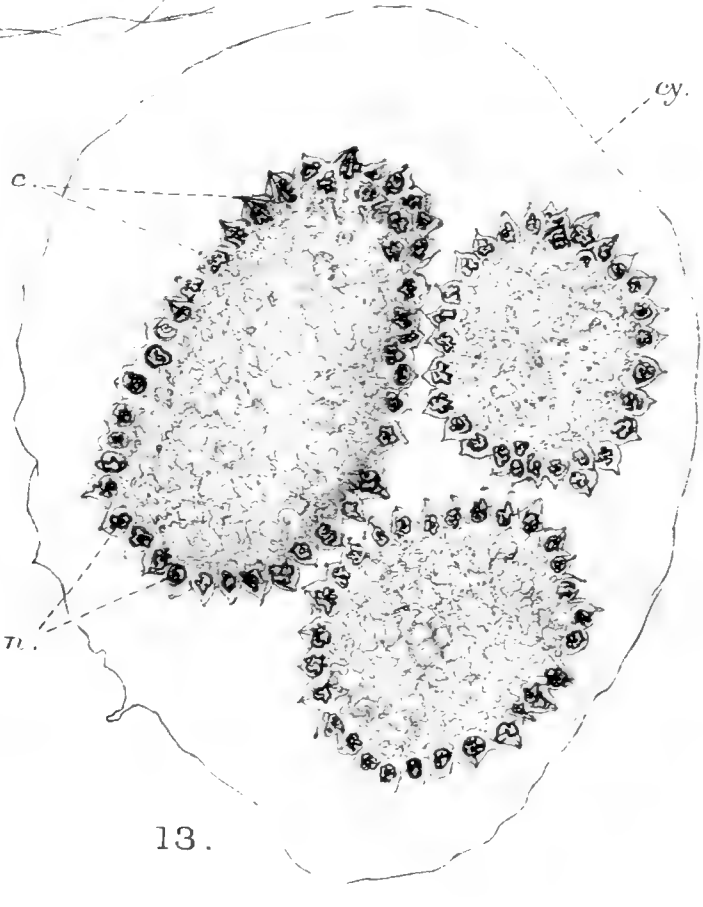

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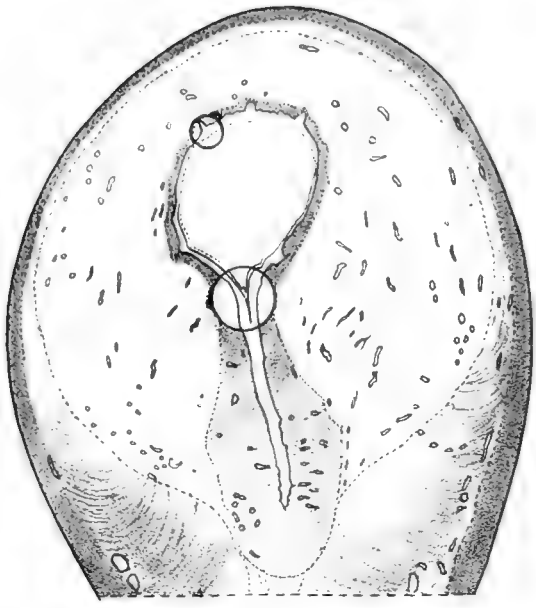

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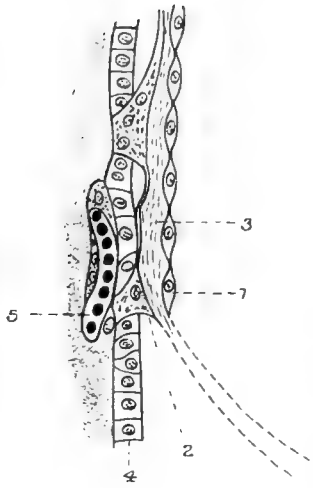

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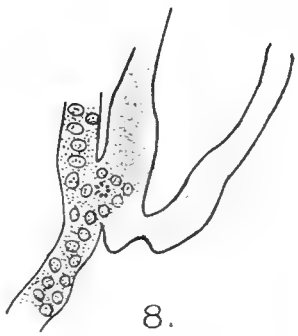

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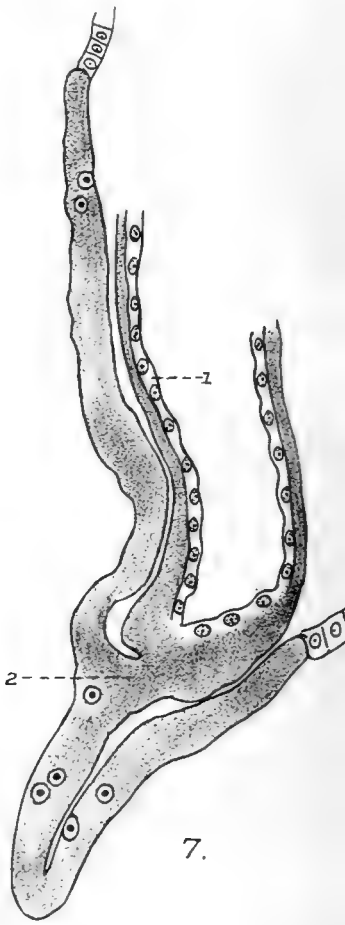

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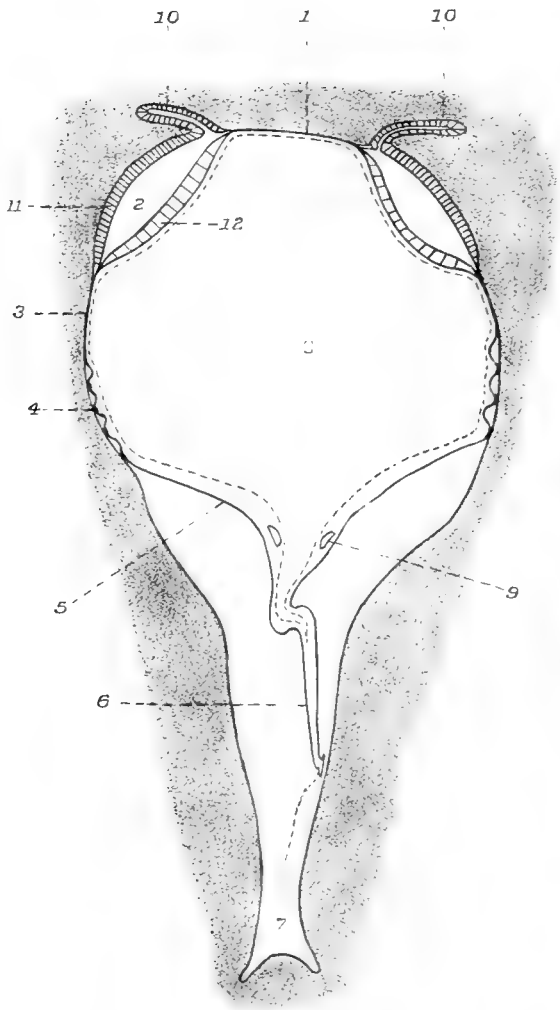

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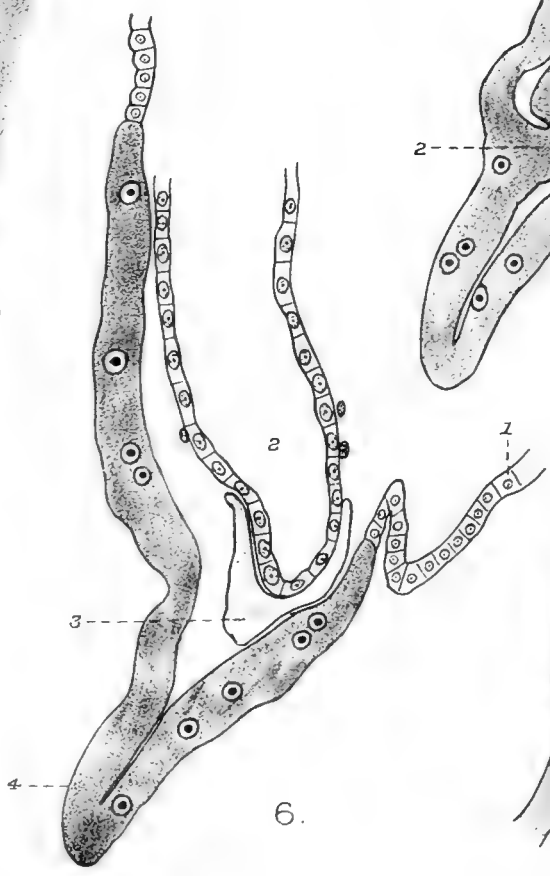

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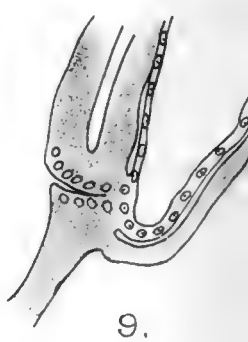

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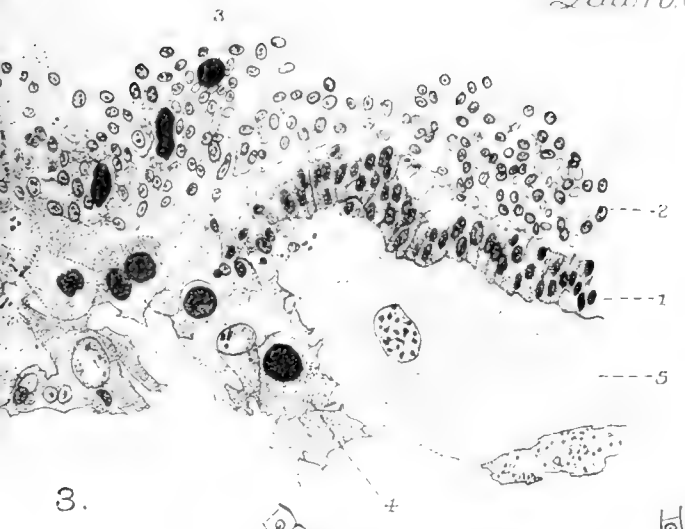

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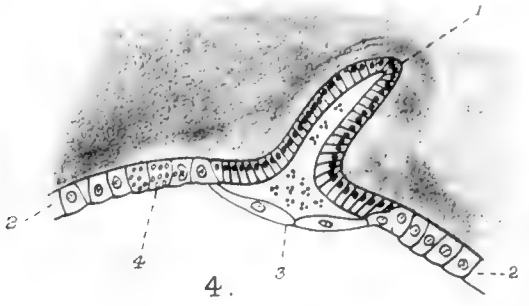

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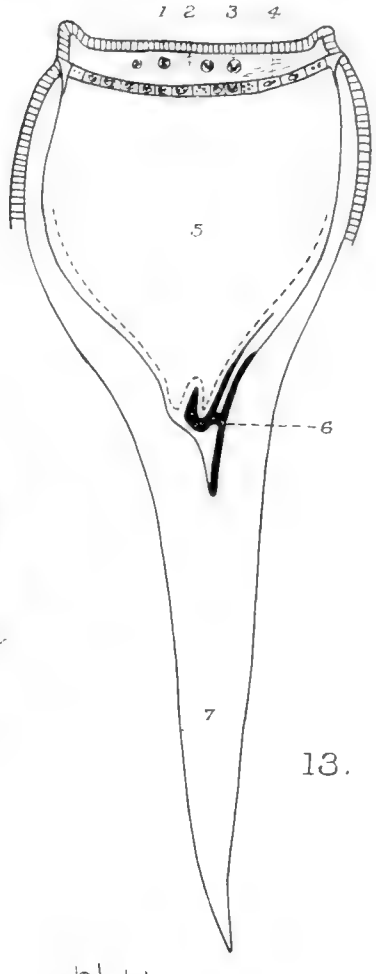

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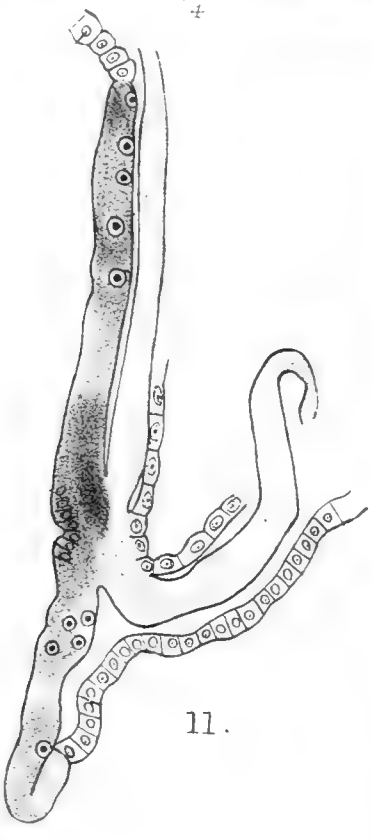

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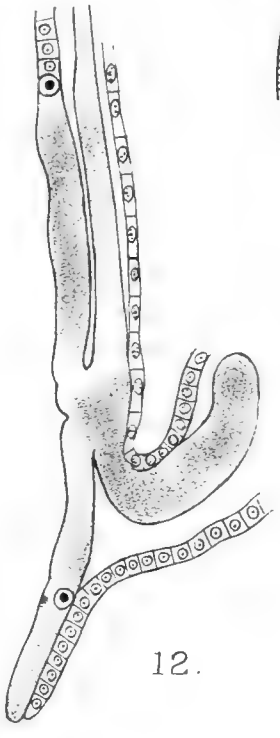

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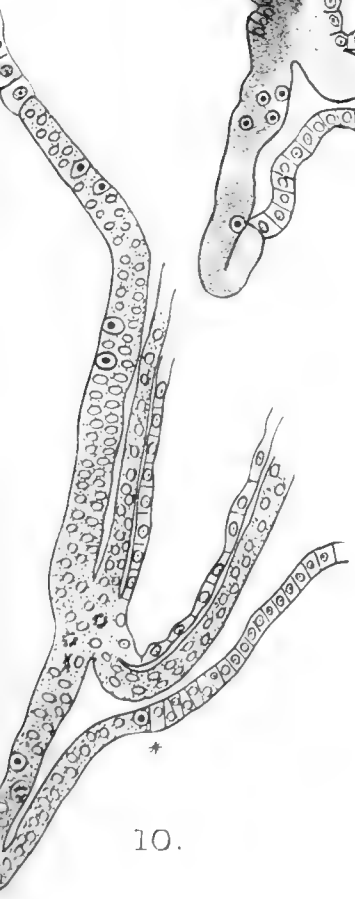

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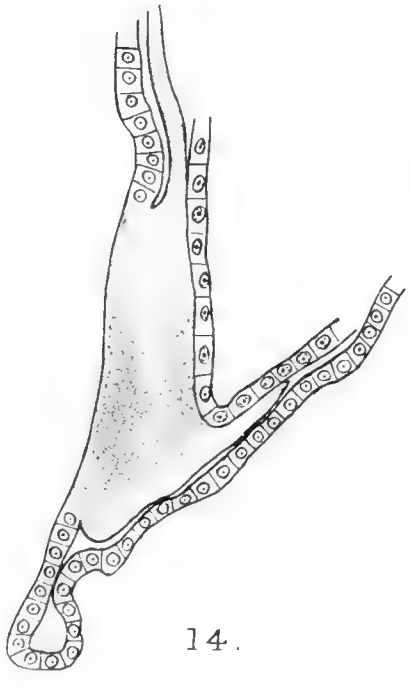

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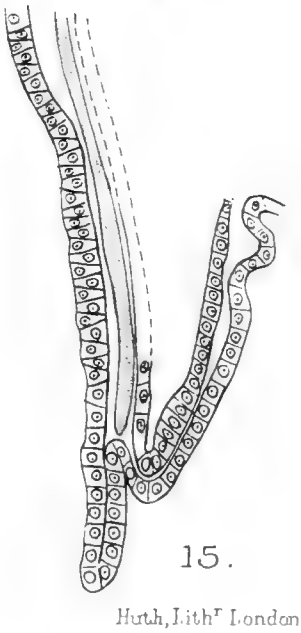

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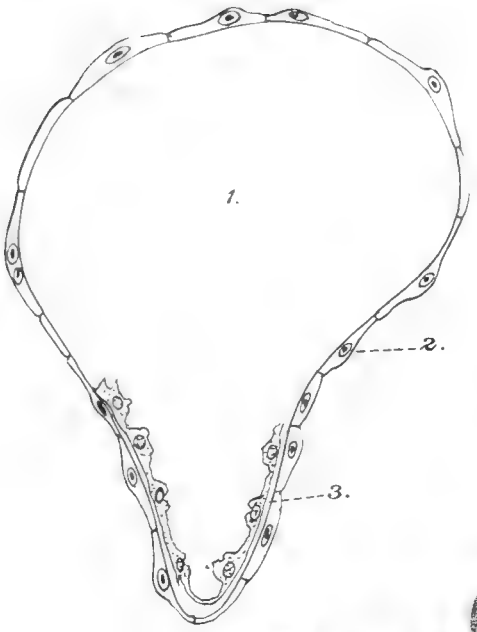

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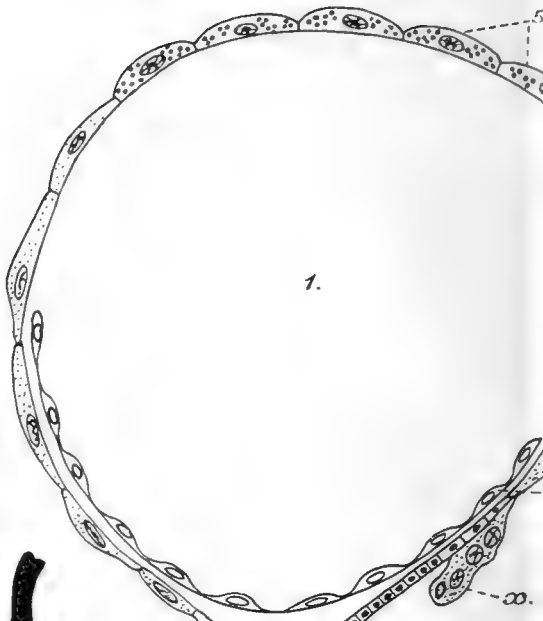

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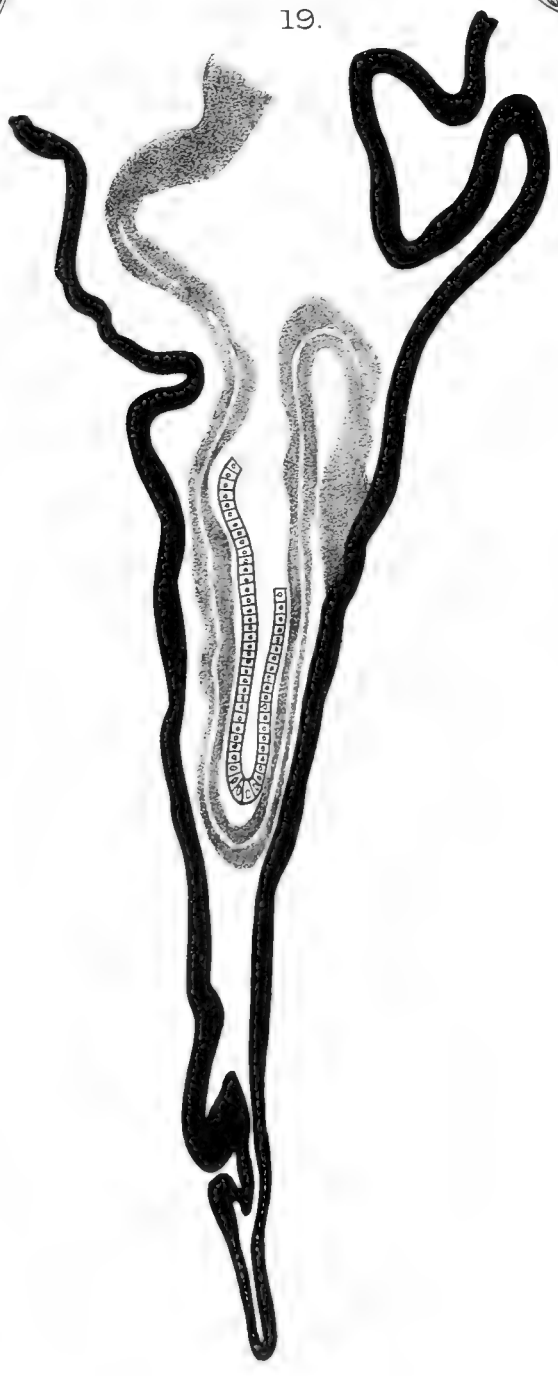

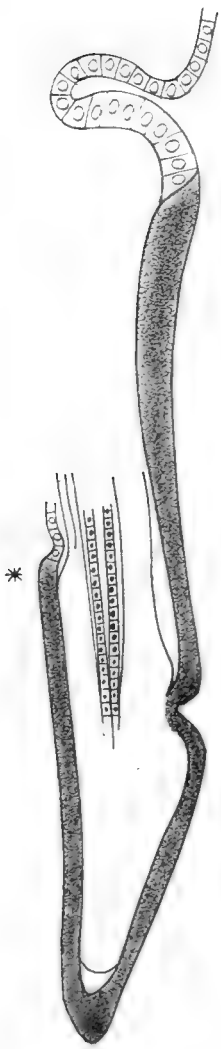

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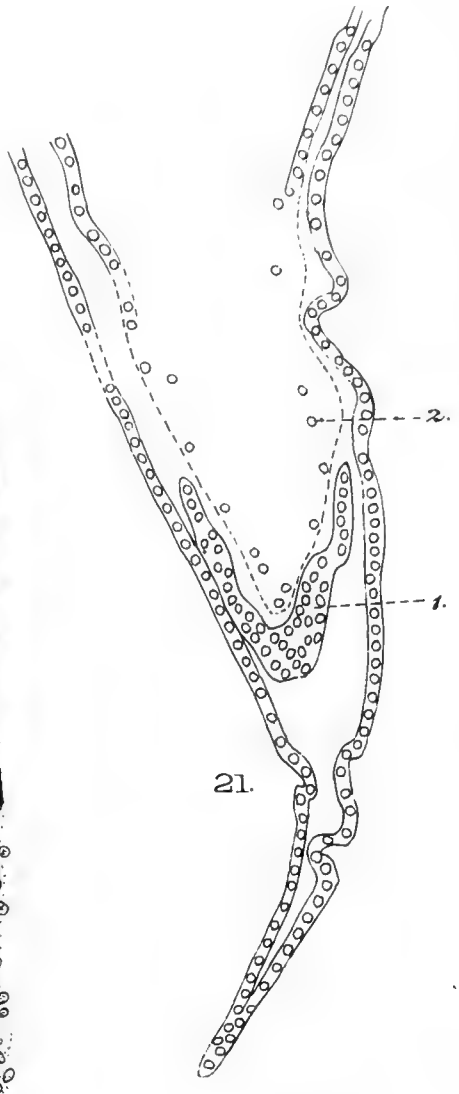

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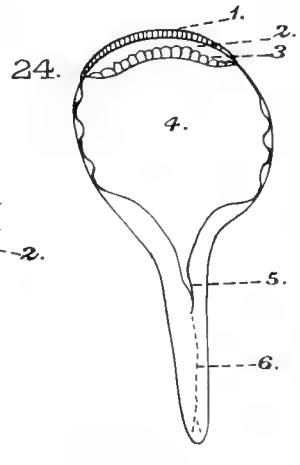

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Huth. Lith^r London.

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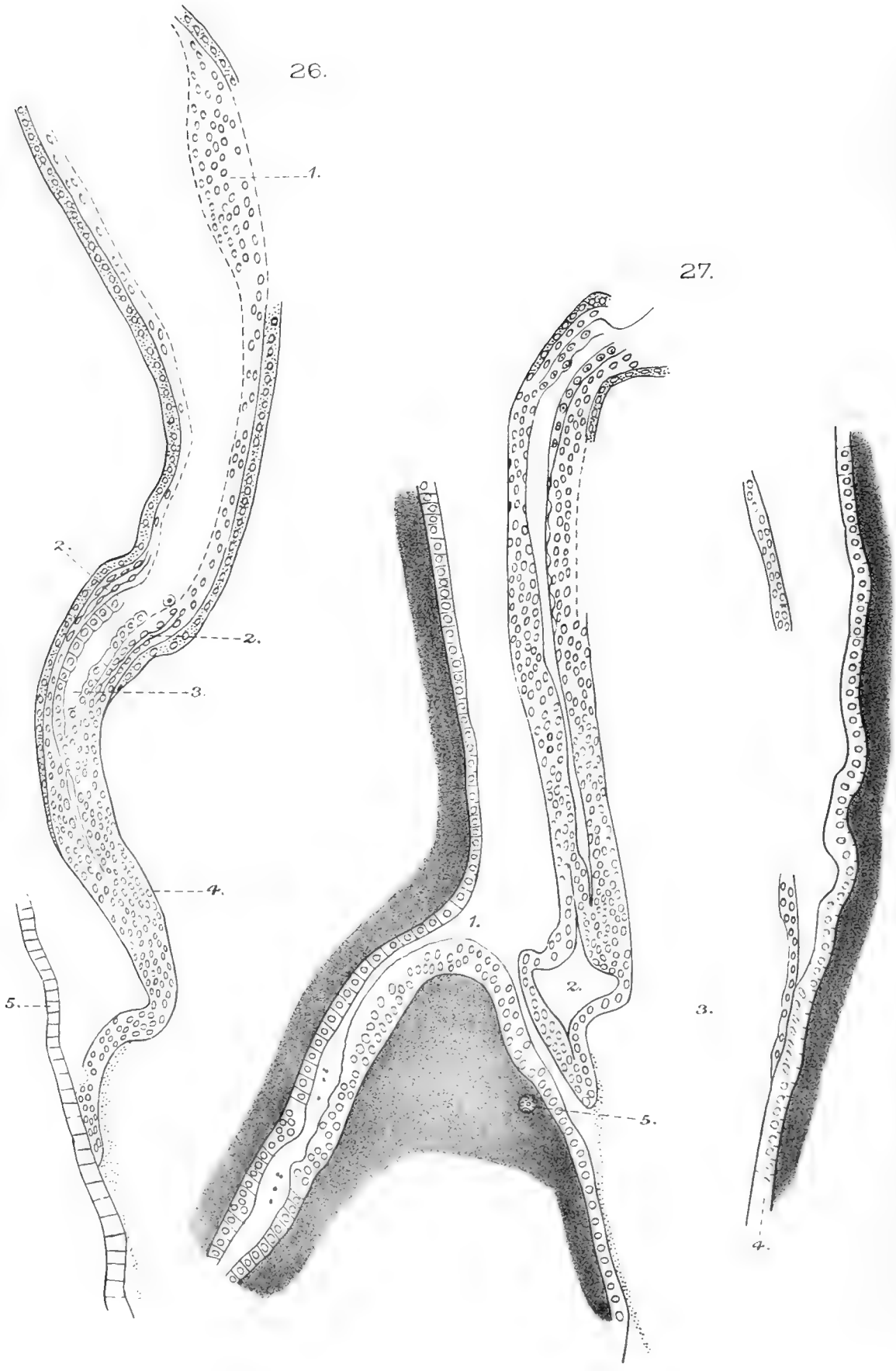
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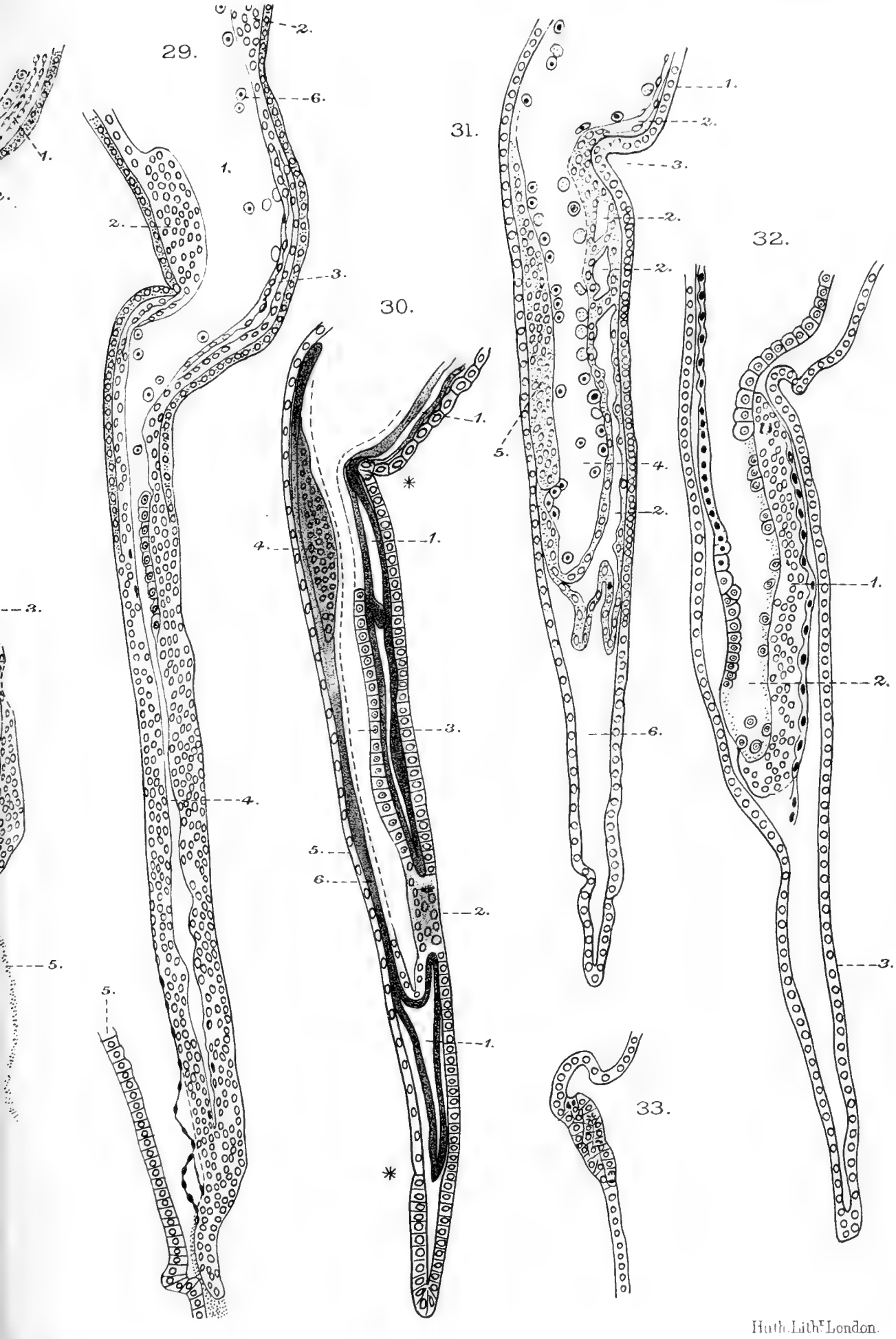

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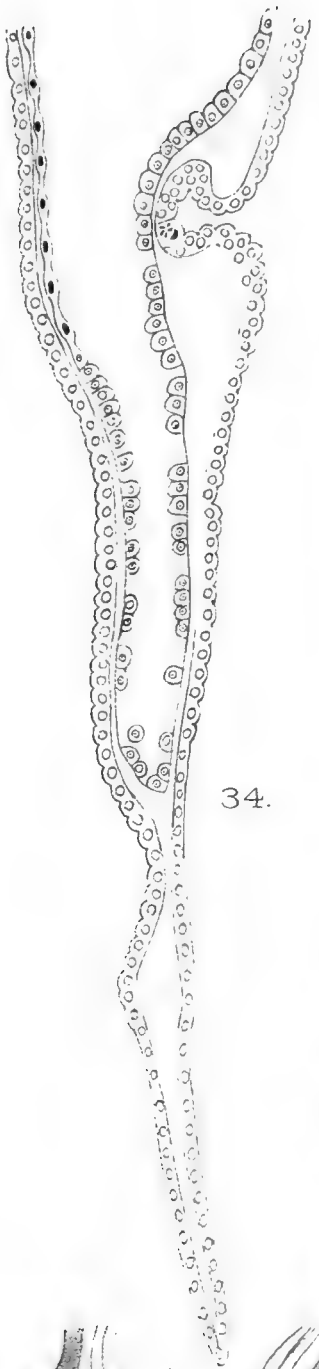

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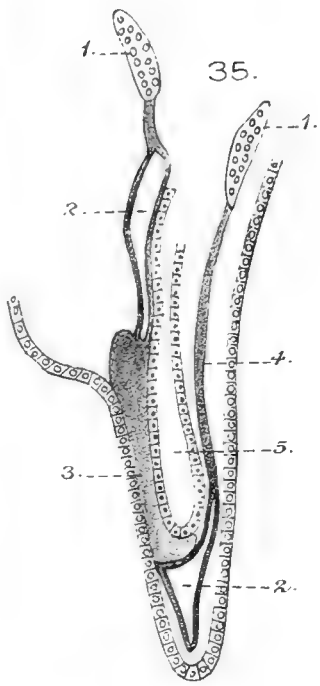

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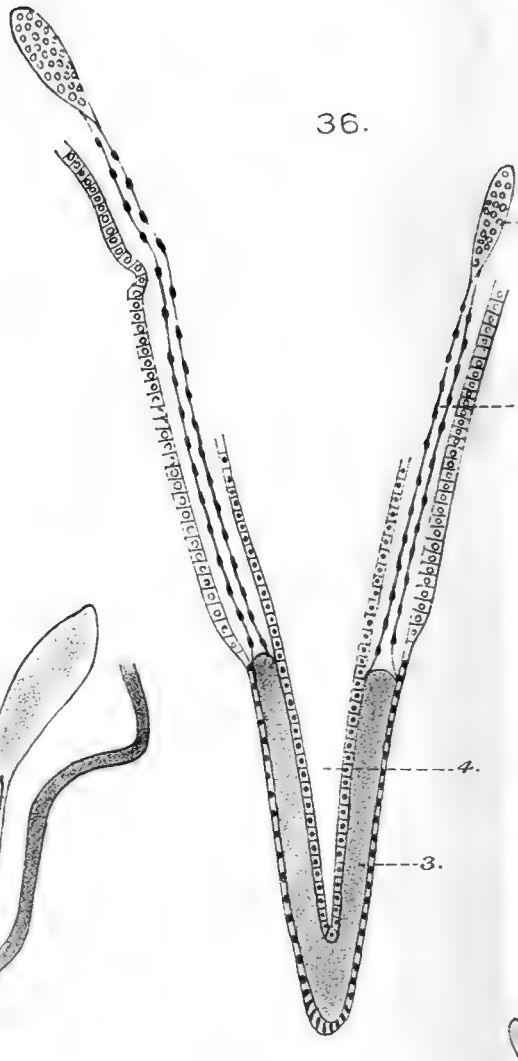

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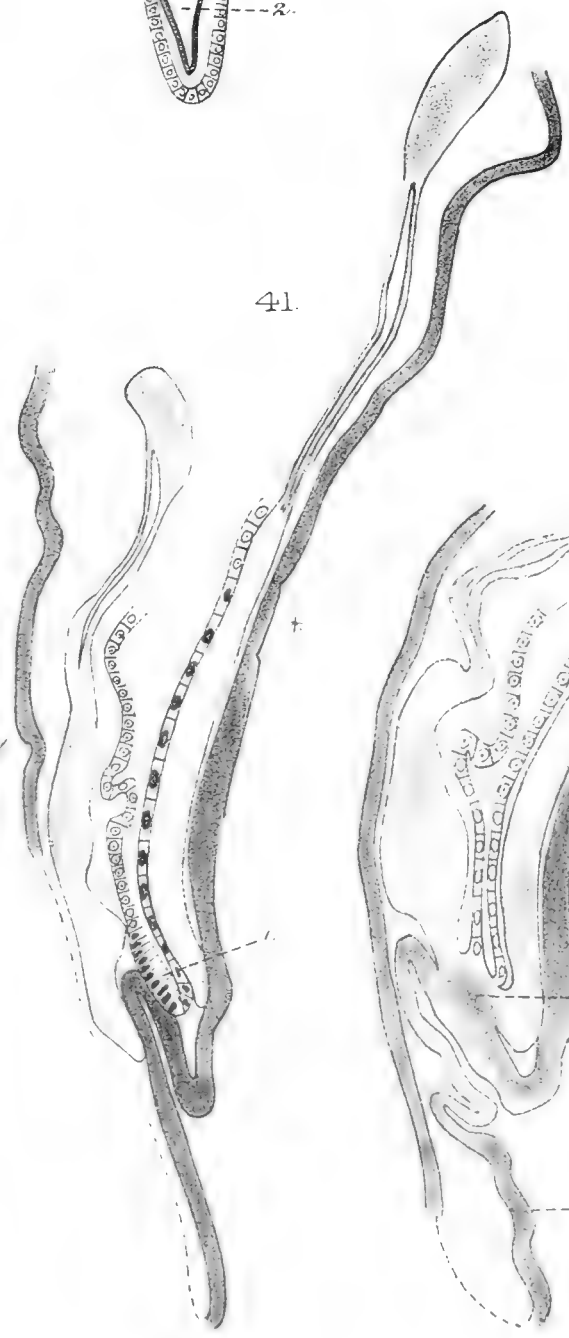

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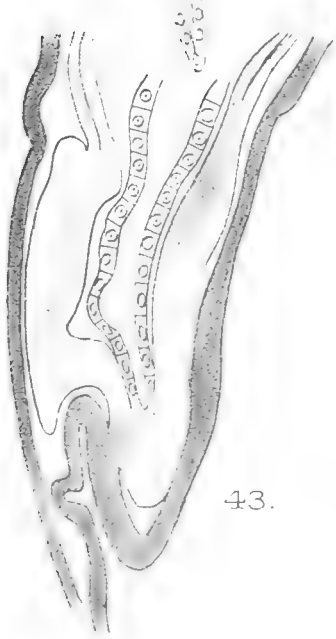

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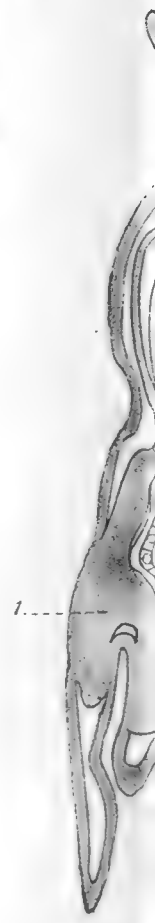

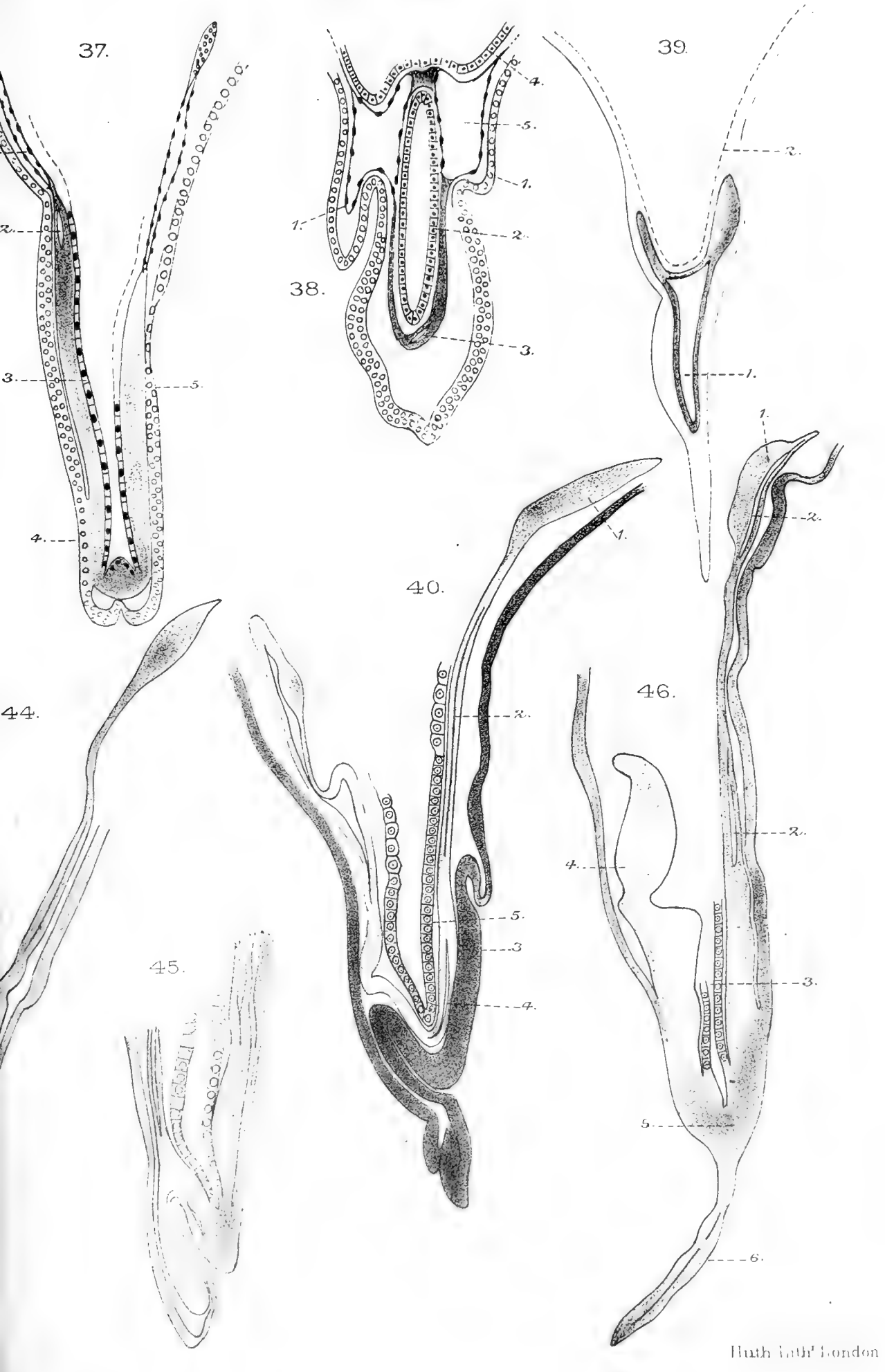

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S PLACENTA.


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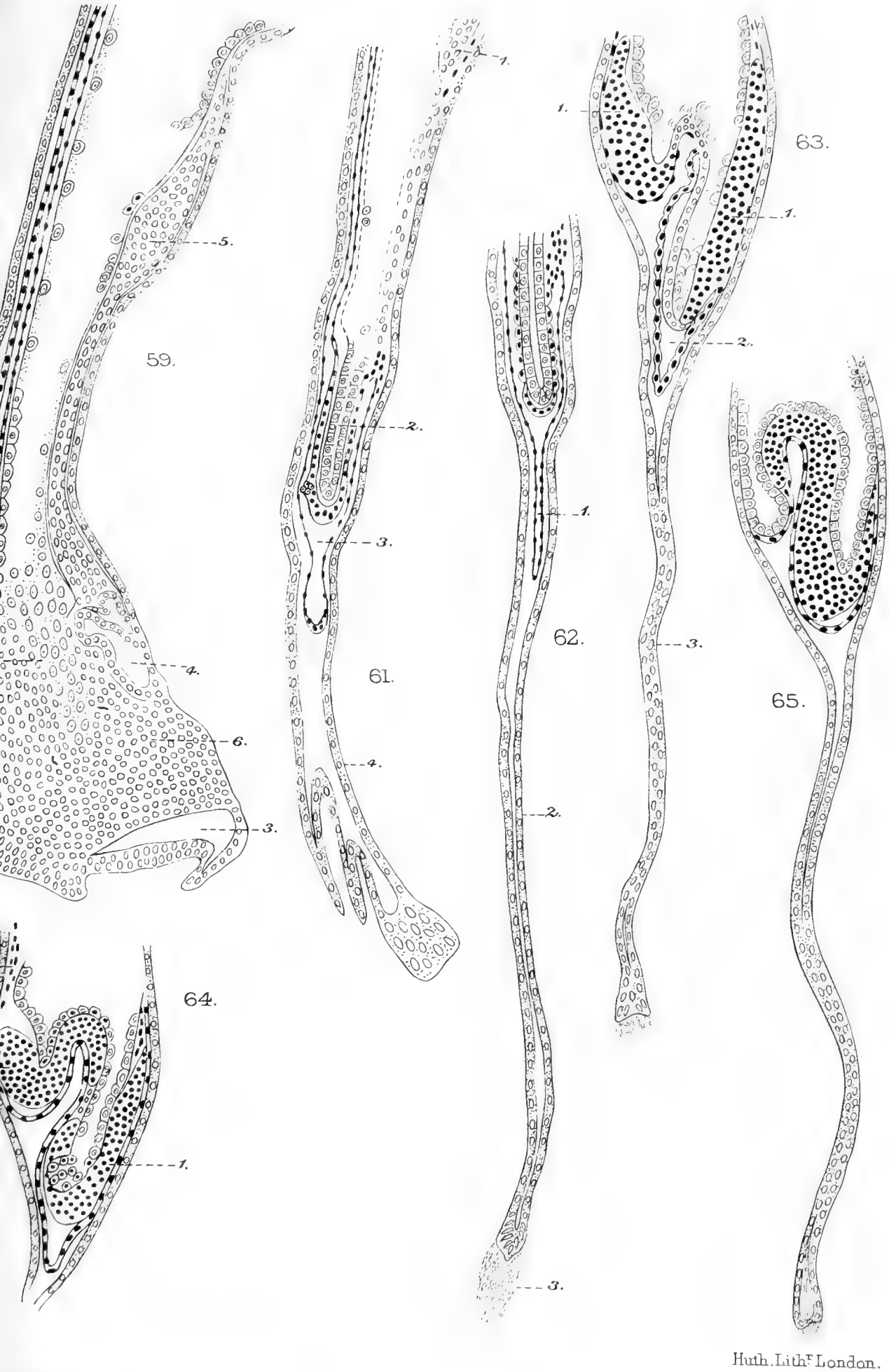

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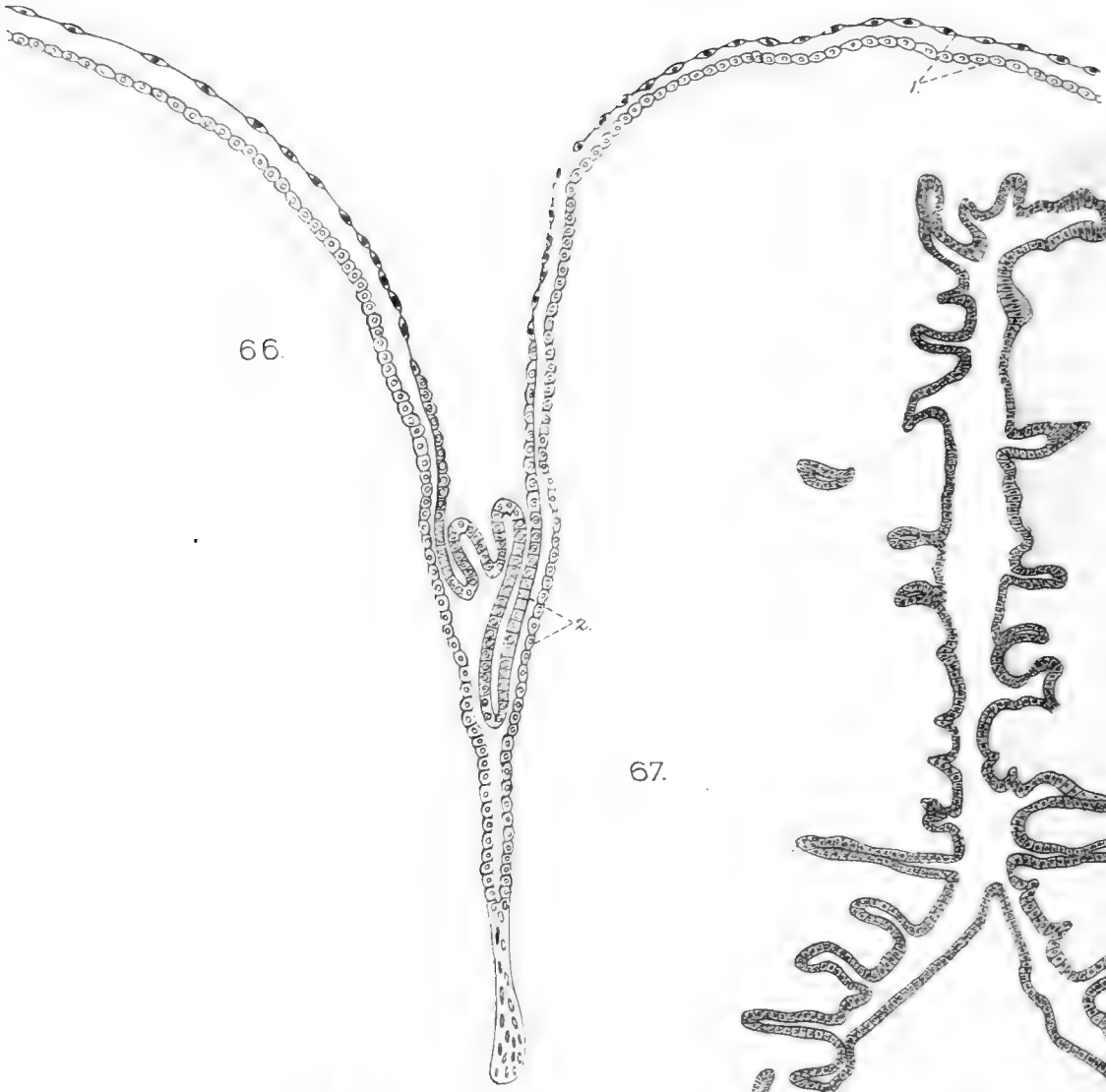
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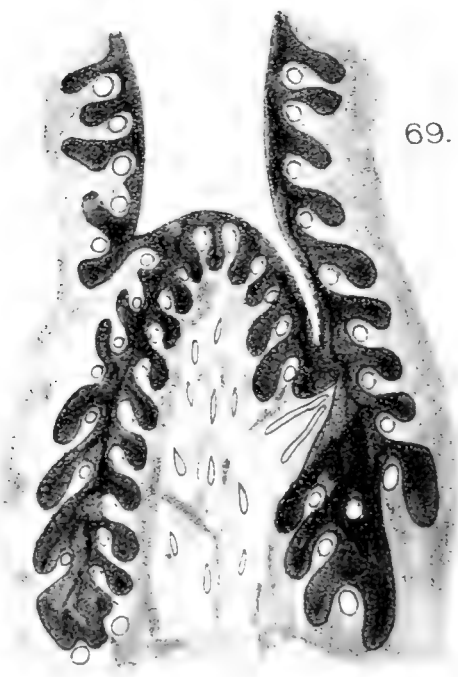
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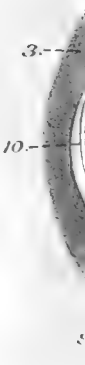
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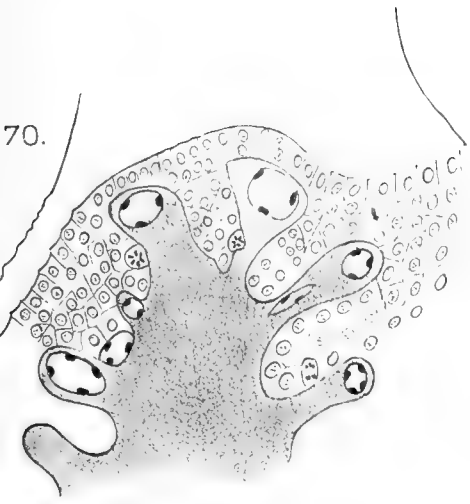

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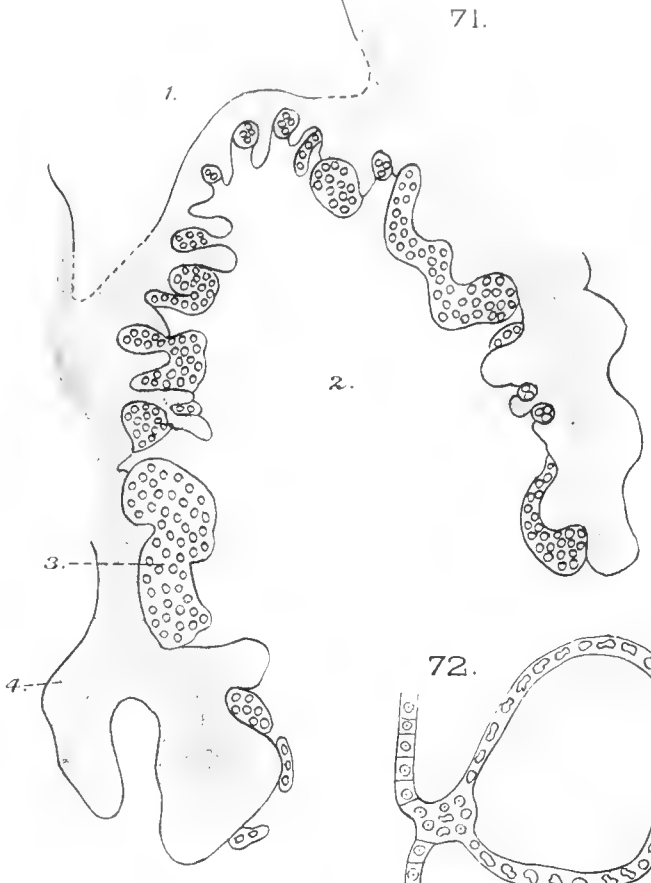

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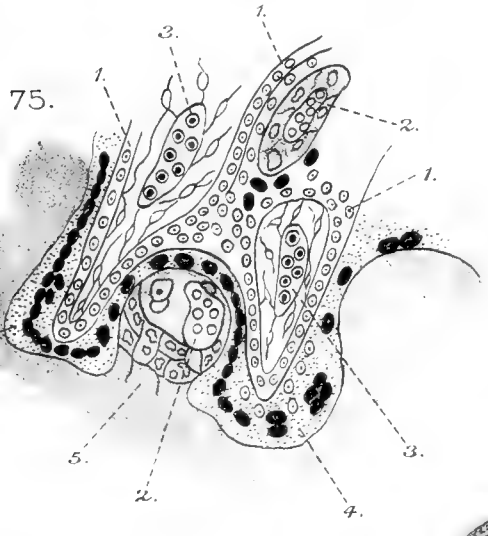

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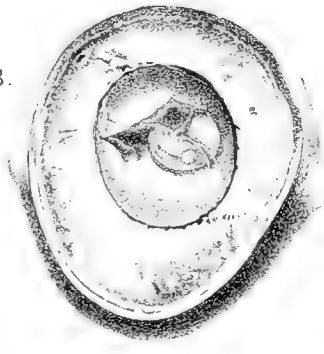

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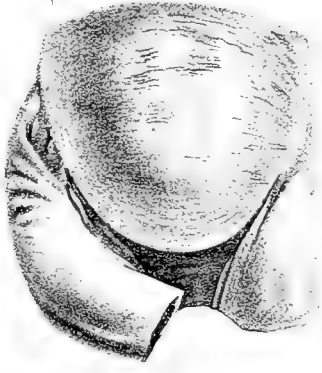

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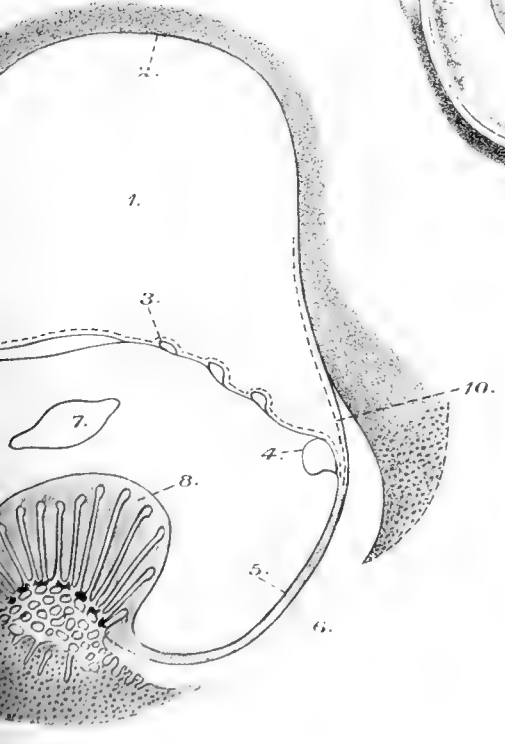

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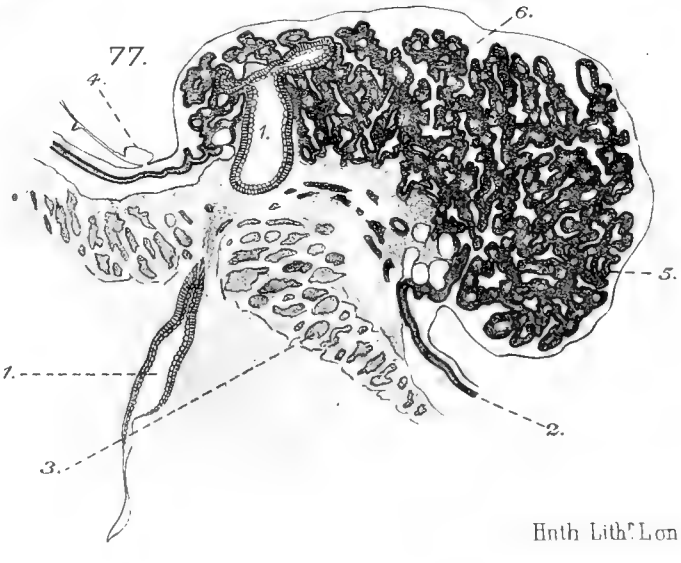

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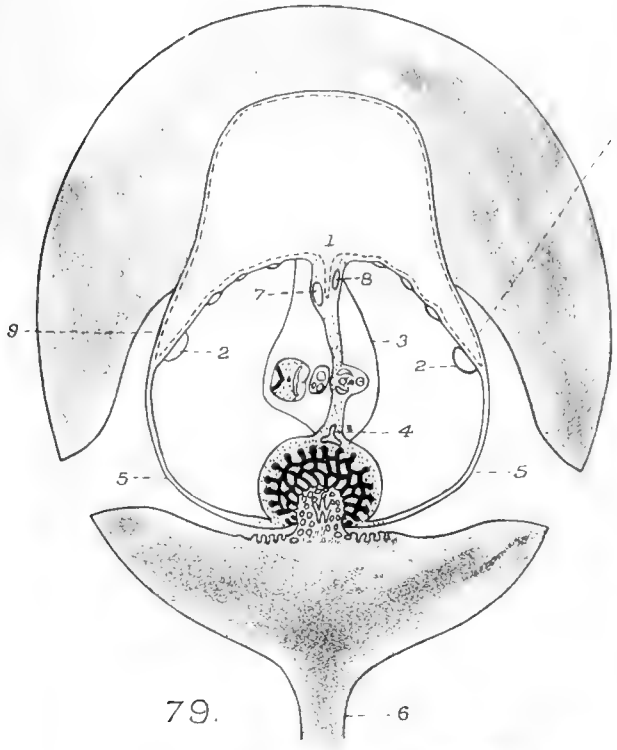

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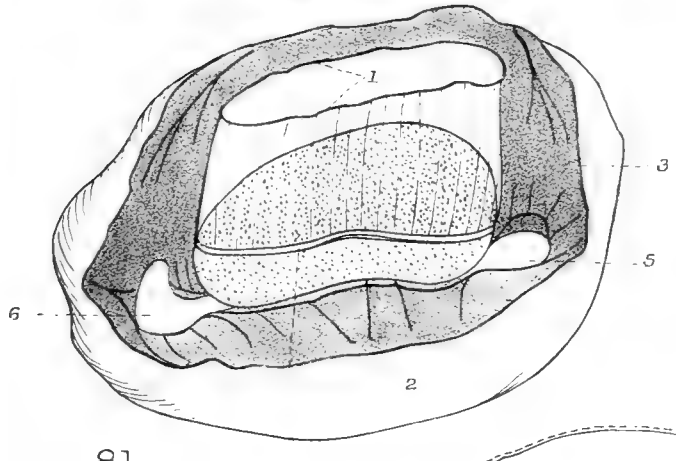

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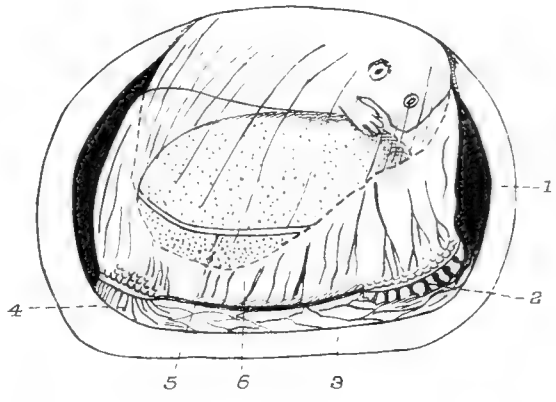

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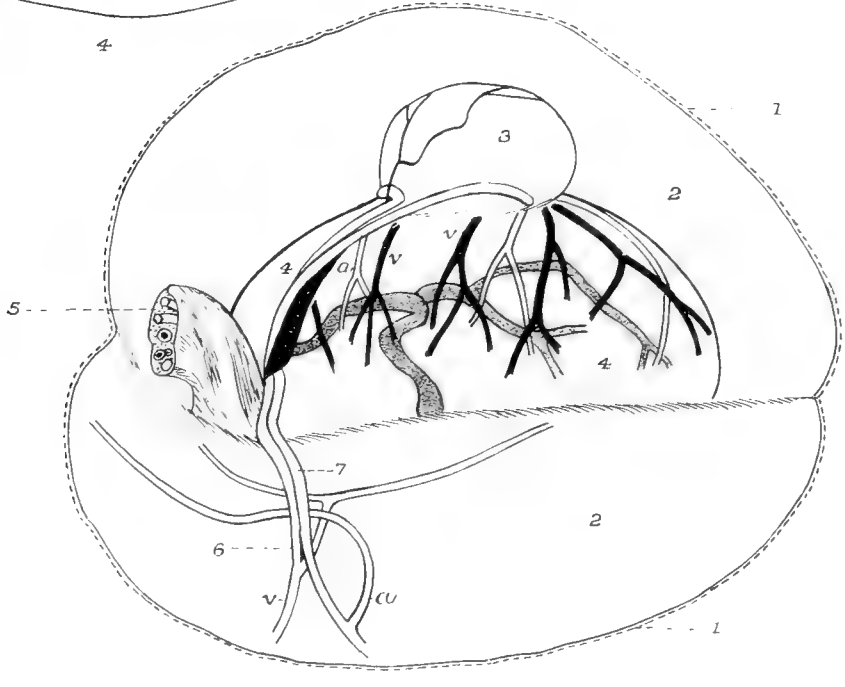

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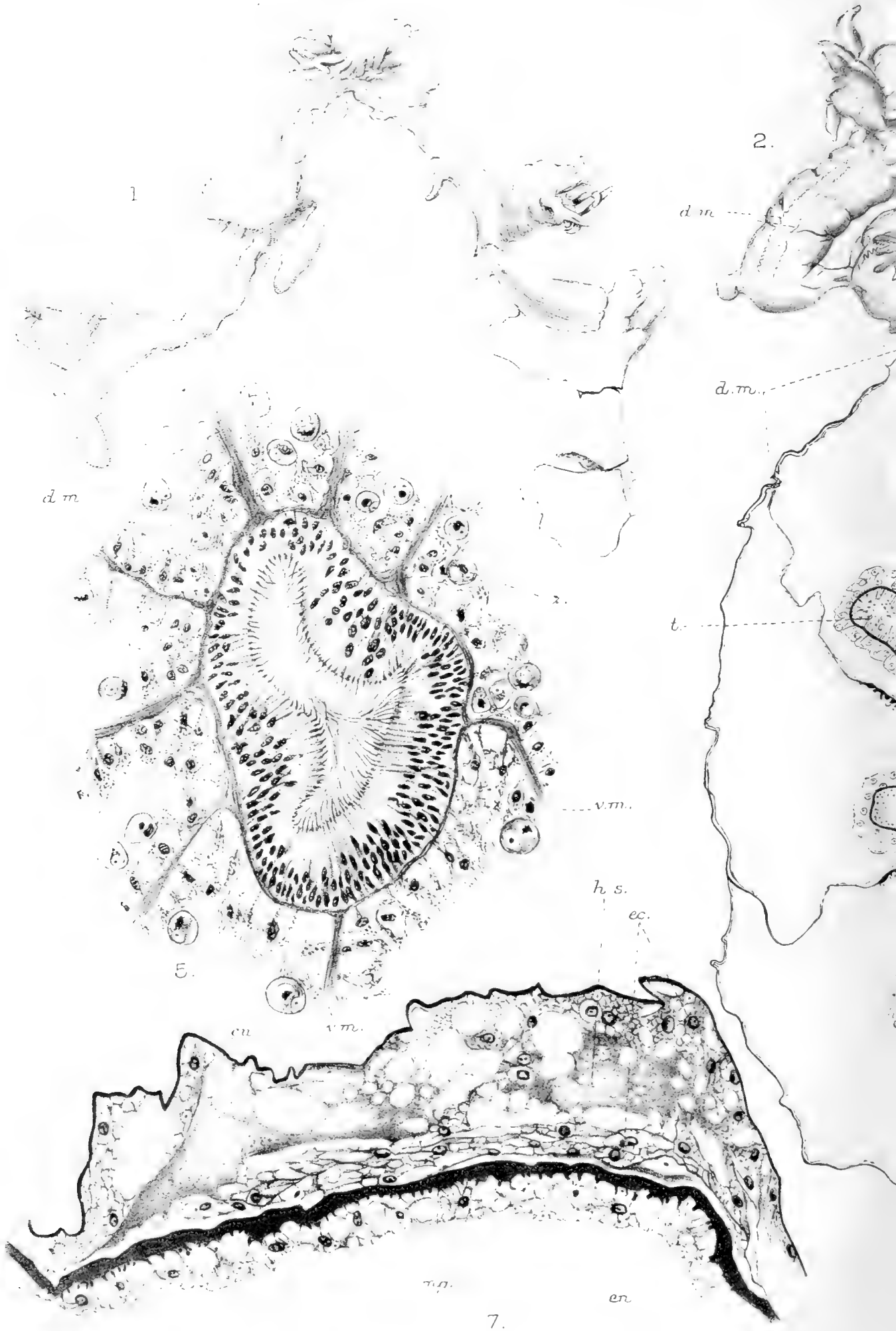

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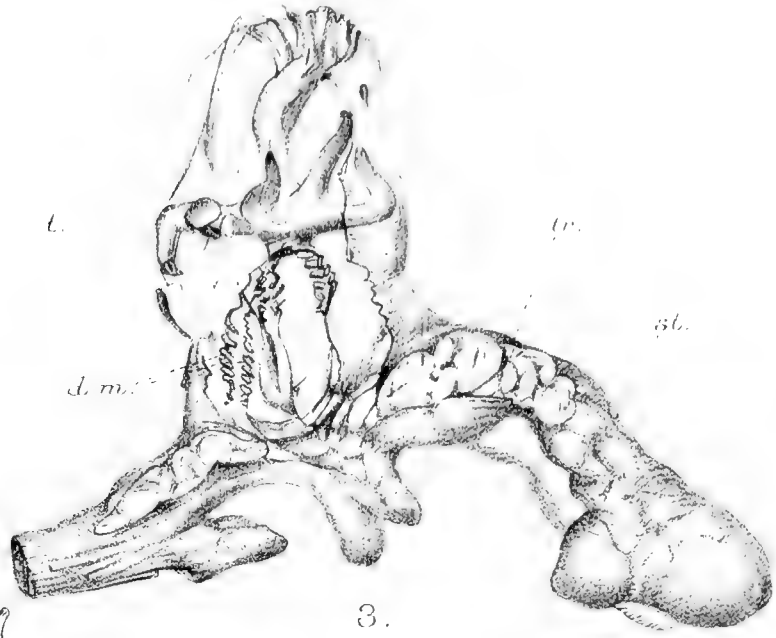

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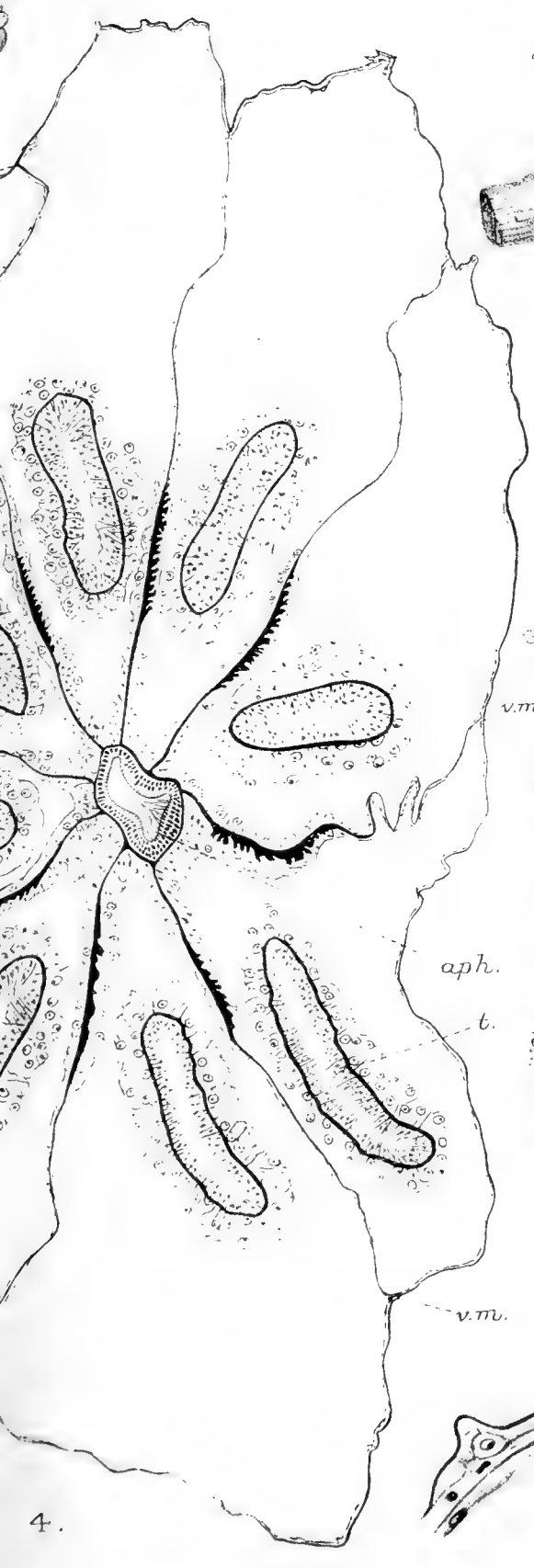

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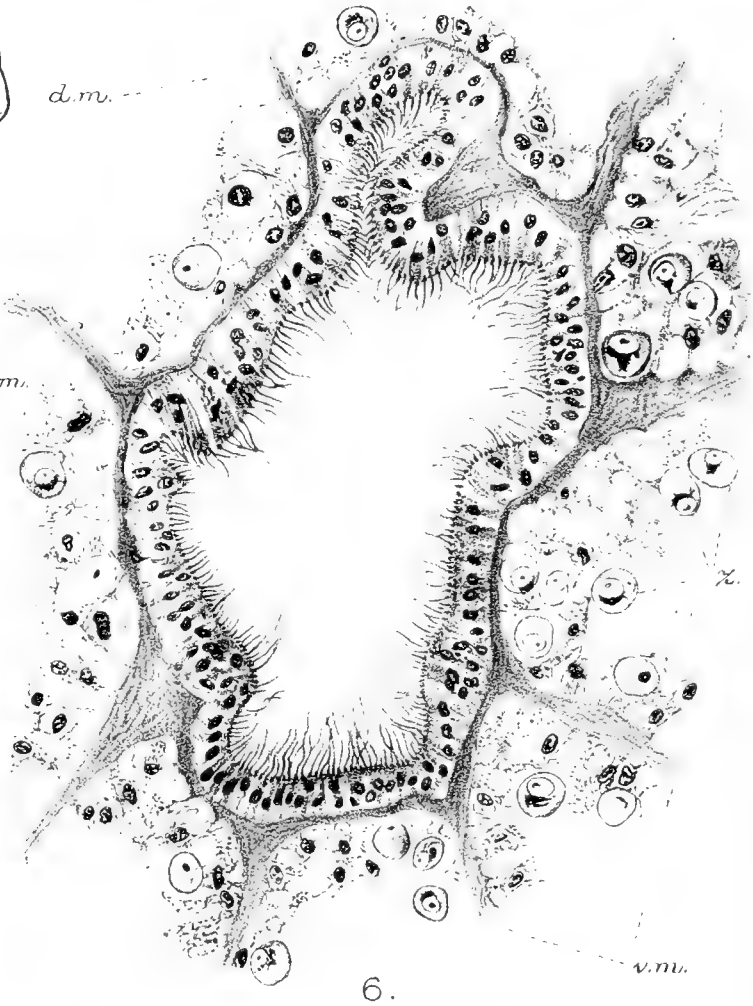

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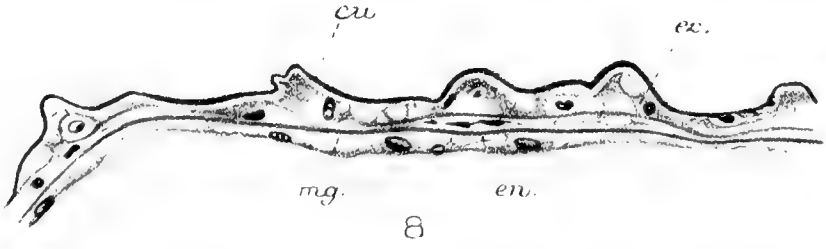

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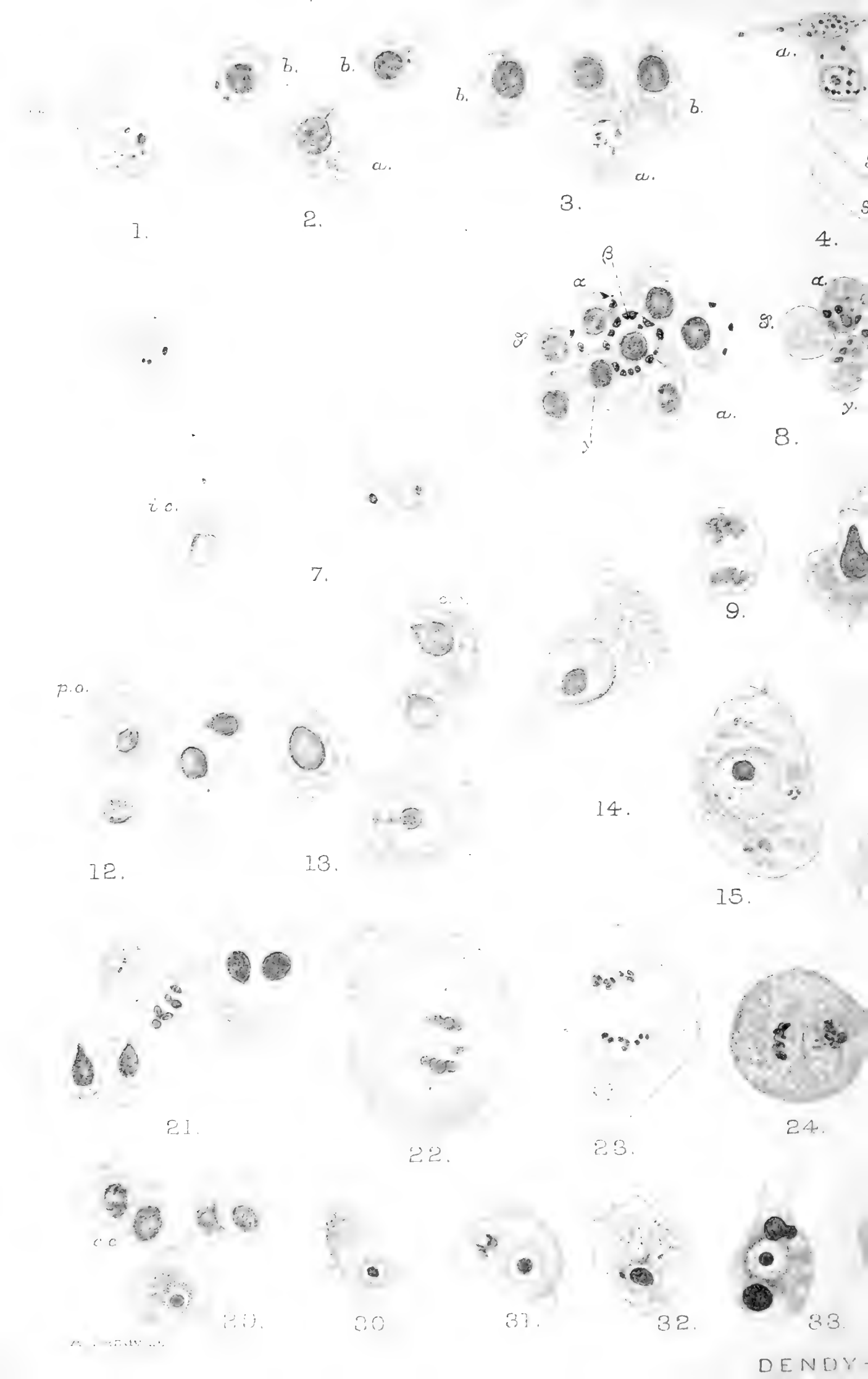
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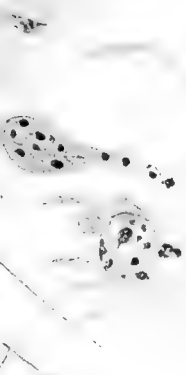

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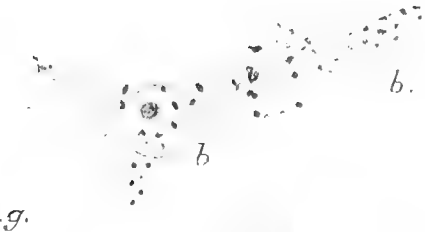

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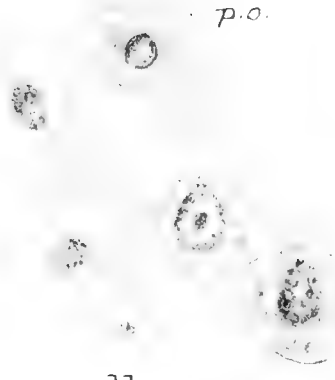

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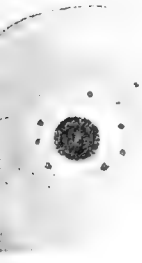

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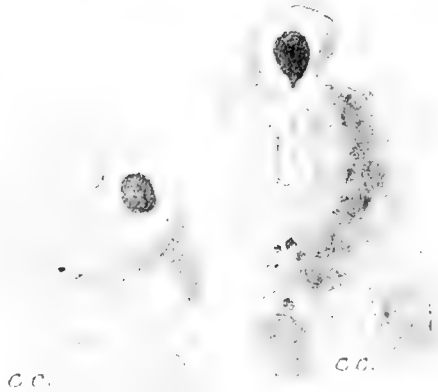

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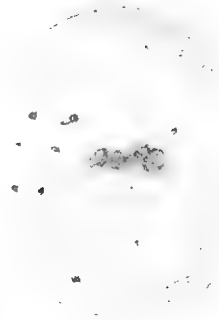

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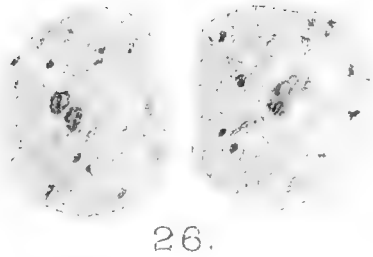

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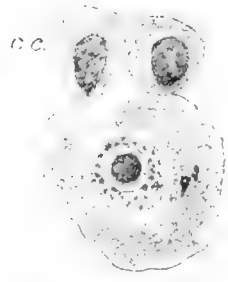

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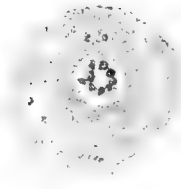

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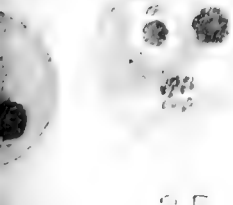

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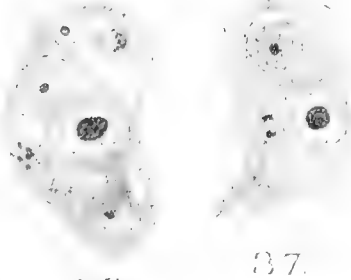

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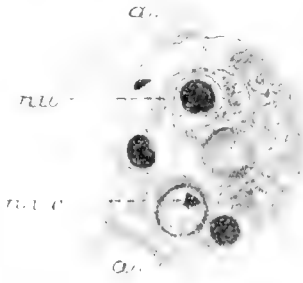

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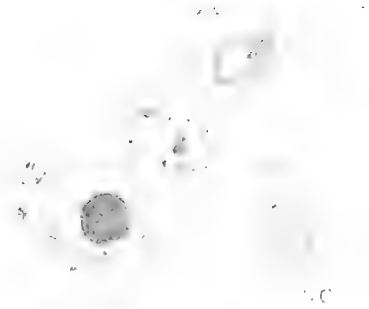

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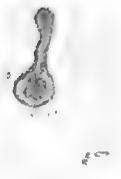

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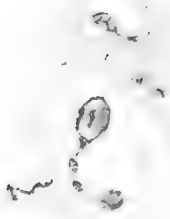

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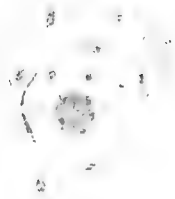

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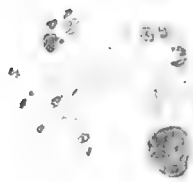

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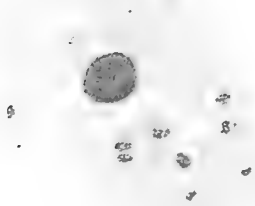

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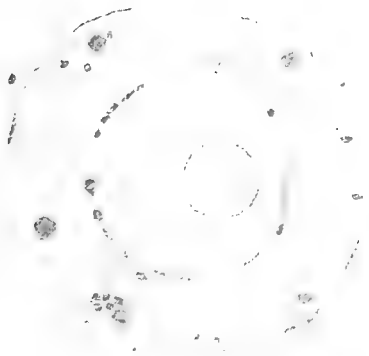

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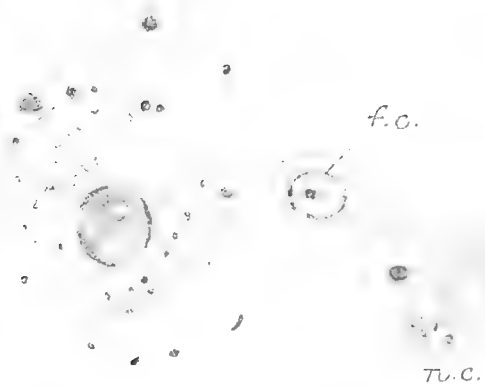

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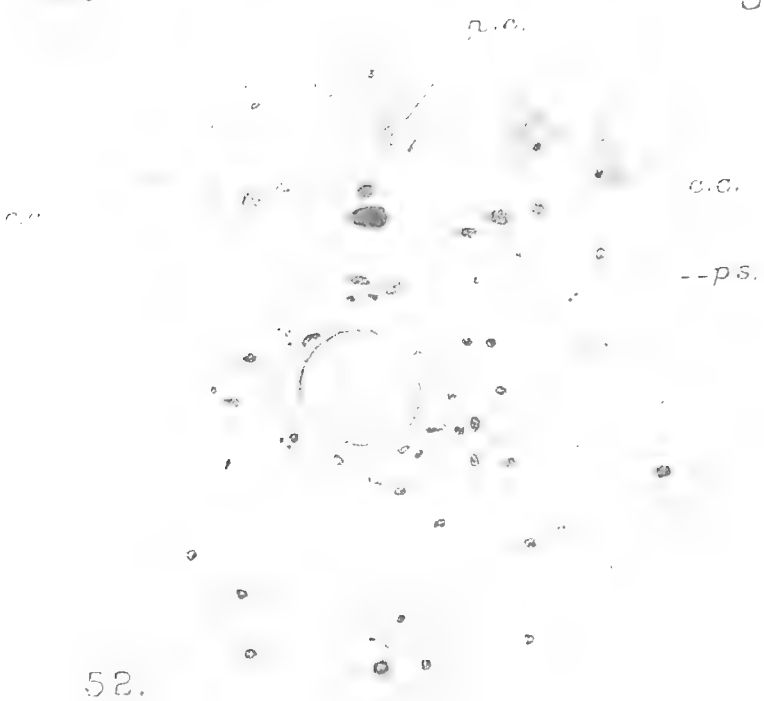

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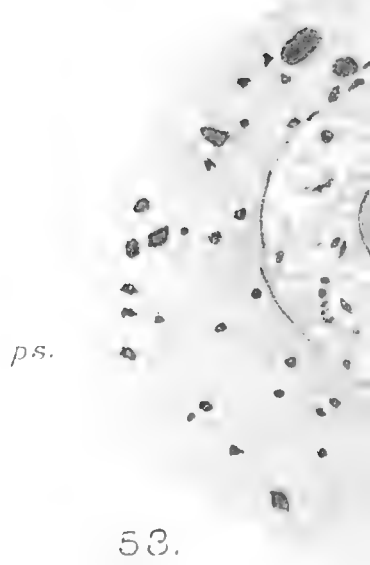

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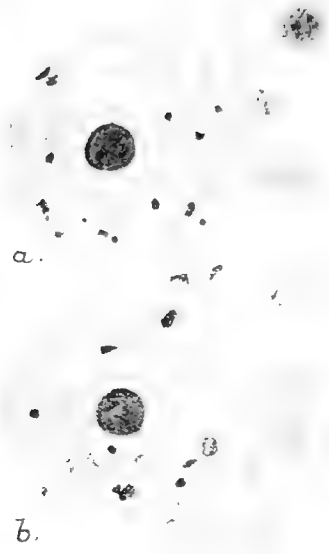

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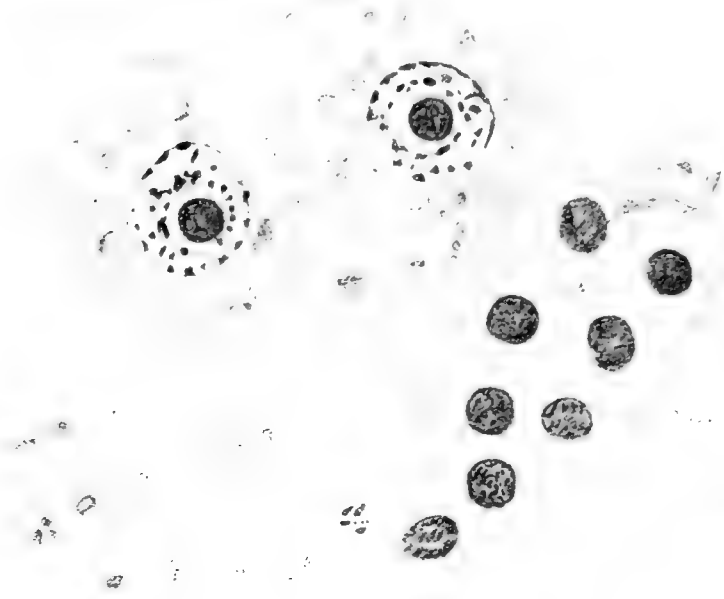

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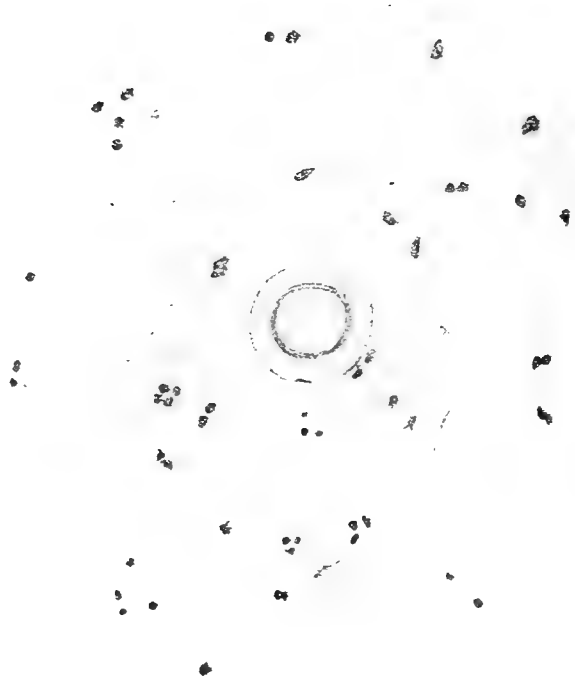

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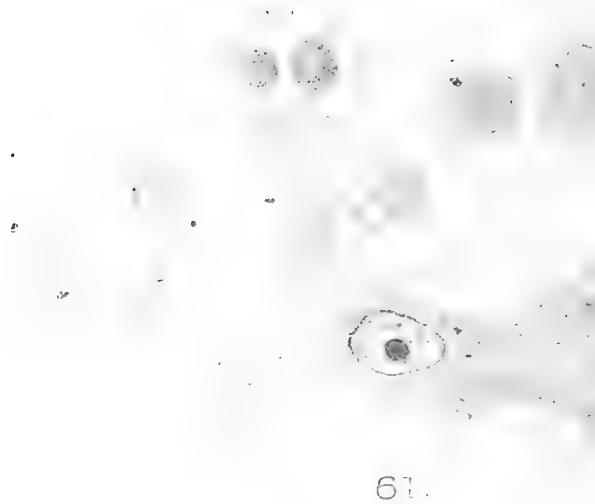

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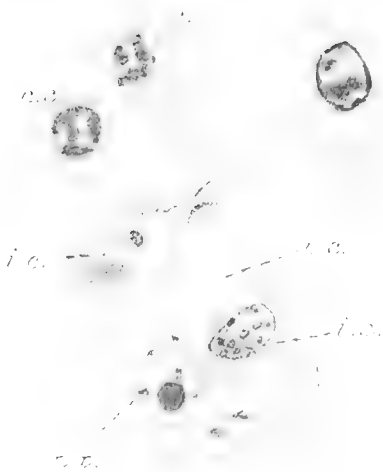

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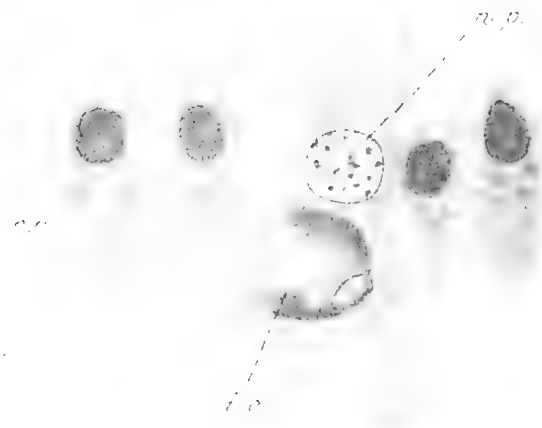

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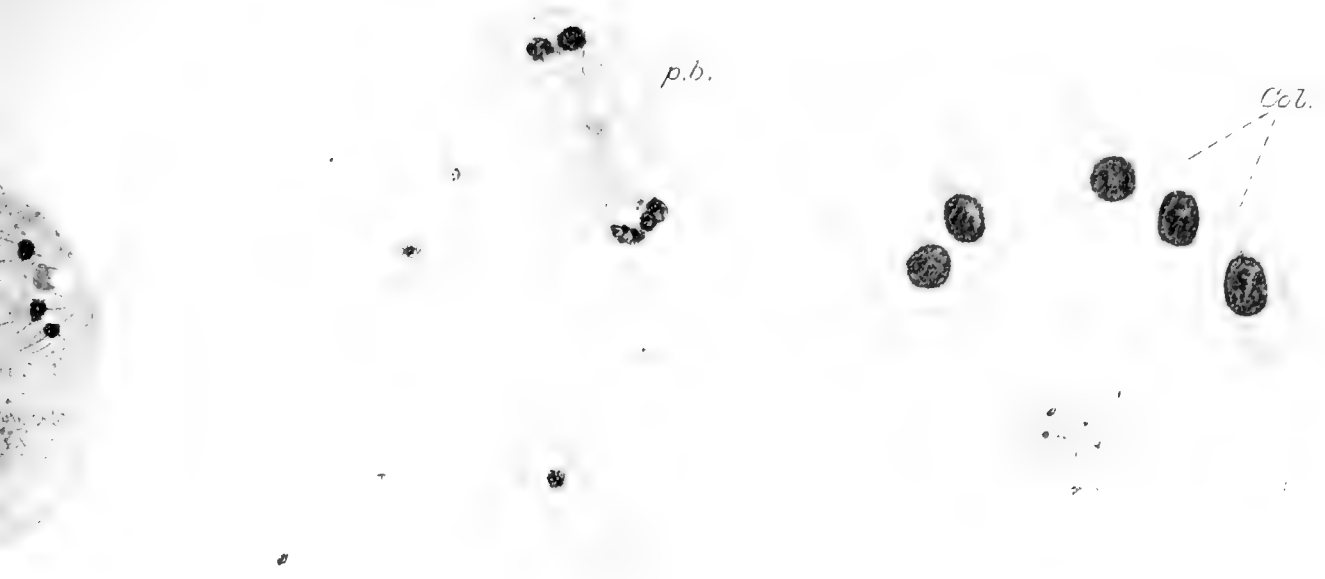

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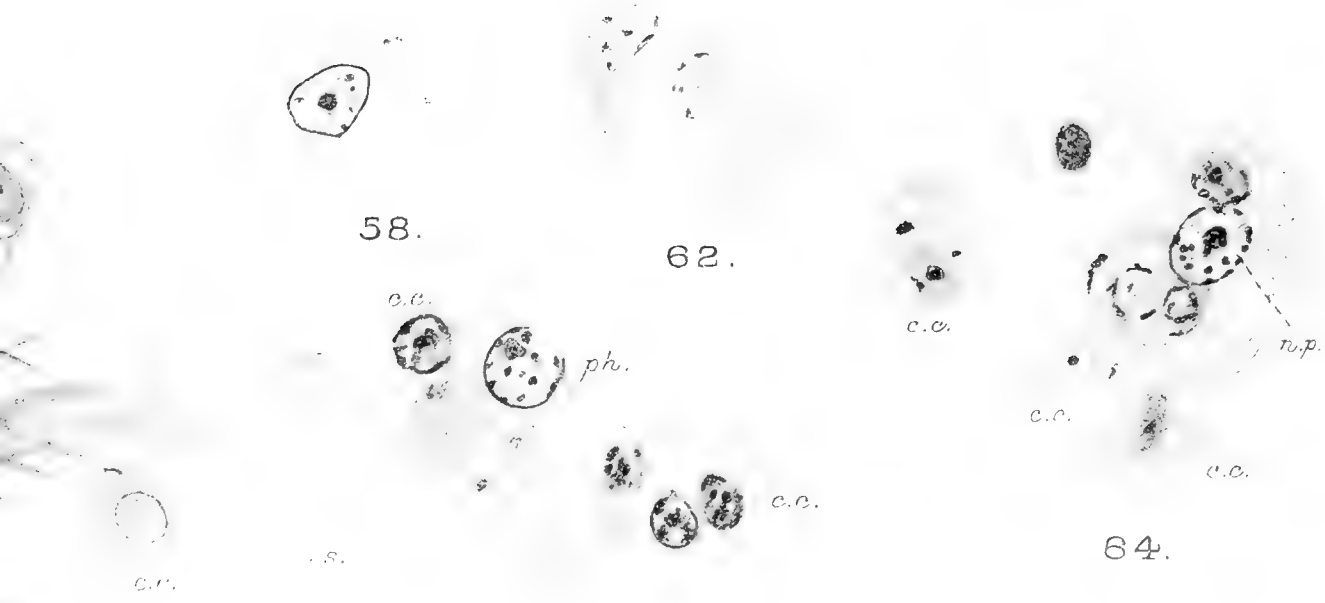

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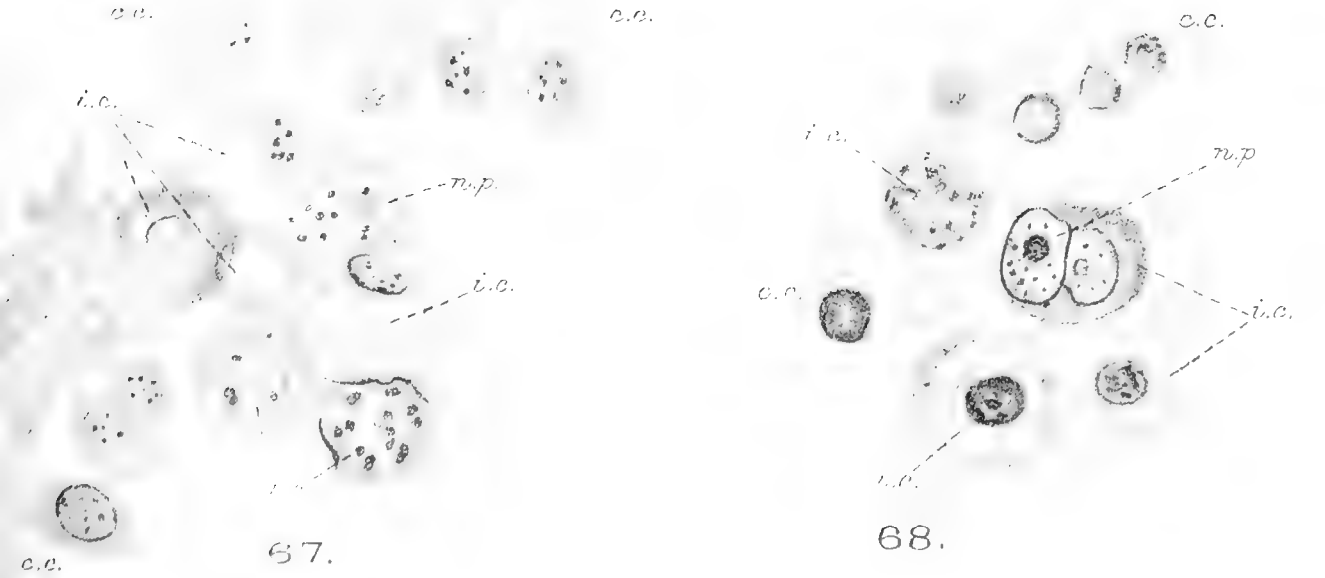

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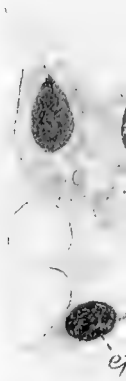

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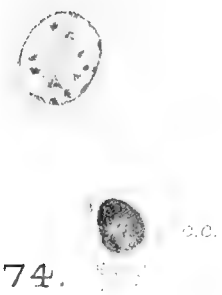

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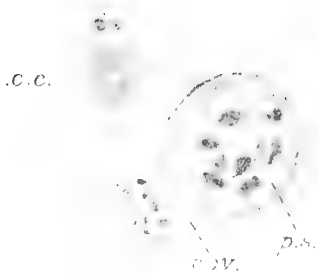

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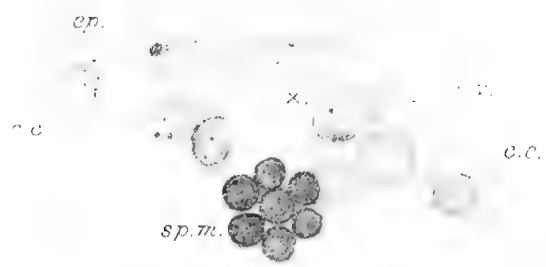

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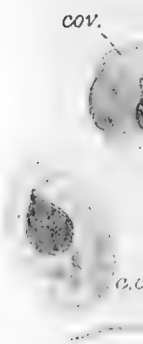

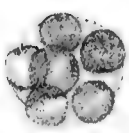

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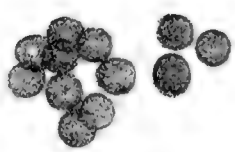

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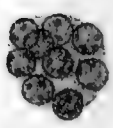

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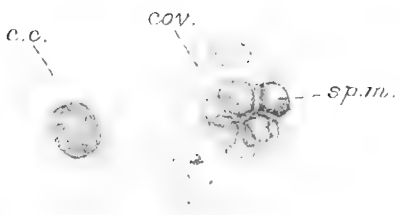

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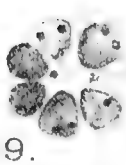

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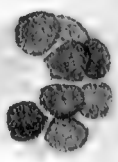

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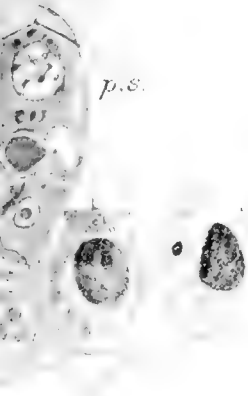

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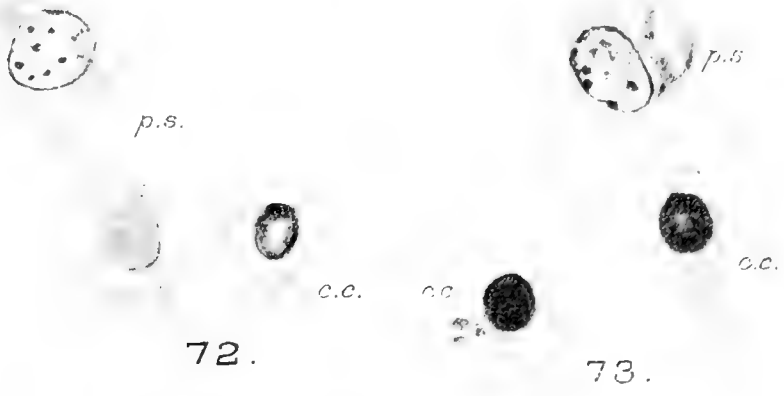

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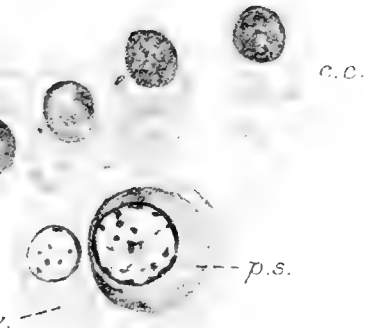

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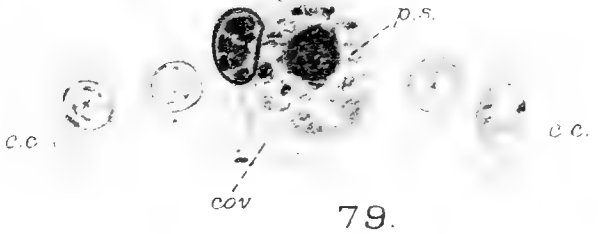

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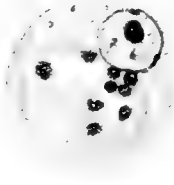

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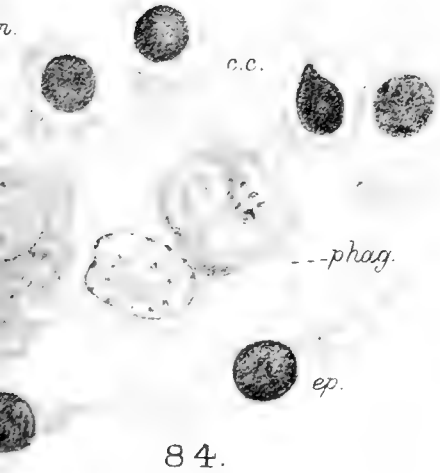

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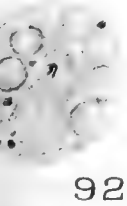

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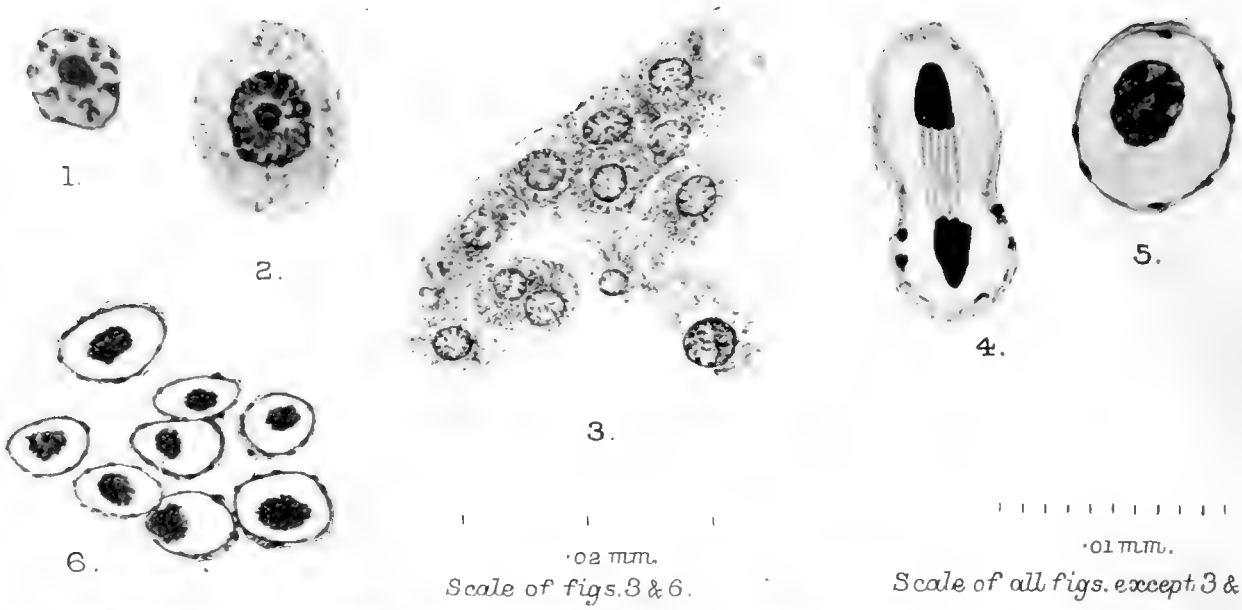
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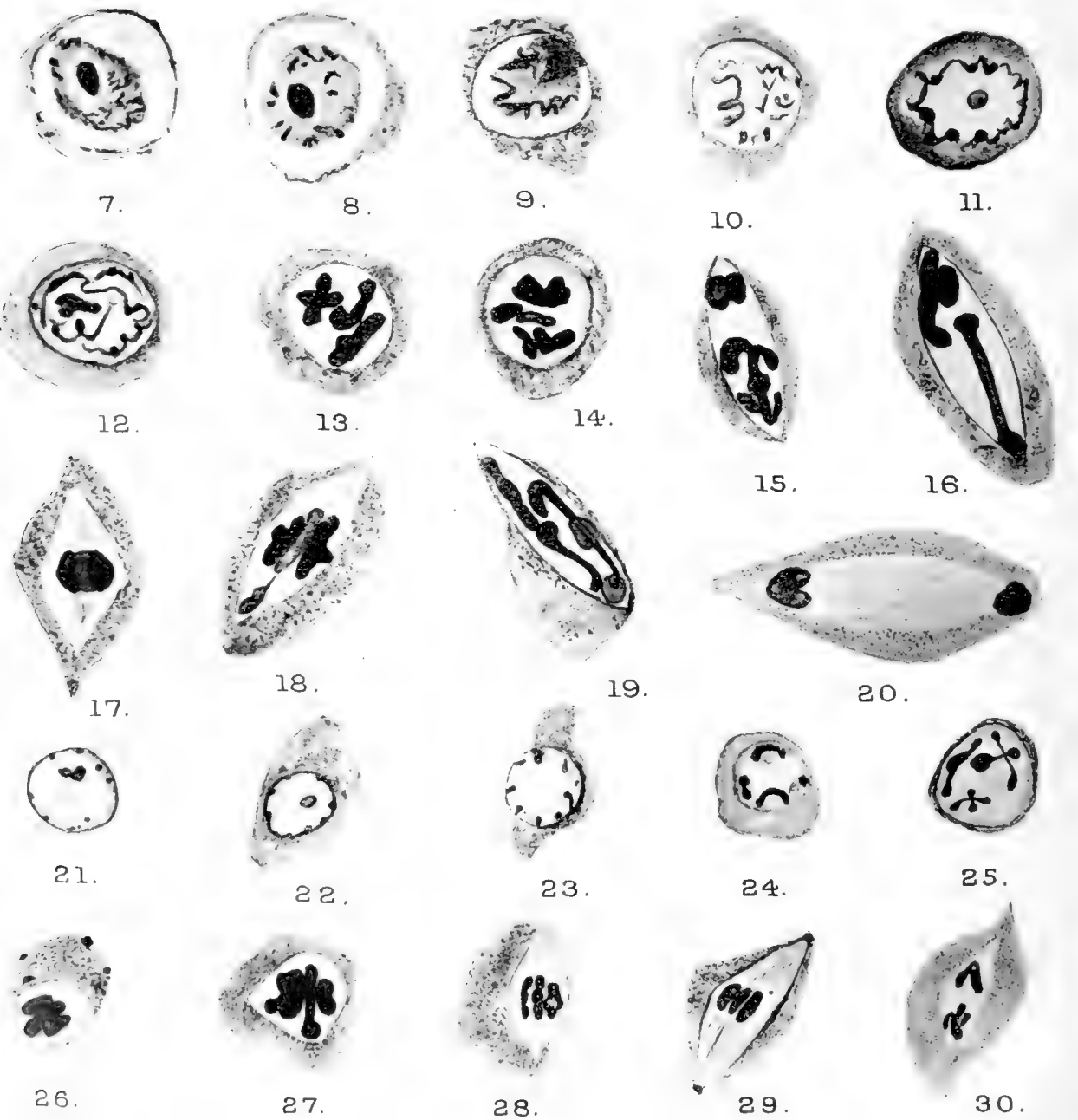
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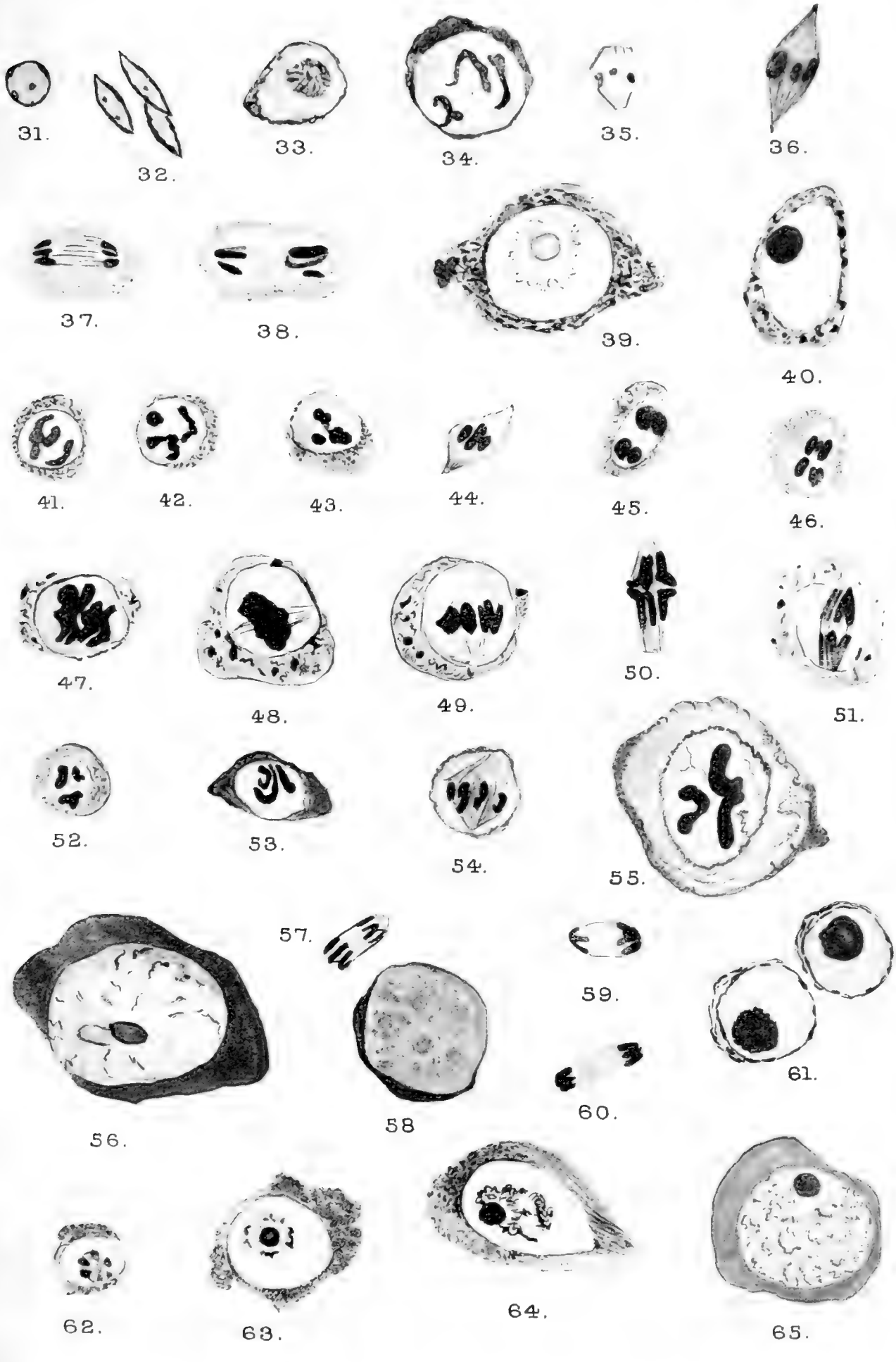
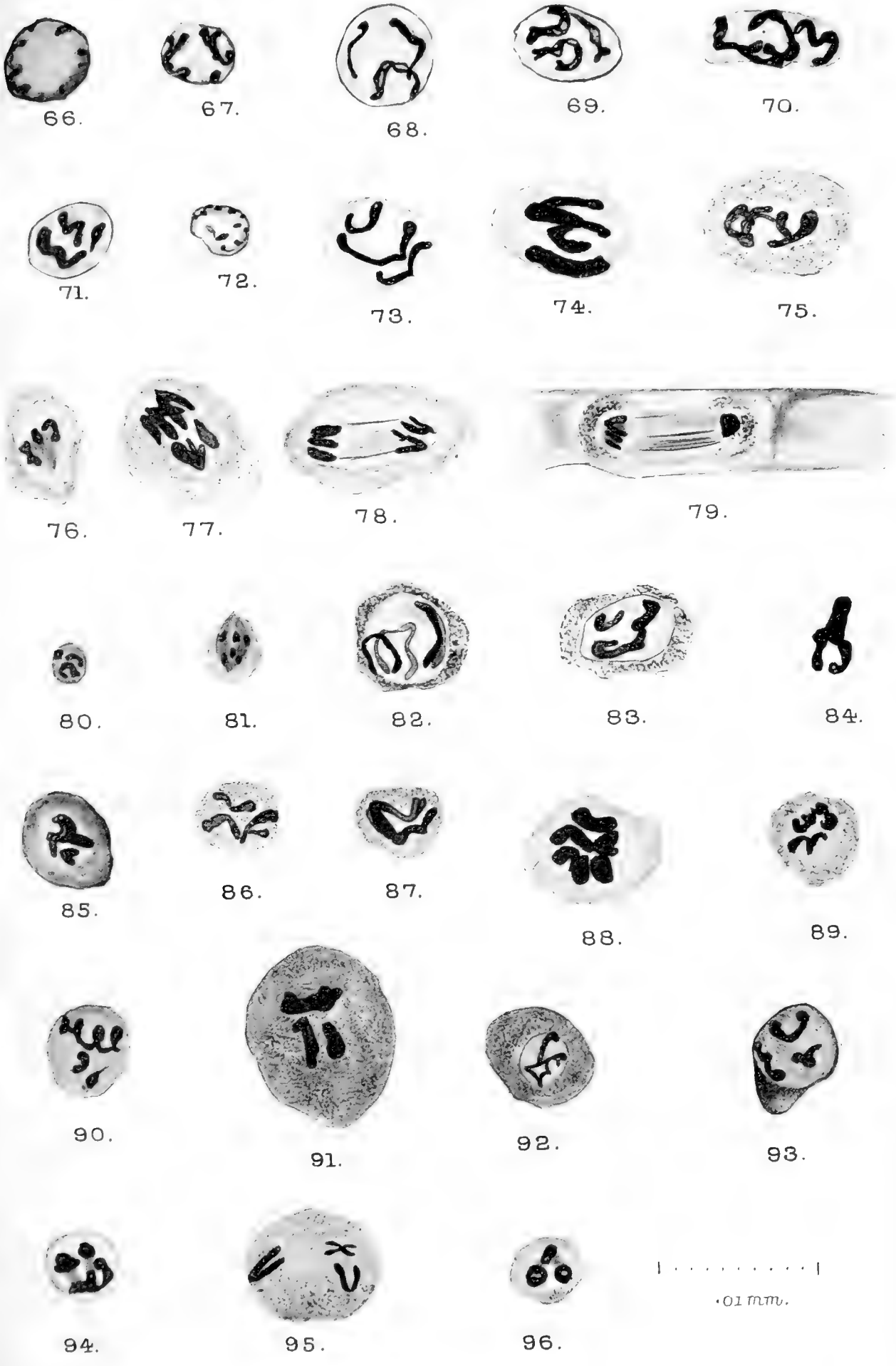


OMES OF CULEX.



M. TAYLOR—CHROMOSOMES OF CULEX.

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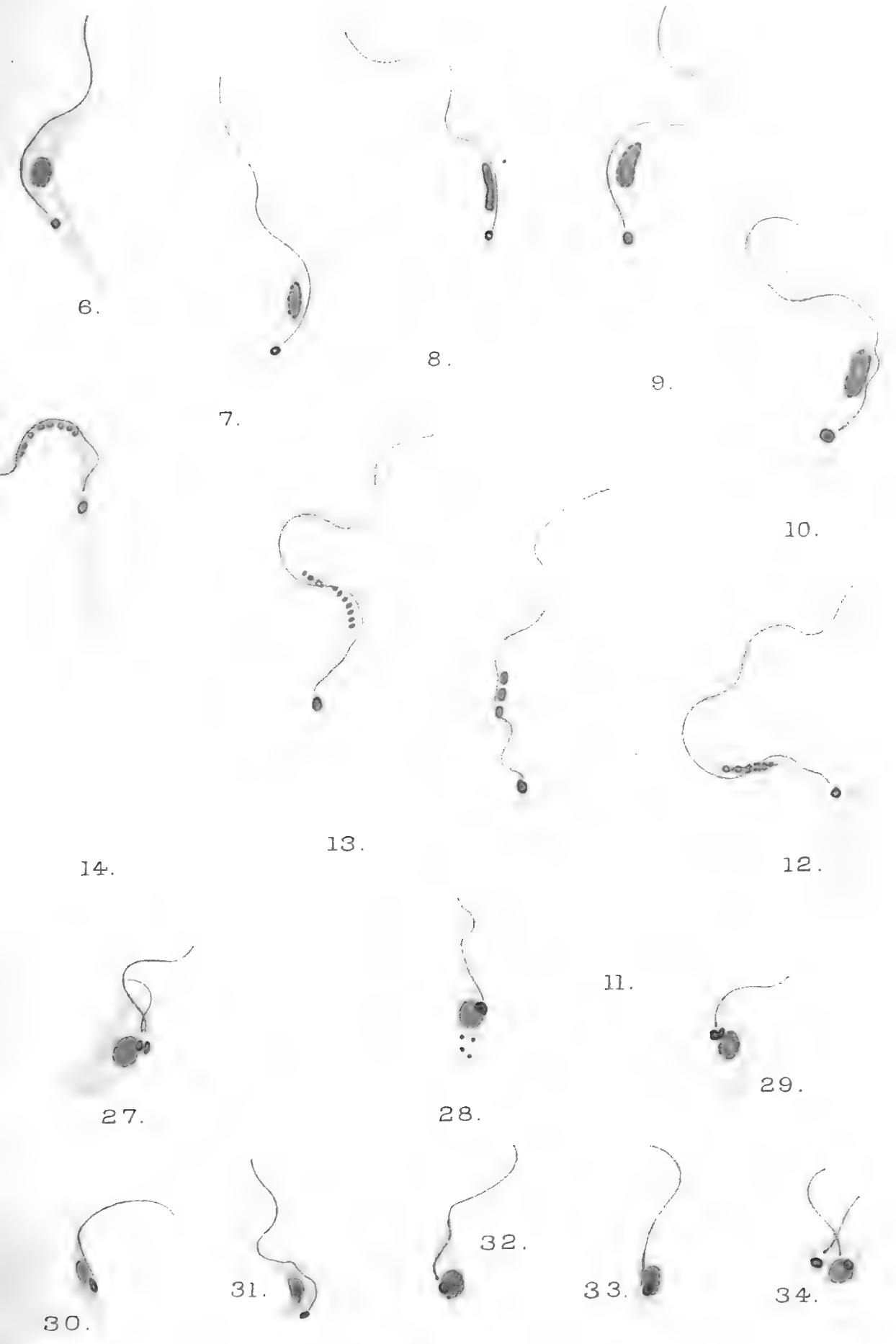

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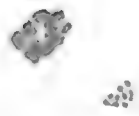

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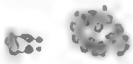

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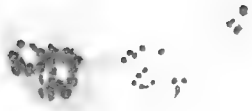

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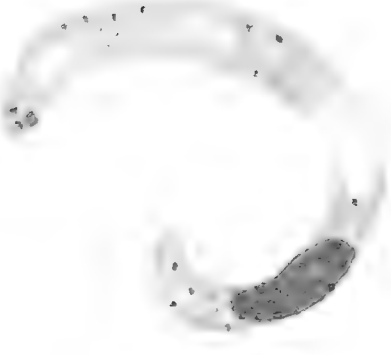

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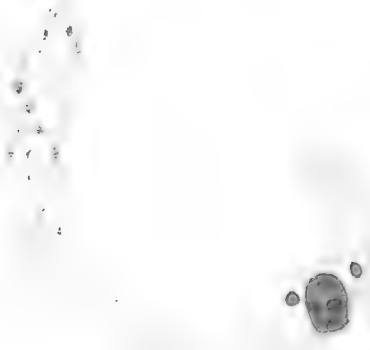

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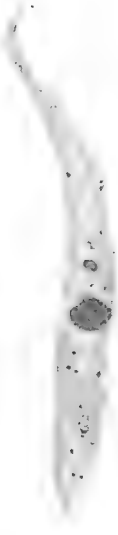
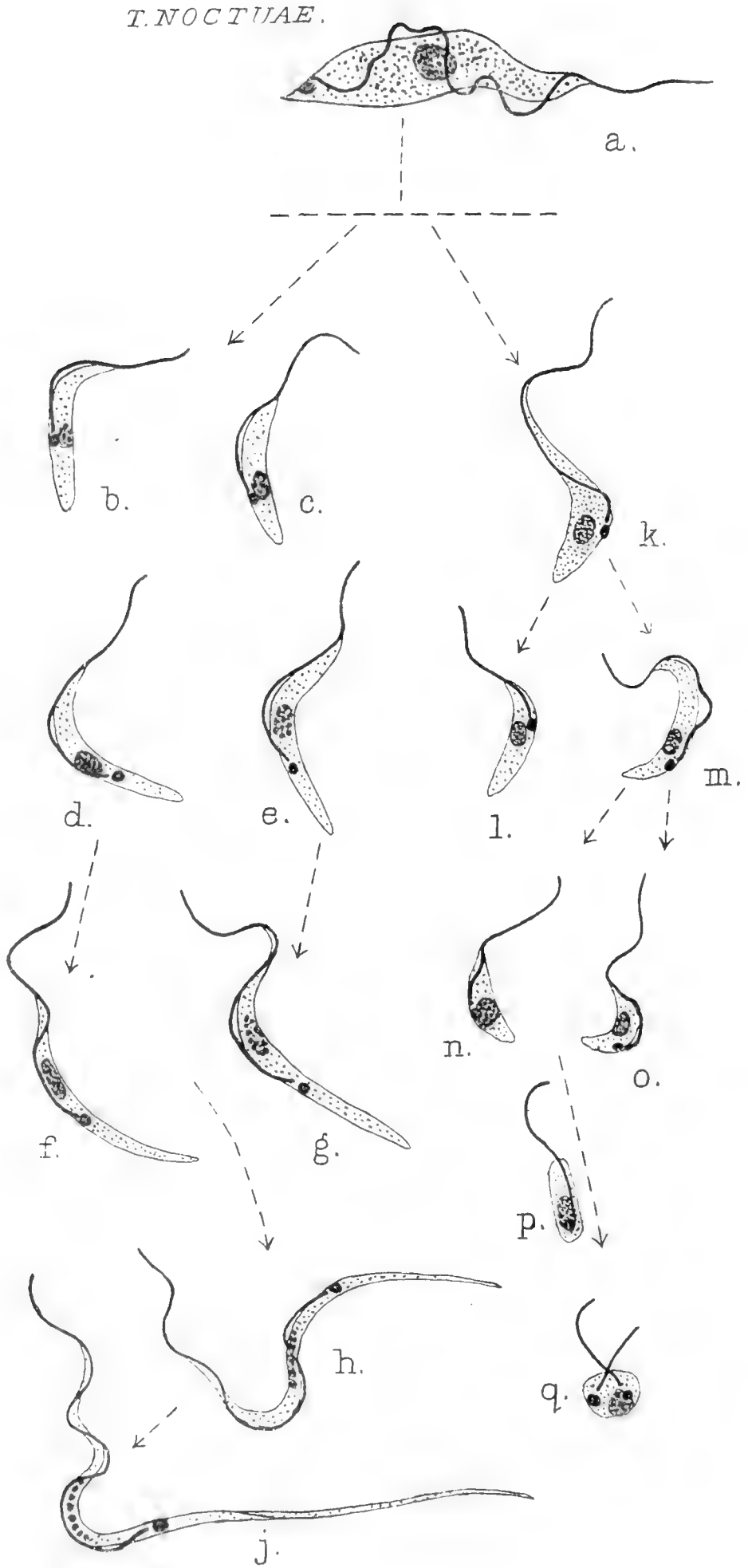


47.

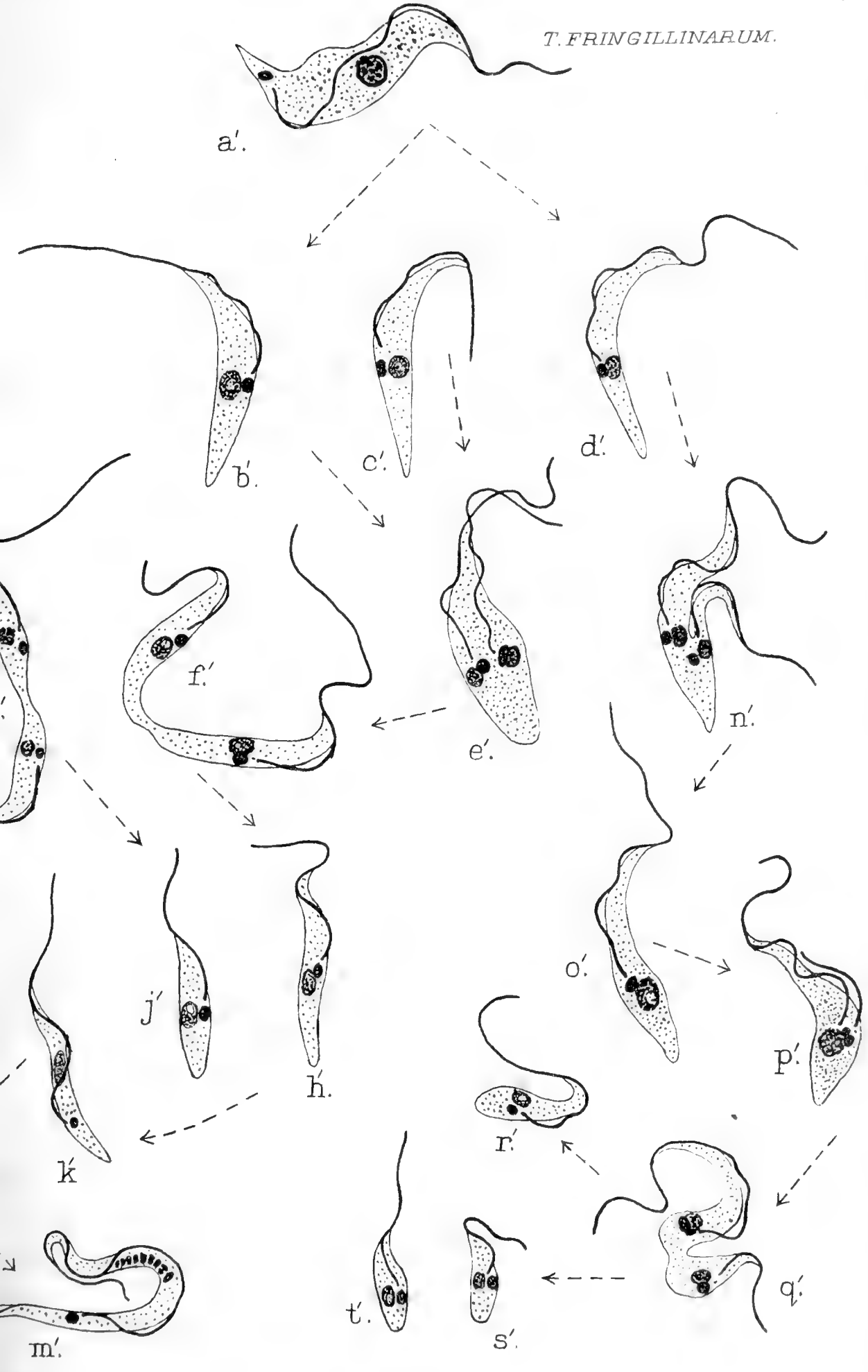
Huth, London.

WOODCOCK — TRYPANOSOMA.



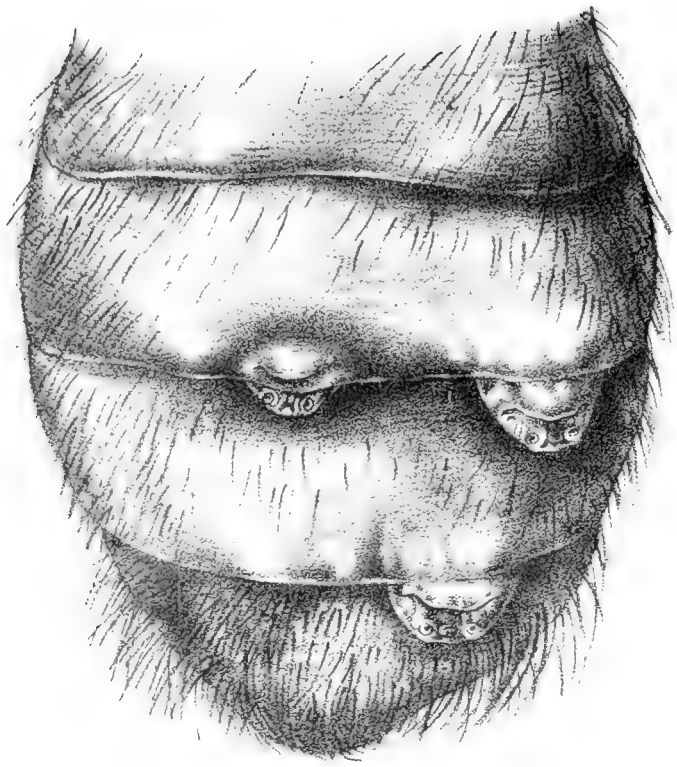
DEVELOPMENT IN THE MOSQUITO.

WOODCOCK



DEVELOPMENTAL STAGES IN CULTURES.

RYPANOSOMA.



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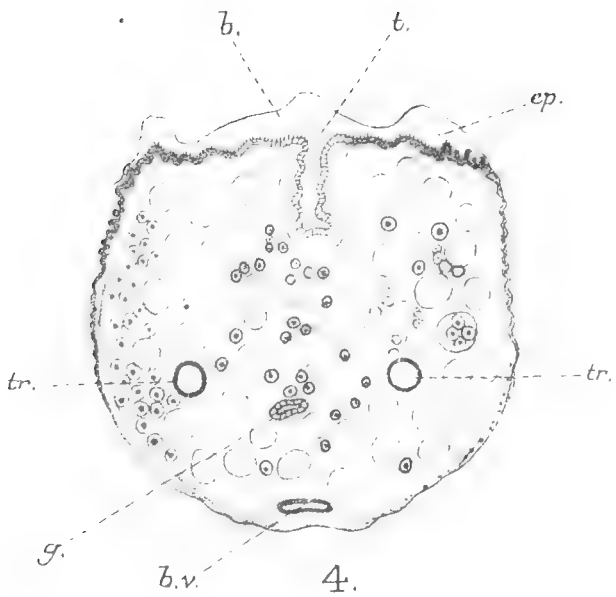

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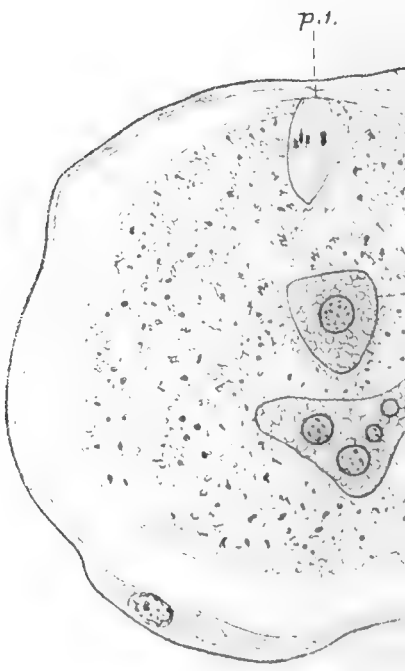

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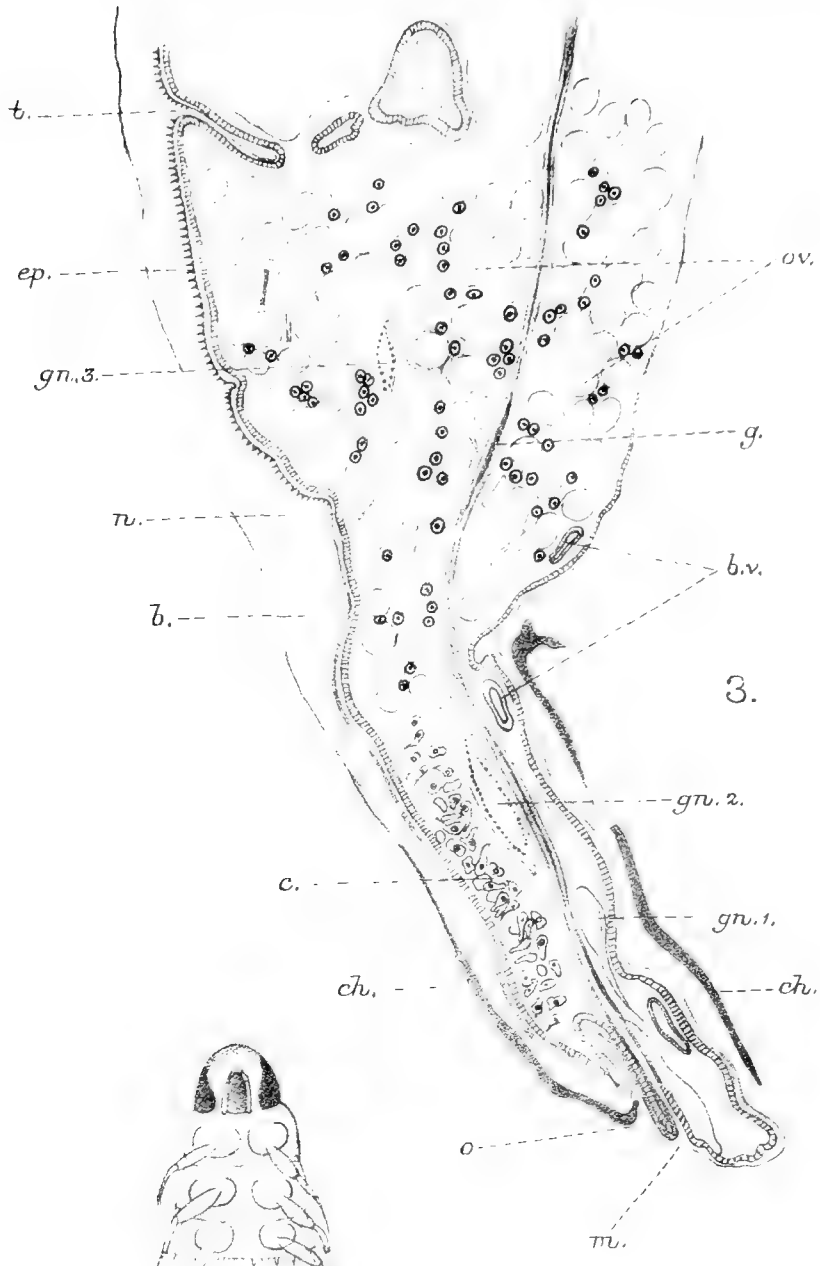

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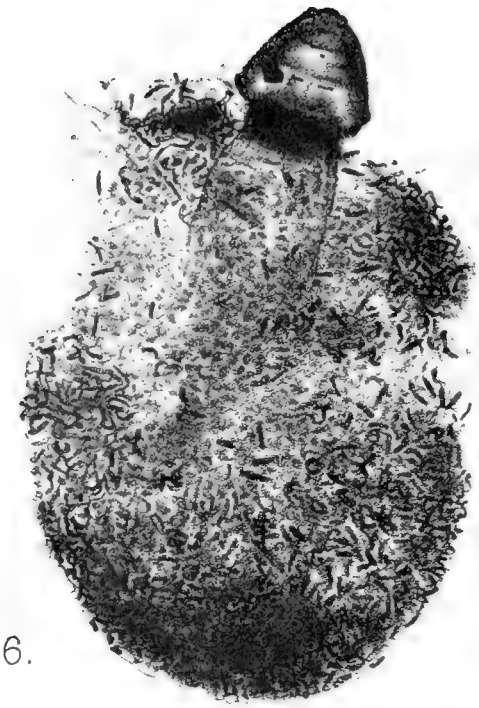

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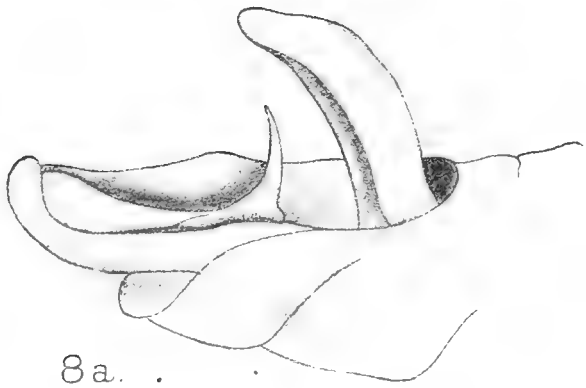

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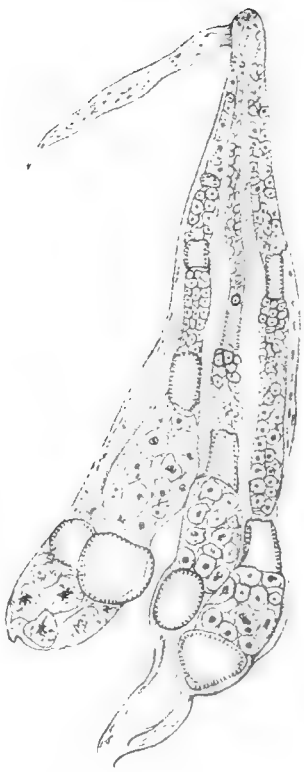

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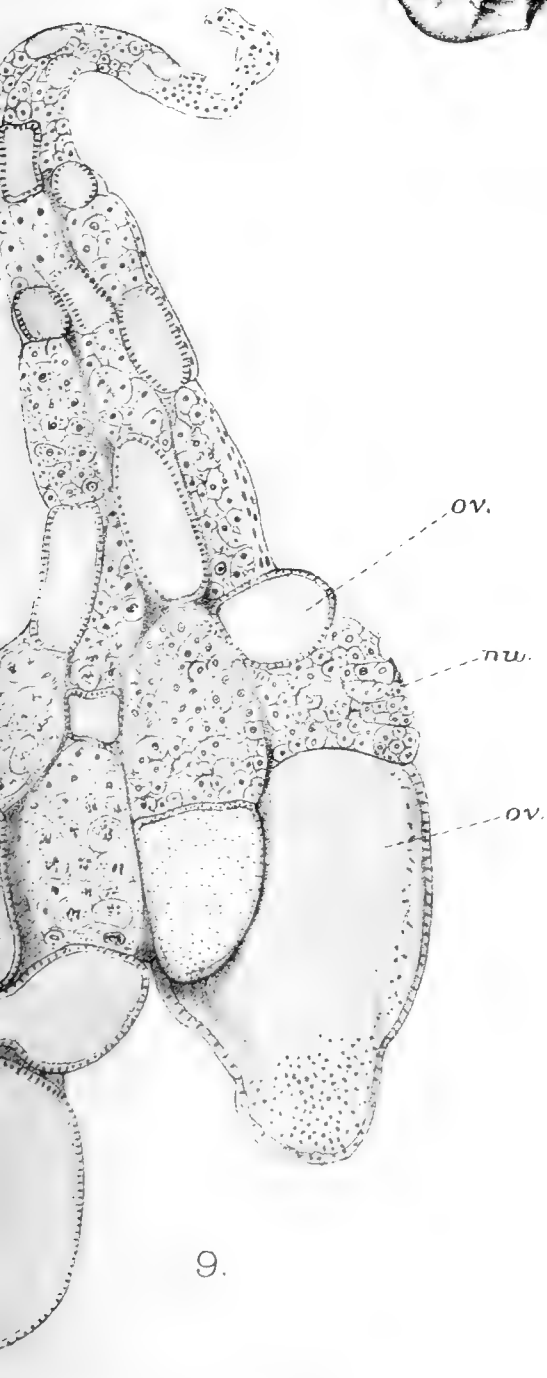

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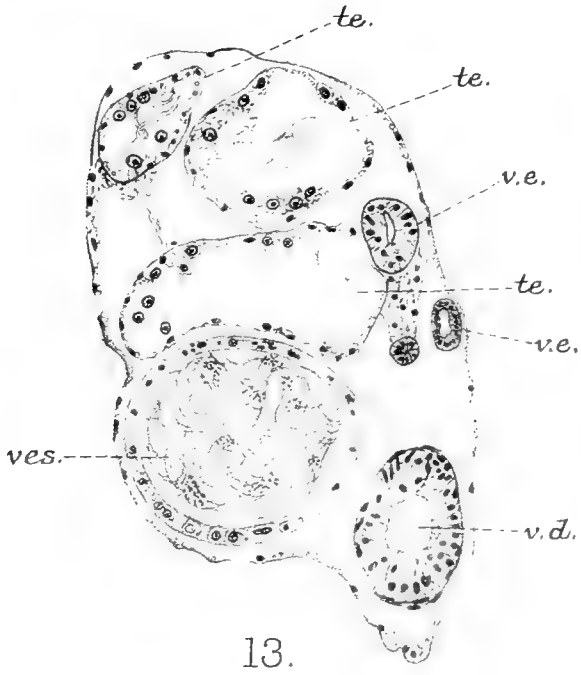

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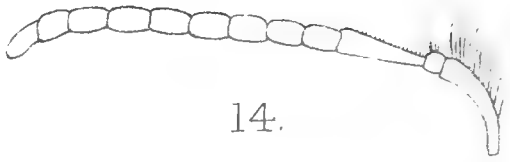

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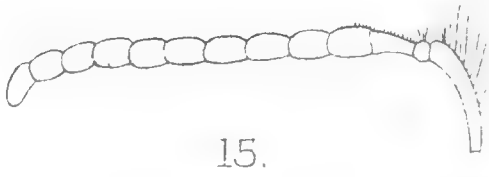
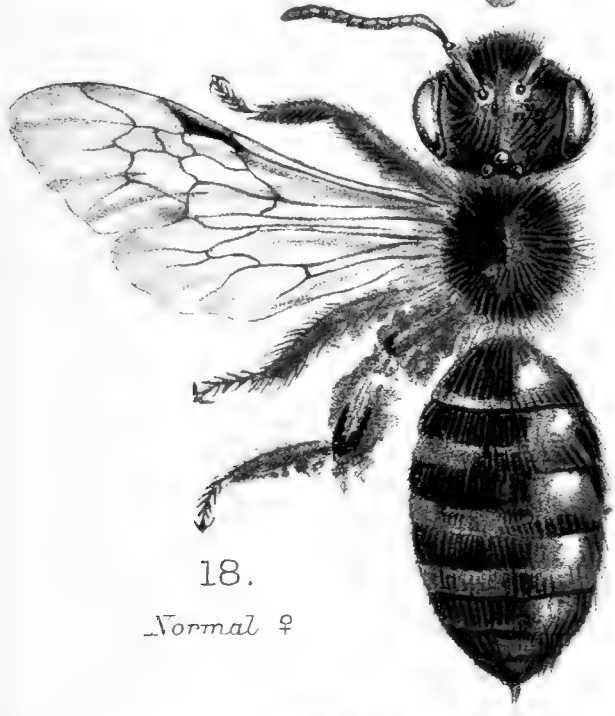


15.

16.
Normal ♂

17.
Stylopised ♂



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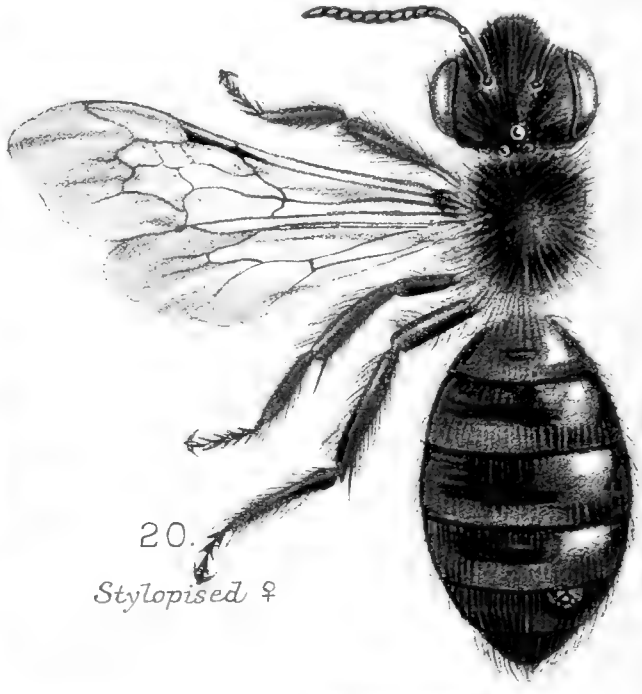


20.
Stylopised ♀

19.
Normal ♀

21.
Stylopised ♀

W.G. Browning, del

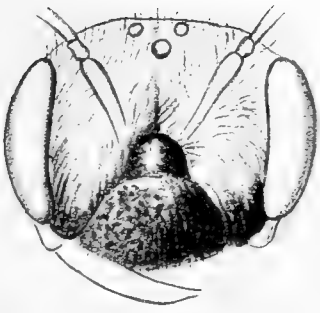
Huth Lith^r London

GEOFFREY SMITH — STYLOPS

22.
Normal ♀

23.
Normal ♂

24.
Stylopised ♀



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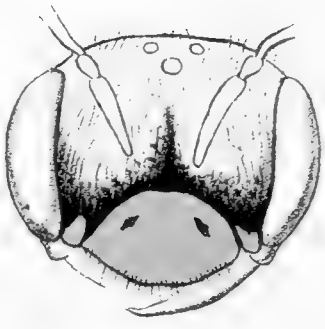

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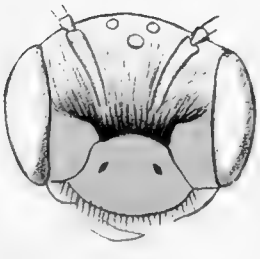

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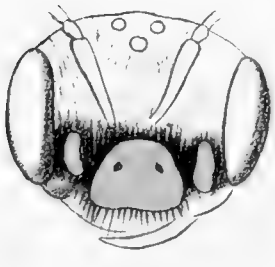
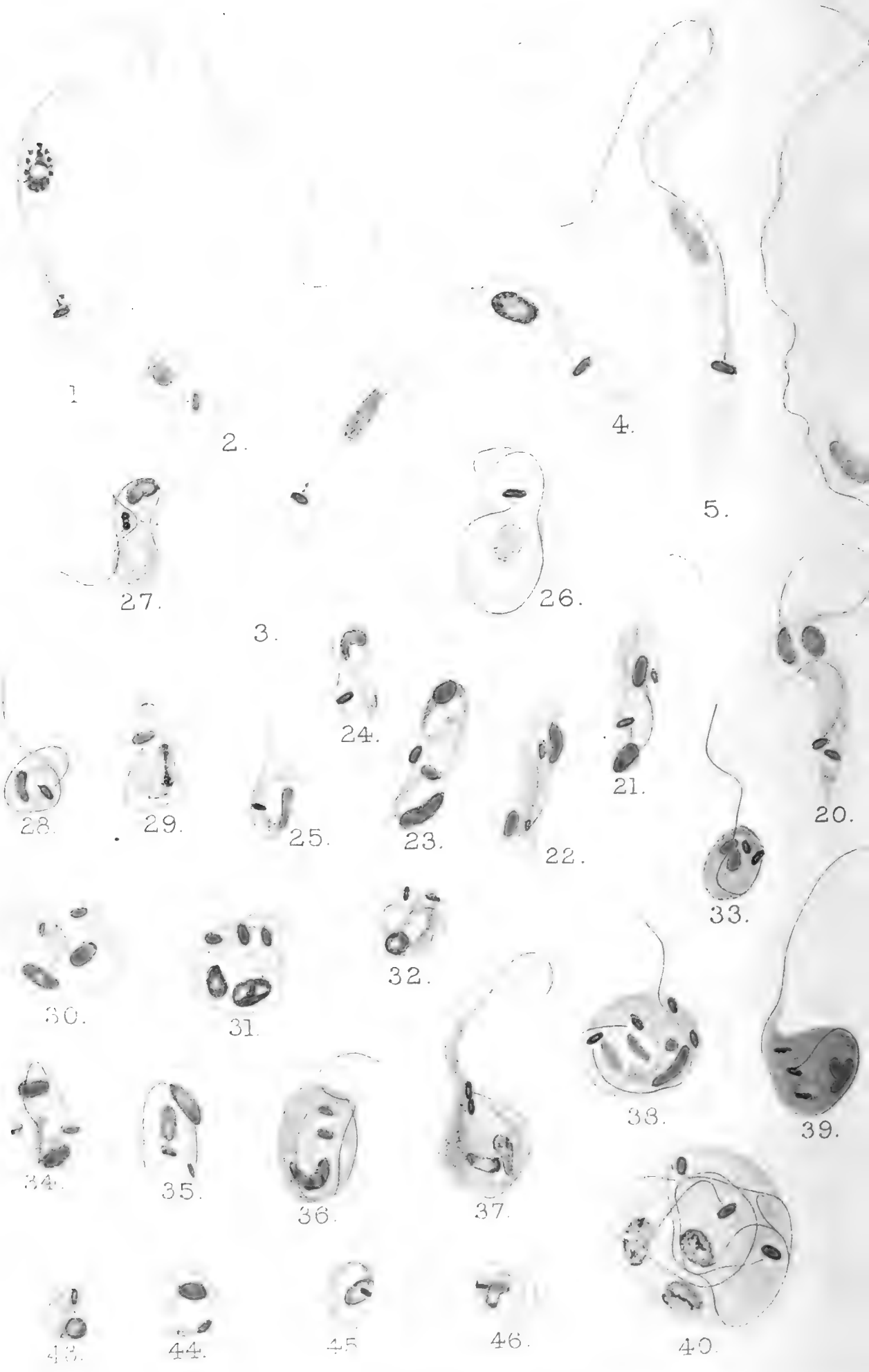


28.
Stylopised ♂

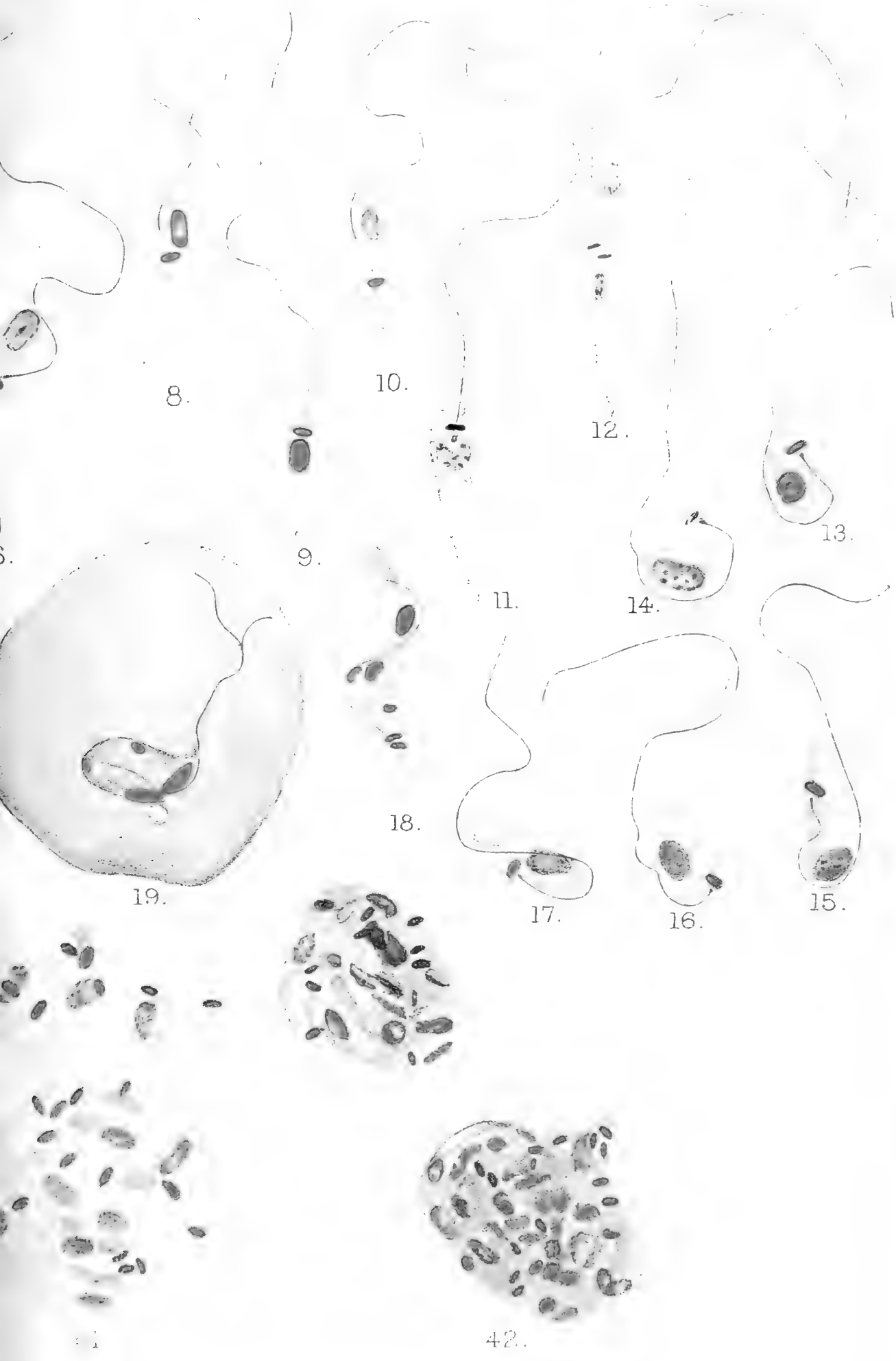
Hanth Lith^r London

GEOFFREY SMITH — STYLOPS.



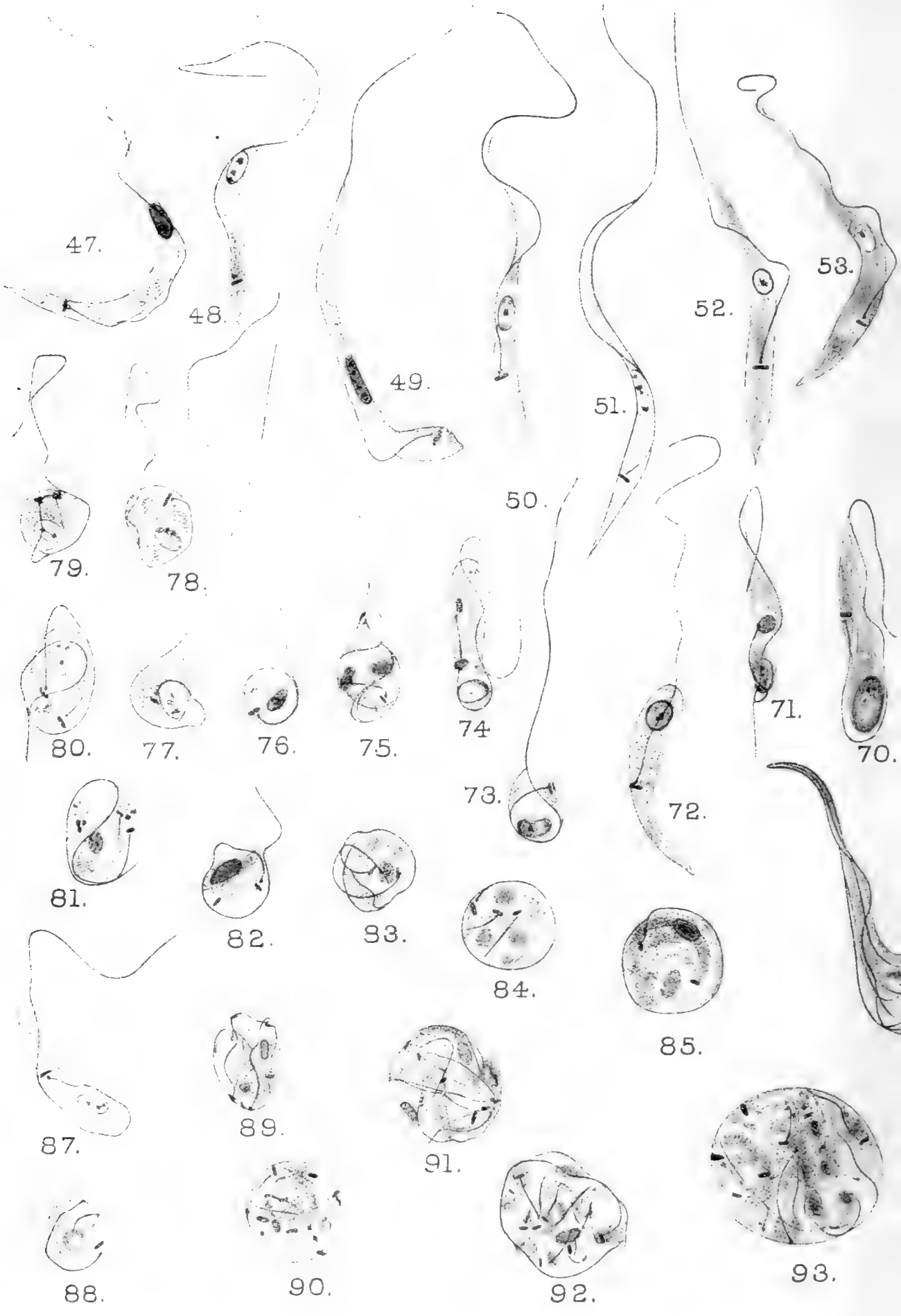
M Rhodes ad nat del.

MINCHIN AND THOMSON—TRYPANOS



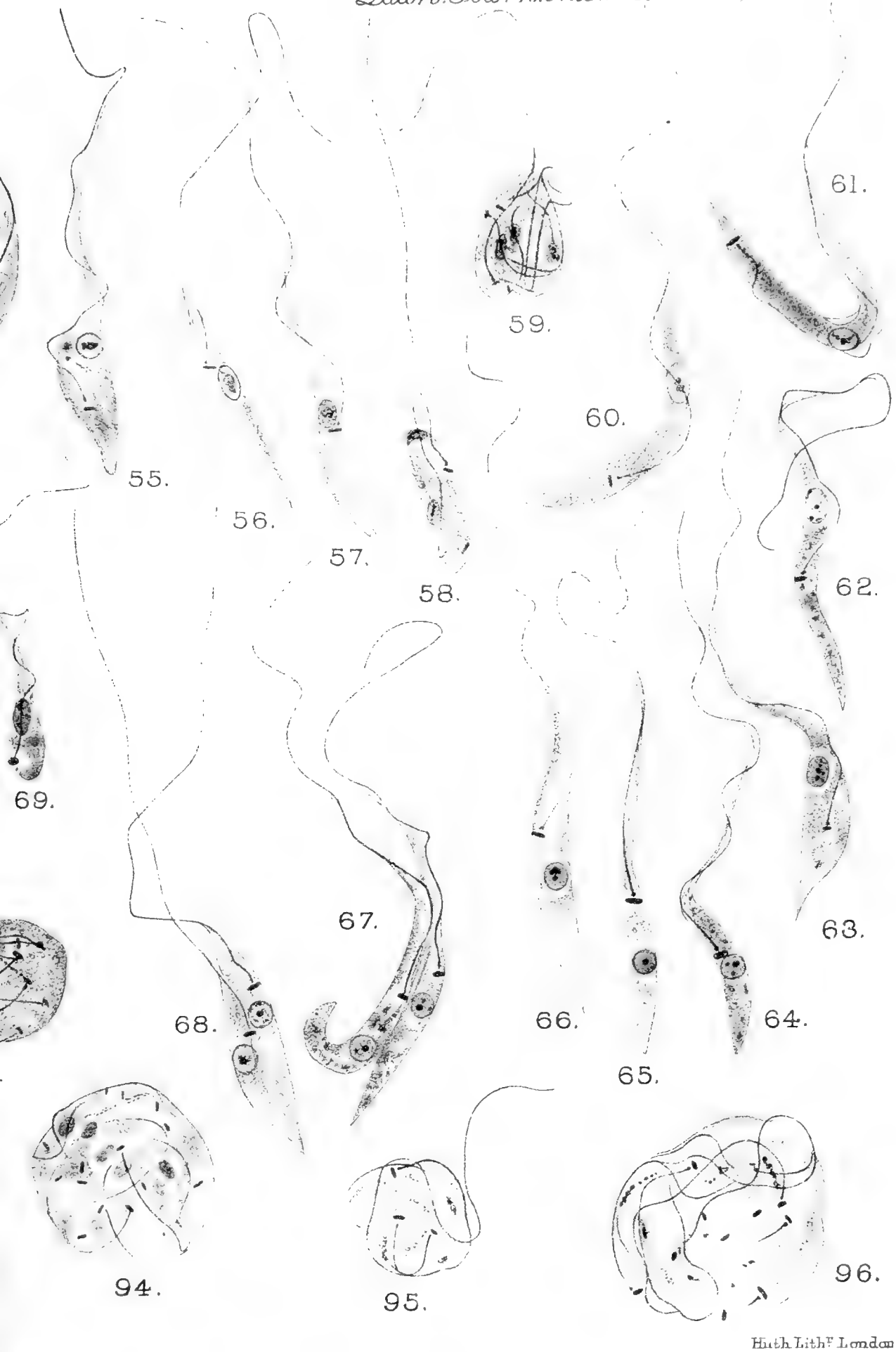
Huth, London.

LEWISI IN THE FLEA—STOMACH—PHASE.



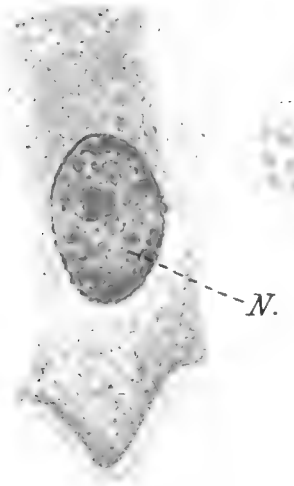
M. Rhodes, ad nat del.

MINCHIN AND THOMSON —TRYPANOSOM



Huth Lith.ᵗ London

EWISI IN THE FLEA— STOMACH—PHASE.



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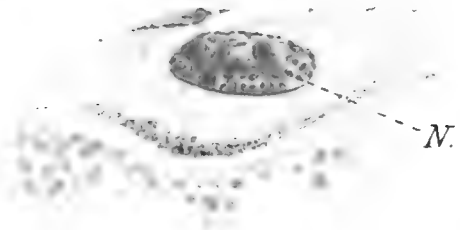

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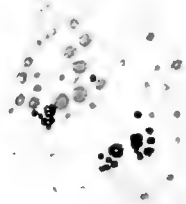

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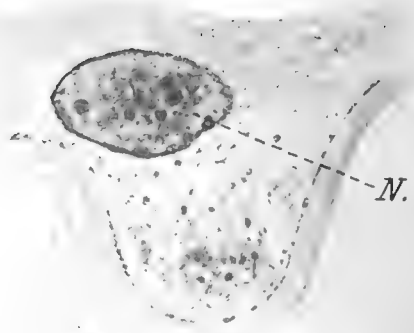
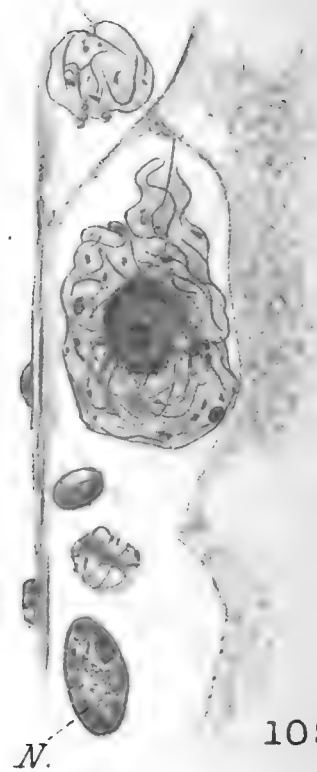


101.

99.

111.

110.



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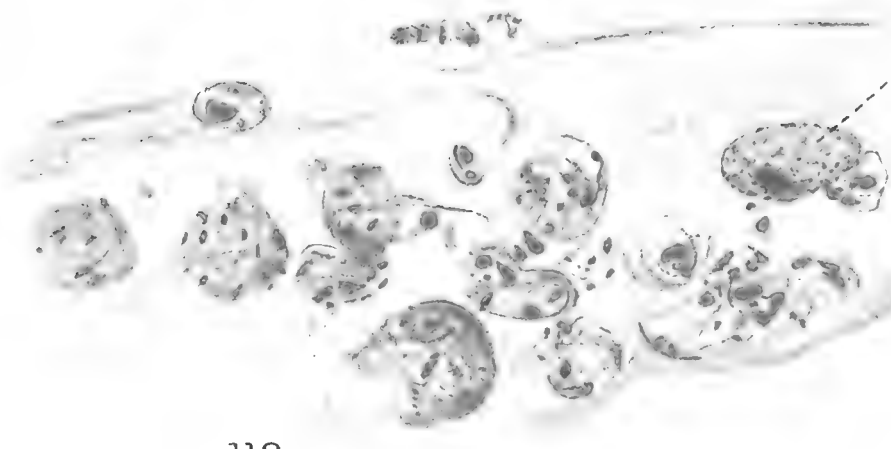

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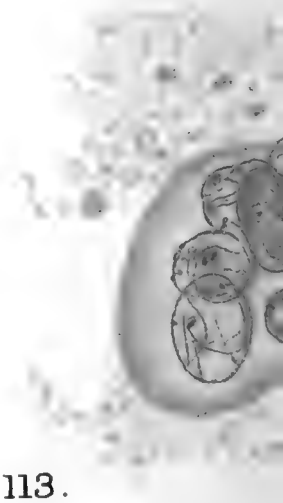
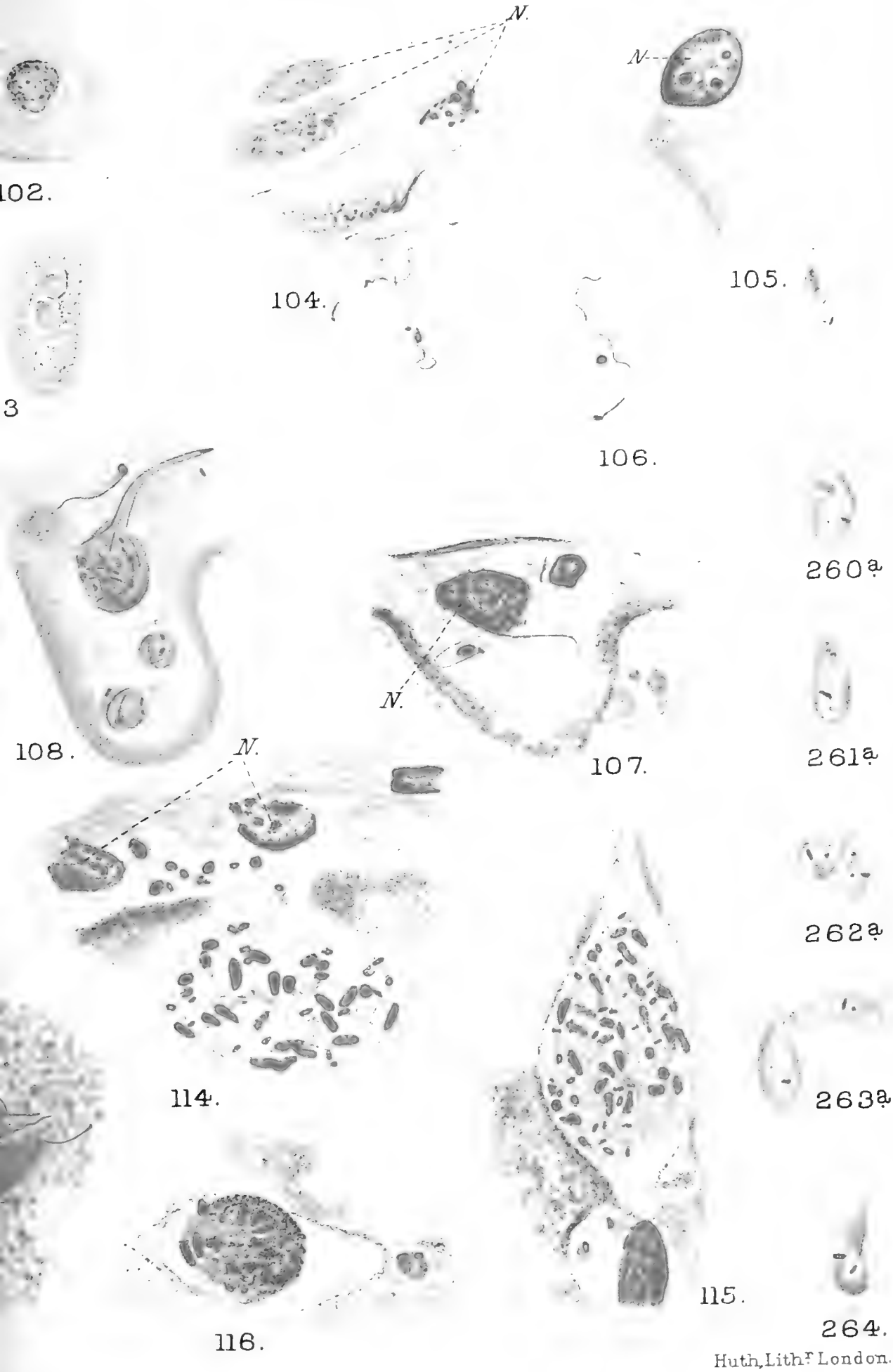


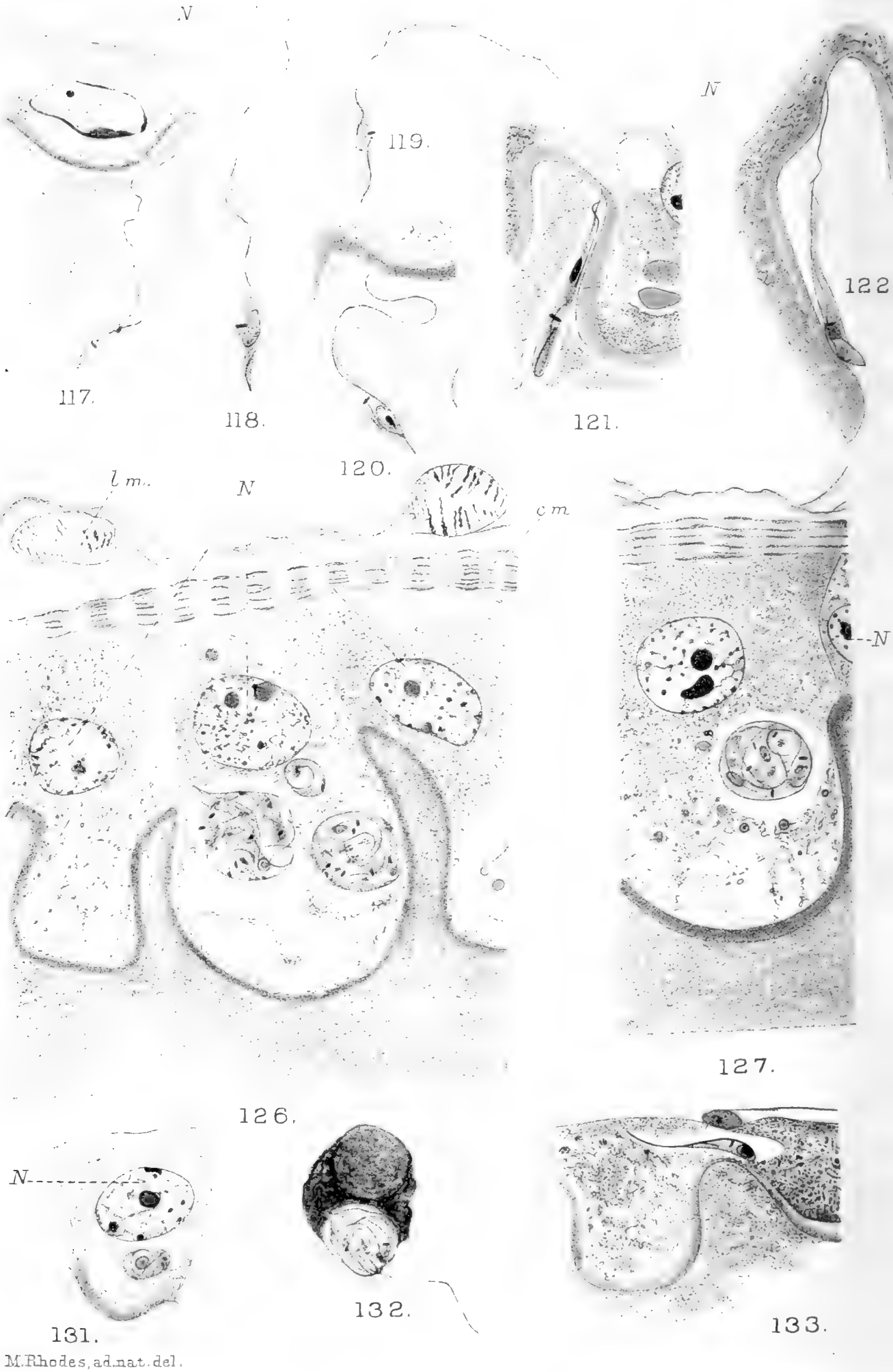
113.

M.Rhodes ad nat. del.

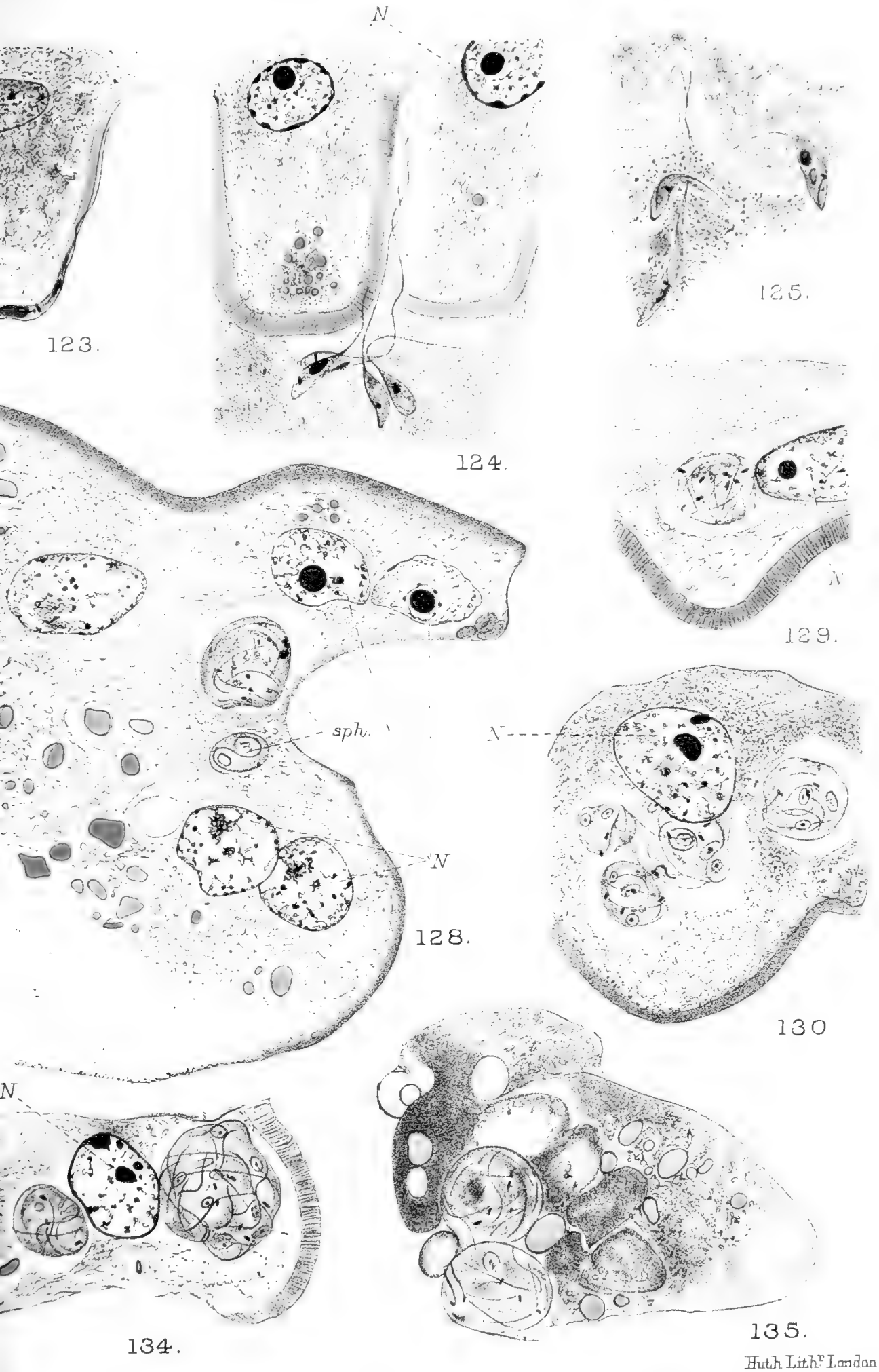


Huth, Lith^r London.

EWISI IN THE FLEA—STOMACH—PHASE.

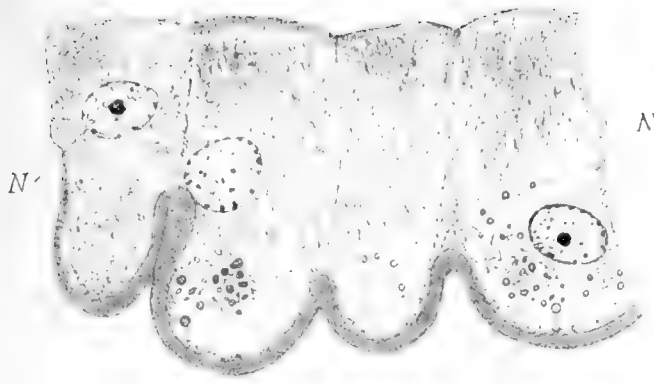


M.Rhodes, ad.nat.del.



Huth Lith^F London

LEWISI IN THE FLEA—STOMACH—PHASE.



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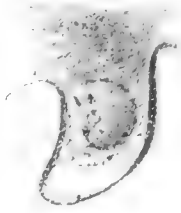

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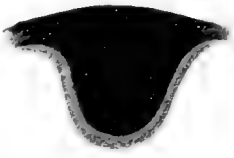

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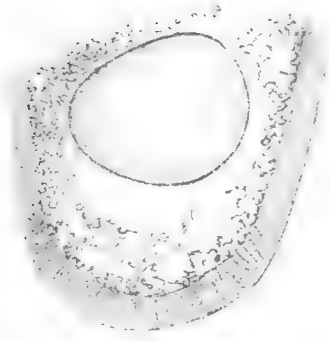

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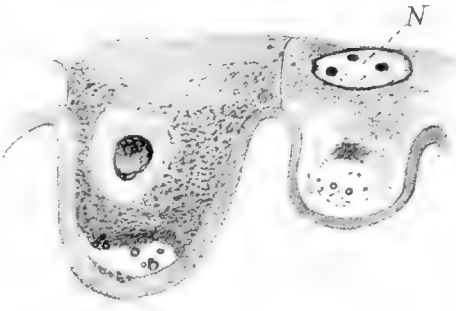

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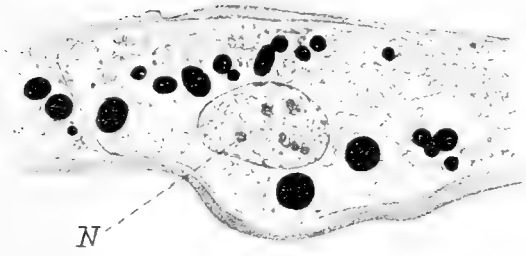

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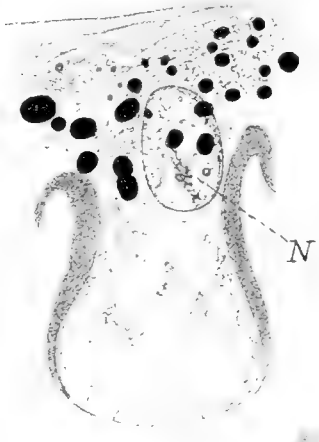

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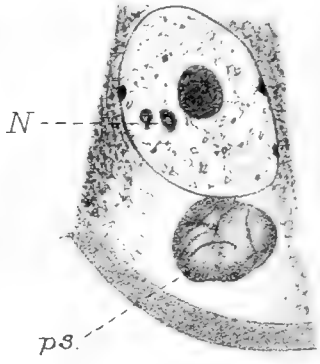

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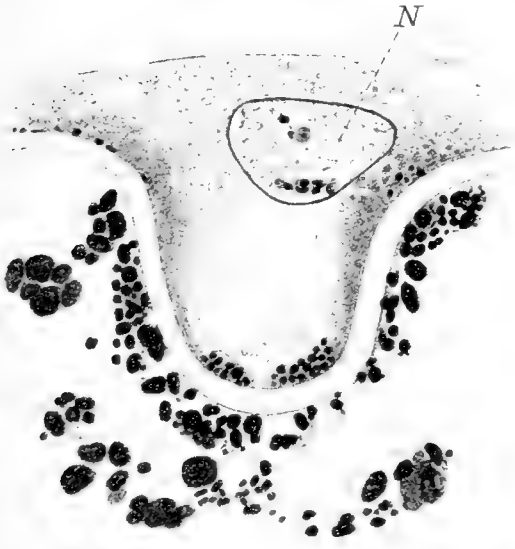

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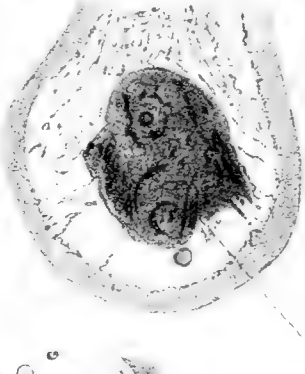

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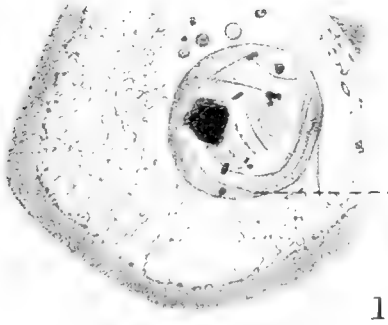
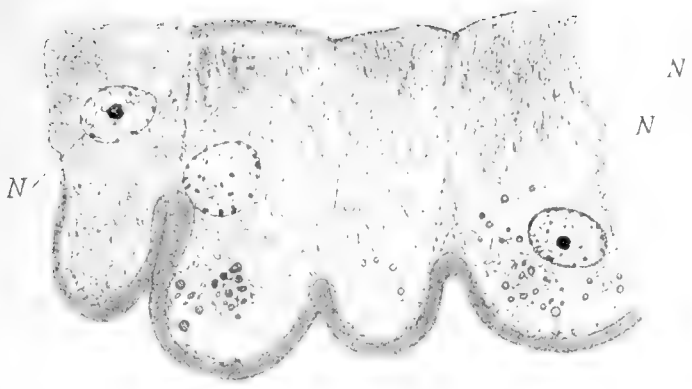


148.

M. Rhodes, ad. nat. del.

Enth Lith^r London

MINCHIN AND THOMSON — EPITHELIUM OF THE STOMACH OF THE FLEA.



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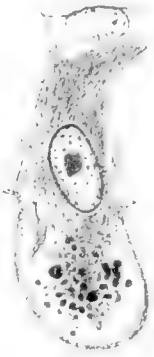

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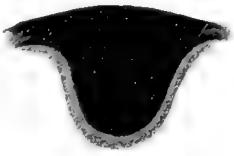

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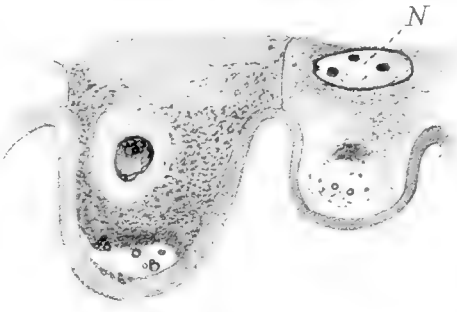

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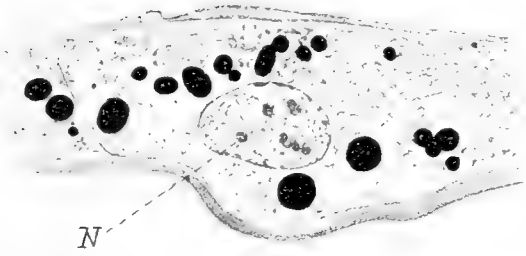

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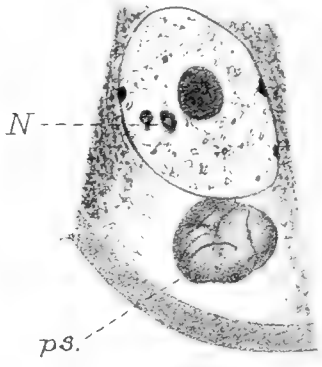

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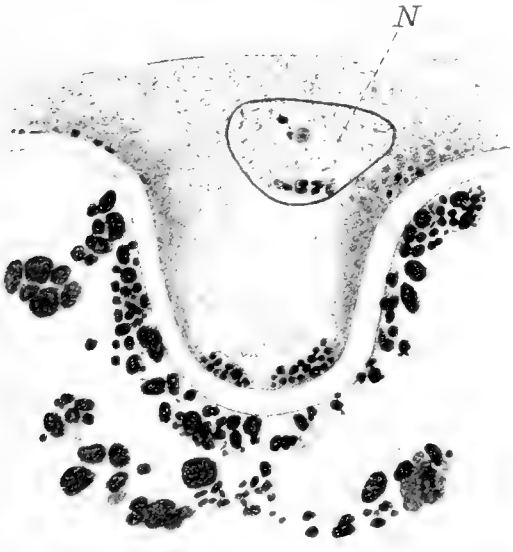

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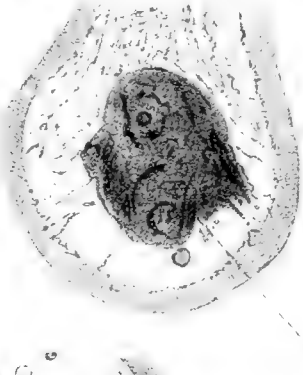

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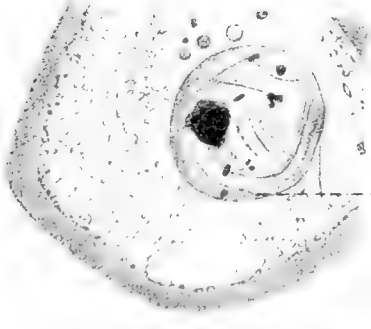
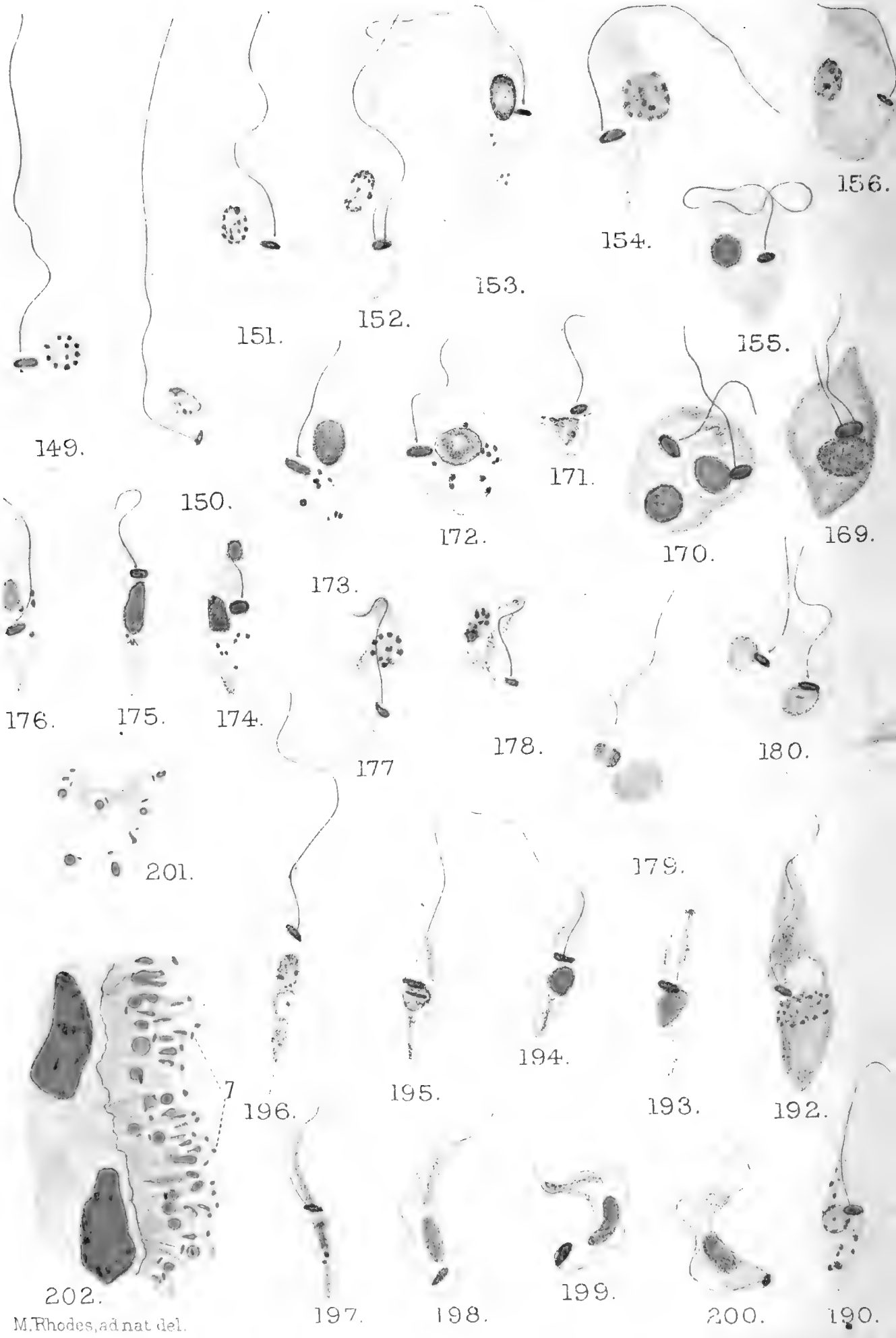


148.

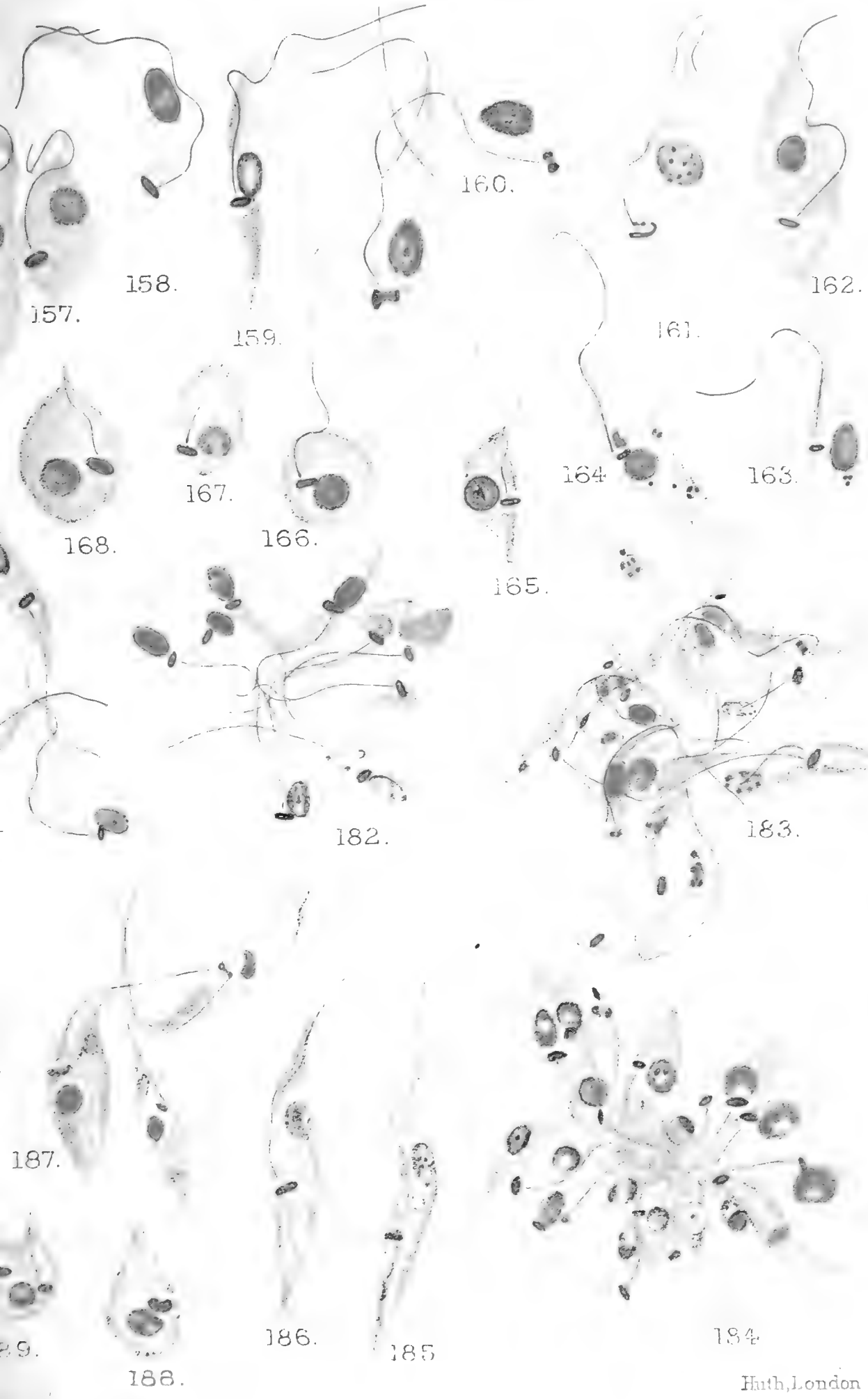
M. Rhodes, ad. nat. del.

Hnth Lith^r London

MINCHIN AND THOMSON—EPITHELIUM OF THE STOMACH OF THE FLEA.

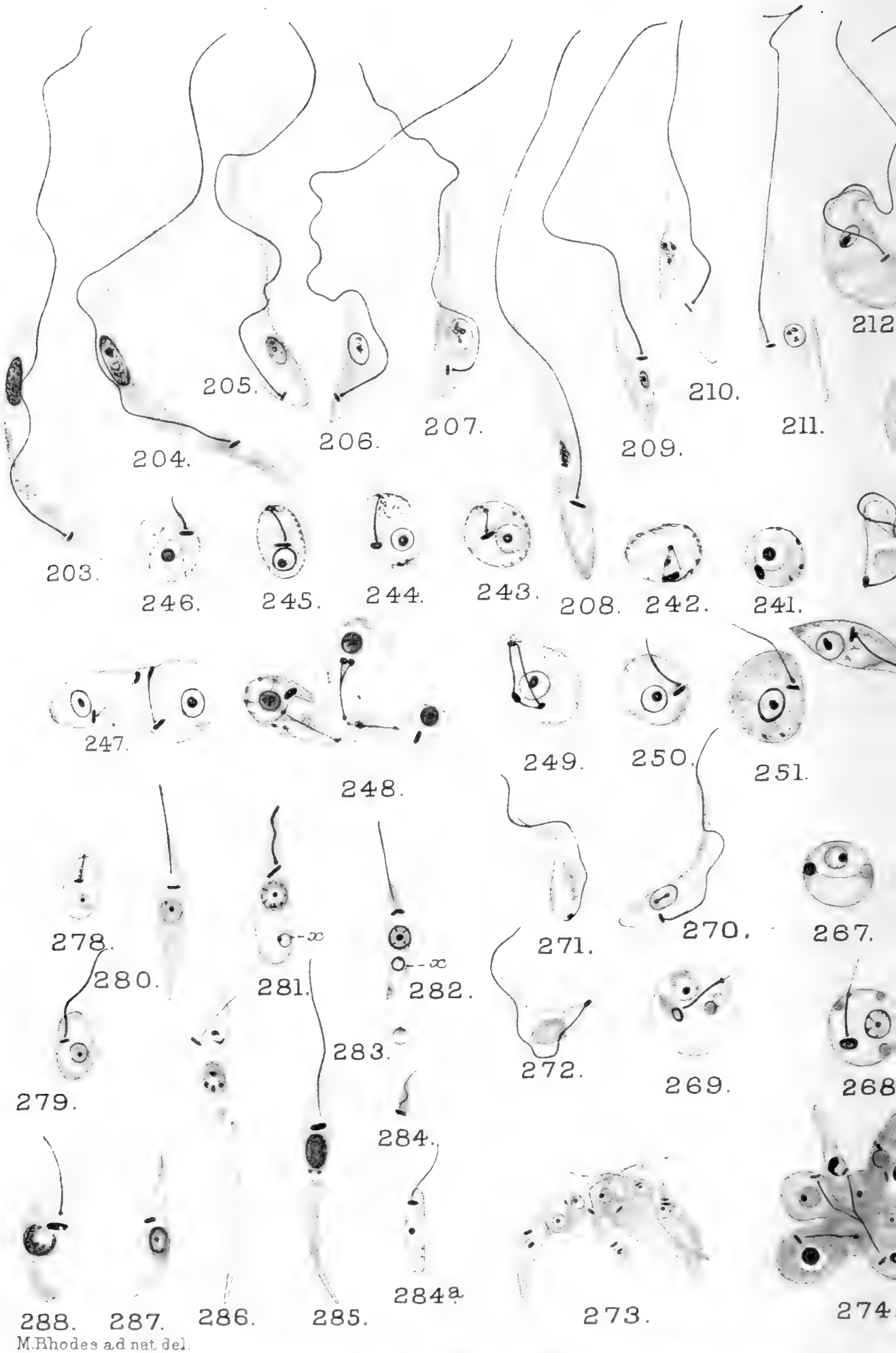


MINCHIN AND THOMSON — TRYPANOSOMA



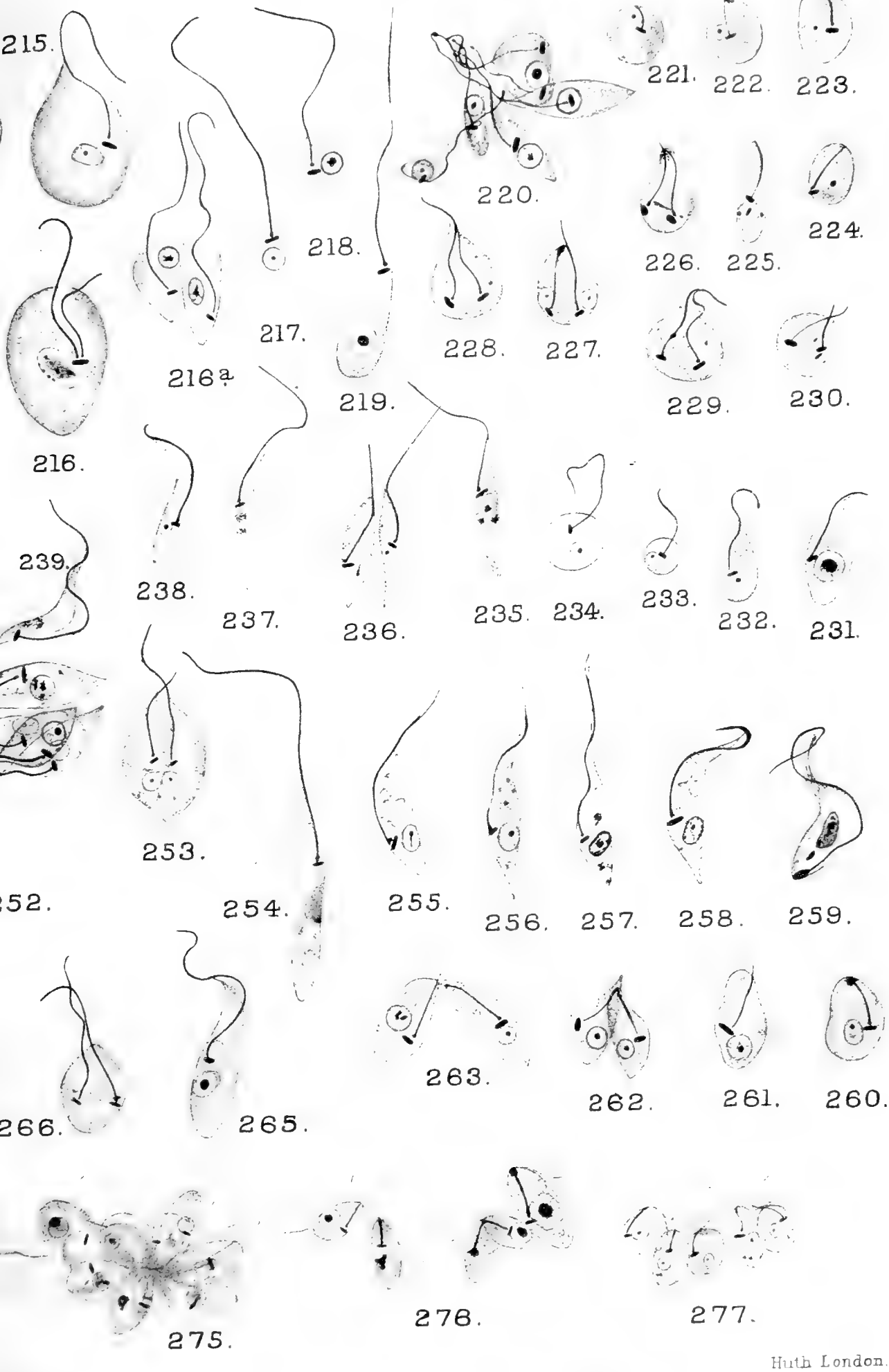
Huth, London

WISI IN THE FLEA — RECTAL PHASE



M.Rhodes ad nat del.

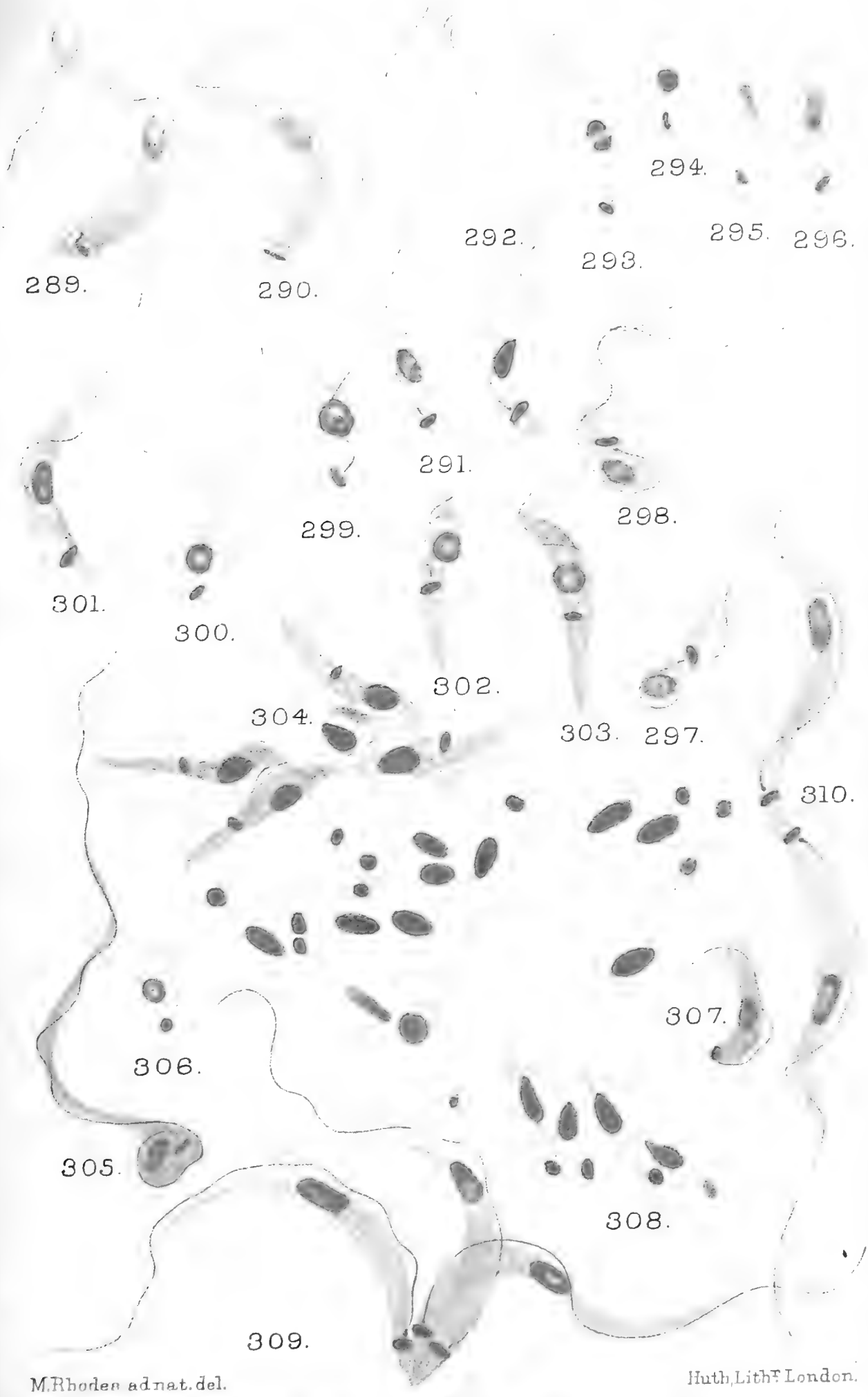
MINCHIN AND THOMSON—TRYPANOSOMA LEWISI & LE



Huth London.

MONAS PATTONI IN THE FLEA—RECTAL PHASE.

For Figs. 260a, 261a, 262a, 263a, 264, see Pl. 38.

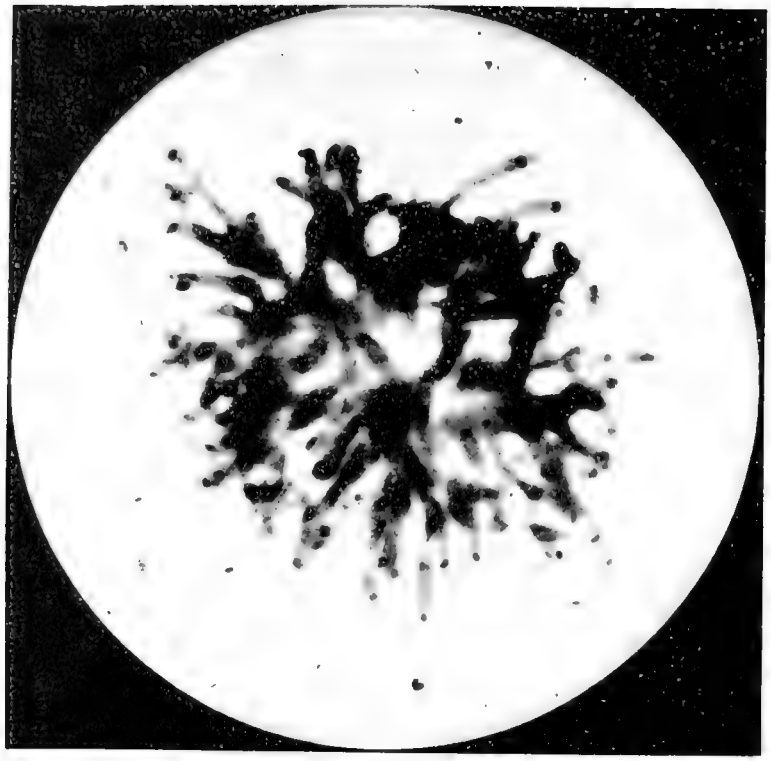


M. Rhoden ad nat. del.

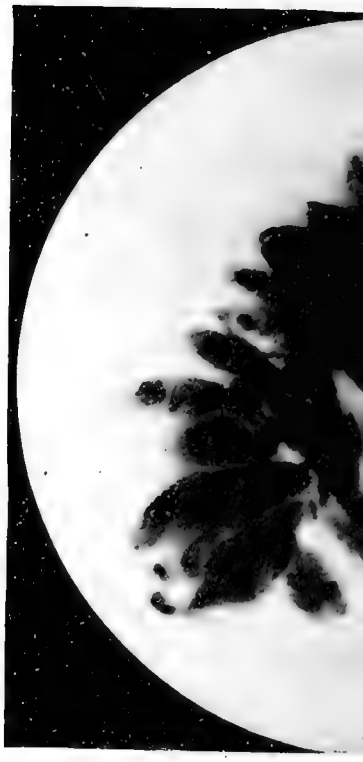
Huth, Lith^r London.

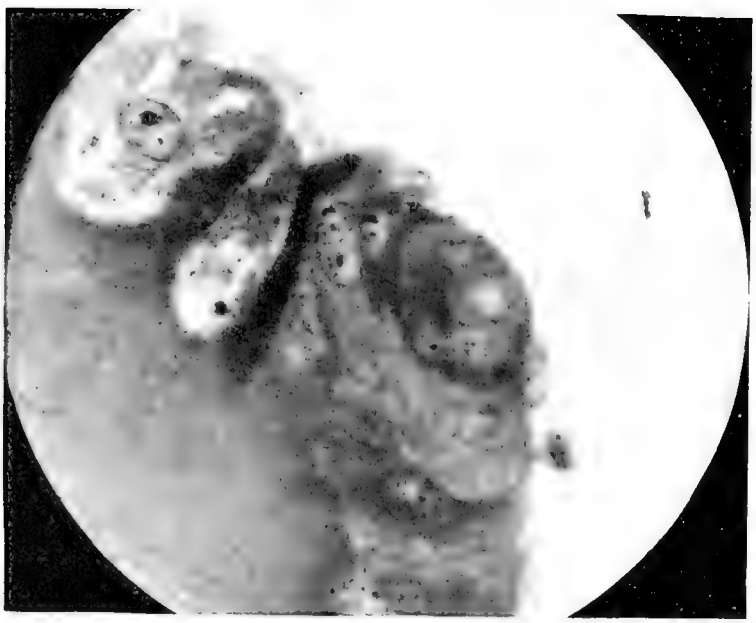
MINCHIN AND THOMSON — TRYPANOSOMA LEWISI IN THE FLEA.

DEGENERATIVE FORMS.



311.





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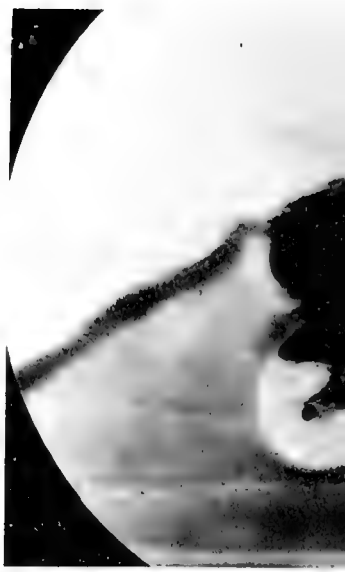

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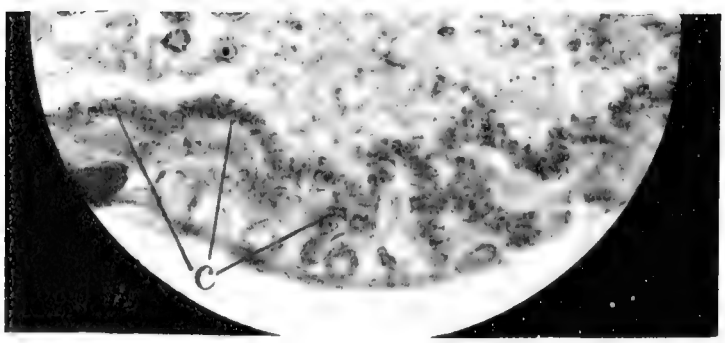
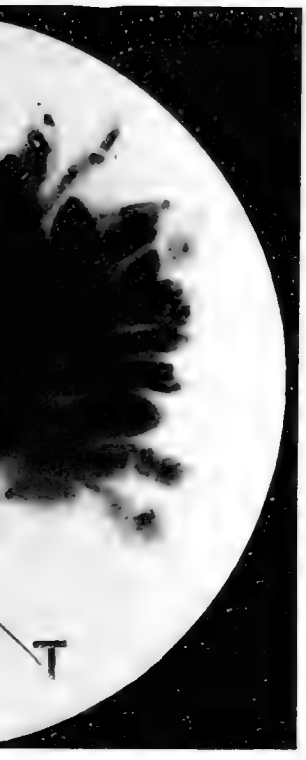
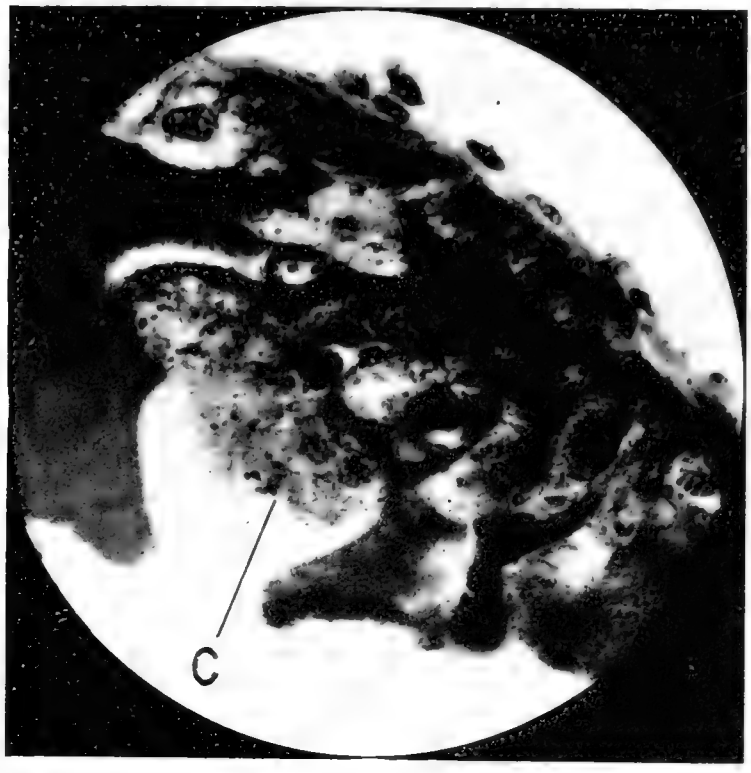


317.

31





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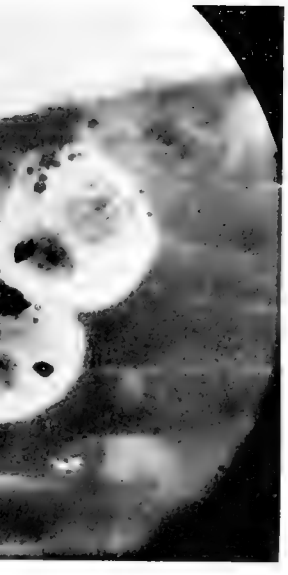

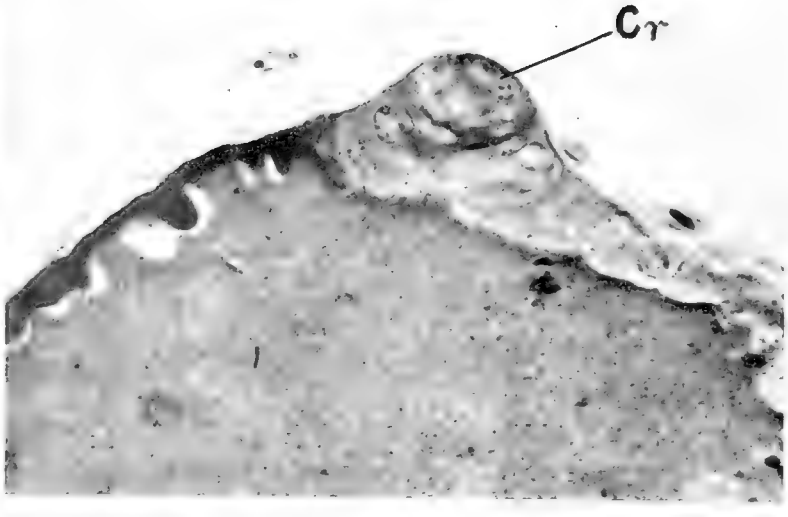

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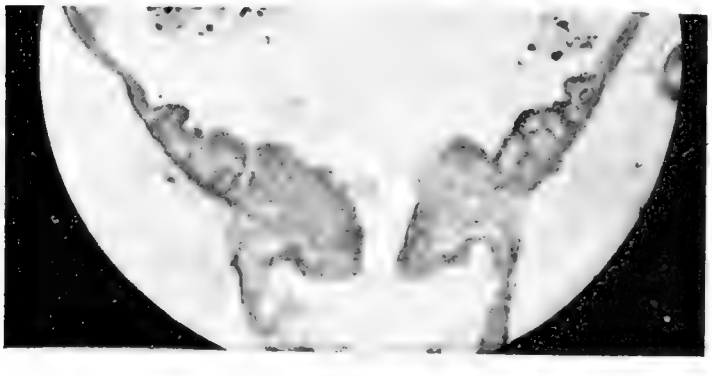
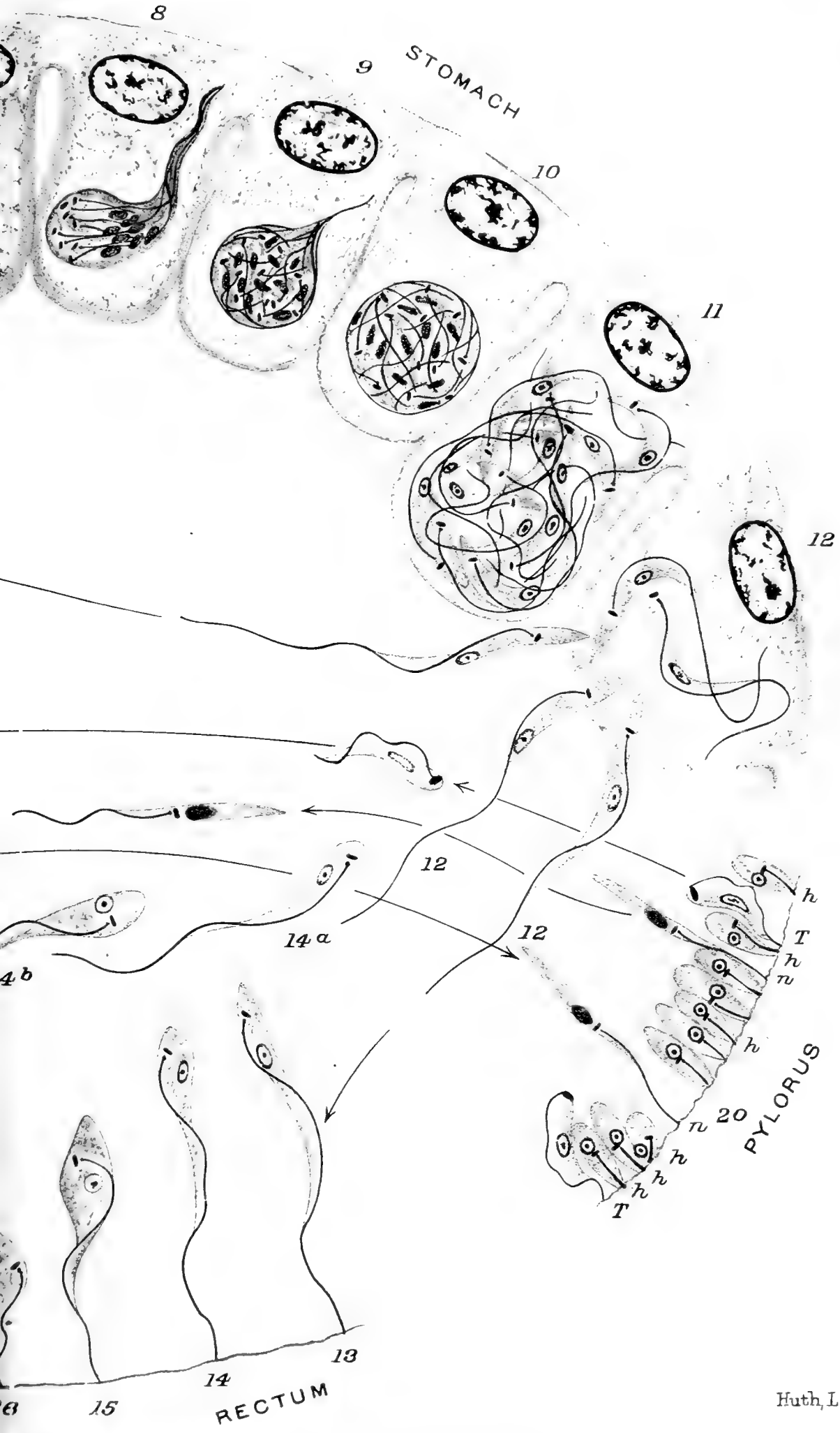


319. Huth, coll.

OMA LEWISI IN THE FLEA.

MINCHIN AND THOMSON—LIFE—CYCL



Huth, London.

TRYPANOSOMA LEWISI IN THE FLEA.