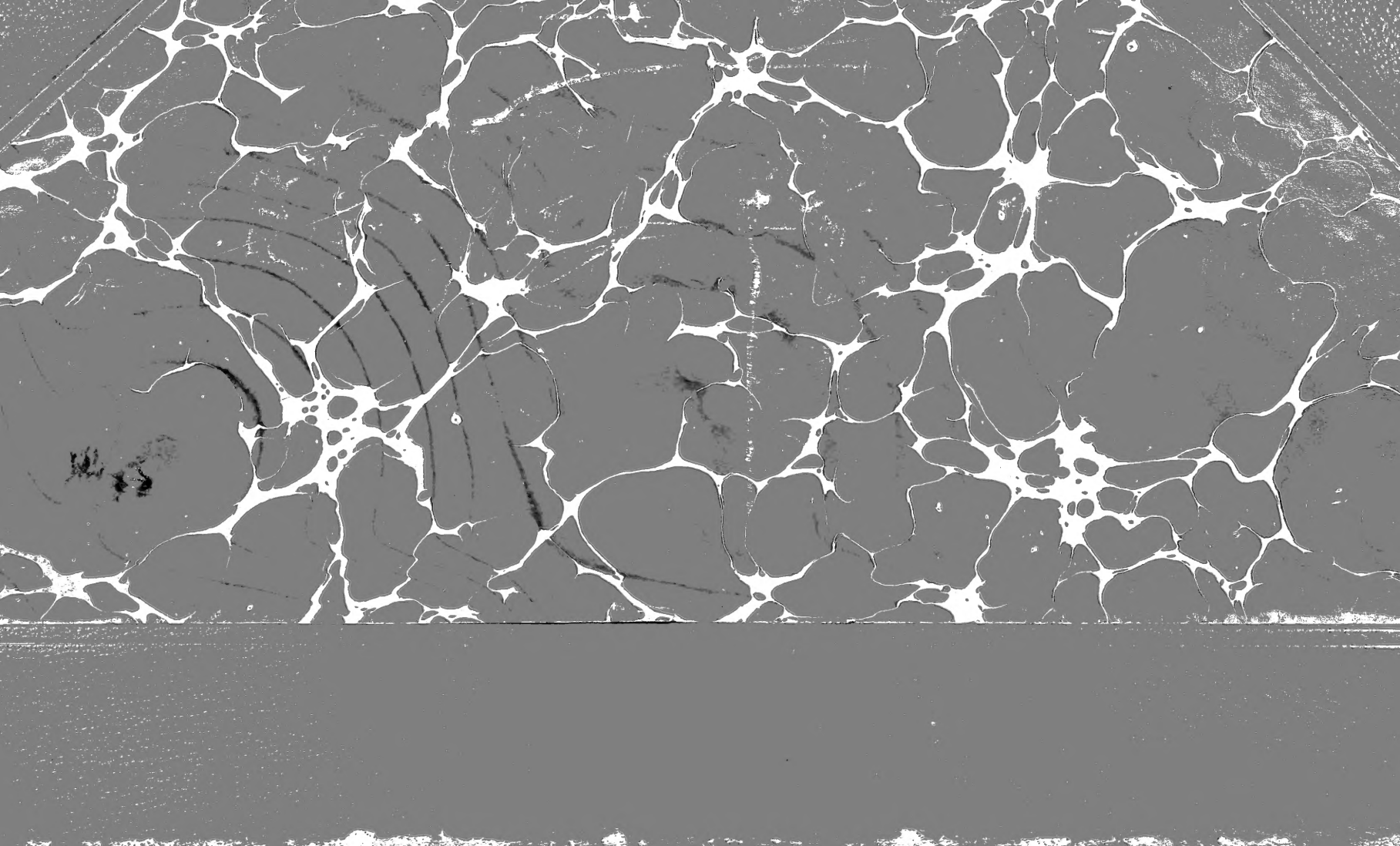


QUARTERLY JOURNAL

OF

MICROSCOPICAL SCIENCE.

EDITED BY

E. RAY LANKESTER, M.A., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND OF THE ACADEMY OF THE LINCEI OF ROME, AND OF THE AMERICAN ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE ROYAL ACADEMY OF BELGIUM; HONORARY MEMBER OF THE NEW YORK ACADEMY OF SCIENCES, AND OF THE CAMBRIDGE PHILOSOPHICAL SOCIETY, AND OF THE ROYAL PHYSICAL SOCIETY OF EDINBURGH, AND OF THE BIOLOGICAL SOCIETY OF PARIS, AND OF THE CALIFORNIA ACADEMY OF SCIENCES OF SAN FRANCISCO; FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE AMERICAN PHILOSOPHICAL SOCIETY;
DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE FULLERIAN PROFESSOR OF PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN;
LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

FELLOW AND TUTOR OF TRINITY COLLEGE, CAMBRIDGE;

AND

SYDNEY J. HICKSON, M.A., F.R.S.,

BEYER PROFESSOR OF ZOOLOGY IN THE OWENS COLLEGE, MANCHESTER.

VOLUME 50.—NEW SERIES.

With Lithographic Plates and Text-Figures

LONDON:
J. & A. CHURCHILL, 7, GREAT MARLBOROUGH STREET.
1906.

CONTENTS.

CONTENTS OF No. 197, N.S., APRIL, 1906.

CONTENTS OF No. 198, N.S., JUNE, 1906.

CONTENTS OF No. 199, N.S., AUGUST, 1906.

CONTENTS OF No. 200, N.S., NOVEMBER, 1906.

New Series, No. 197 (Vol. 50, Part 1). Price 10s. net.

APRIL, 1906.

THE QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE.

EDITED BY

E. RAY LANKESTER, M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND OF THE ACADEMY OF THE LINCEI OF ROME, AND OF THE AMERICAN ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE ROYAL ACADEMY OF BELGIUM; HONORARY MEMBER OF THE NEW YORK ACADEMY OF SCIENCES, AND OF THE CAMBRIDGE PHILOSOPHICAL SOCIETY, AND OF THE ROYAL PHYSICAL SOCIETY OF EDINBURGH, AND OF THE BIOLOGICAL SOCIETY OF PARIS, AND OF THE CALIFORNIA ACADEMY OF SCIENCES OF SAN FRANCISCO; FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE AMERICAN PHILOSOPHICAL SOCIETY; DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE FULLERIAN PROFESSOR OF PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN; LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

FELLOW AND TUTOR OF TRINITY COLLEGE, CAMBRIDGE;

W. F. R. WELDON, M.A., F.R.S.,

LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD; LATE FELLOW OF ST. JOHN'S COLLEGE, CAMBRIDGE;

AND

SYDNEY J. HICKSON, M.A., F.R.S.,

BEYER PROFESSOR OF ZOOLOGY IN THE OWENS COLLEGE, MANCHESTER.

WITH LITHOGRAPHIC PLATES AND TEXT-FIGURES.

LONDON:
J. & A. CHURCHILL, 7 GREAT MARLBOROUGH STREET.
1906.

Adlard and Son, Impr.,] [London and Dorking.

CONTENTS OF No. 197.—New Series.

MEMOIRS:

The Life-Cycle of "Cystobia" irregularis (Minch.), together with Observations on other "Neogamous"[1] Gregarines.[2]

By

H. M. Woodcock, D.Sc.Lond.

With Plates 1—6.

Contents.

Preface.

The Gregarines of Holothurians were first brought to my notice by Professor Minchin, who suggested that I might suit-

[1] From *νεος*, young, and *γαμος*, marriage, on the analogy of Neosporidia. I am indebted to Prof. Minchin for suggesting this appropriate and convenient term.

[2] Thesis approved for the Degree of Doctor of Science in the University of London.

ably devote a portion of my time as Derby Scholar in endeavouring to obtain further stages in the life-history of Cystobia (Gregarina) irregularis. This Gregarine, which is parasitic in Holothuria forskâli,[1] the "cotton-spinner" of our South-Western coast, was originally described by him (**25**), but, in view of the recent important advance in our knowledge of the life-cycle of many Gregarines, it appeared desirable to try and ascertain more fully to what extent Cystobia agrees with, or diverges from, other members of the order.

I applied for, and obtained, the use of a British Association table at Plymouth, which I occupied in the spring and again during the summer of 1902. Some of my time, however, was taken up with other Sporozoan work. Apart from "free" or unencysted parasites, which were usually examined fresh, most of the material collected was preserved at once in different ways and cut, stained, and examined either there or on my return to University College, where all the drawings were made.

I experienced, unfortunately, great difficulty in procuring the hosts, especially in the spring and early summer, when some very important stages in the life of the parasite are undergone. Only too often, moreover, the animals, when obtained, were found to be uninfected. Nevertheless, I have been successful in learning many interesting additional facts with regard to this species of Cystobia—sufficient to enable me to give a fairly complete account of its life-cycle. In addition, I have found another Gregarine, inhabiting Cucumaria pentactes and C. planci, which shows marked peculiarities in its habitat and trophic life and is certainly a distinct species. Owing to the even greater scarcity of this parasite I am unable, in spite of great efforts, to give an equally complete description of this new form. Whereas in C. irregularis, the trophic stages are the more uncommon, in this

[1] This species is generally known as H. nigra, Kinahan, but Koehler (**15**) states that it has been shown to be identical with H. forskâli, De C., and that the latter name takes priority.

case it is the ripe cysts, containing spores, which are extremely rare. For this new species I have already proposed (**40**) the specific name minchinii, after Professor Minchin, in recognition of his earlier work on C. irregularis.

Since publishing my preliminary note on the trophic phases of C. irregularis and C. minchinii, I have been able to examine the spores of the latter. Mainly as a result, I have come to the conclusion that these two parasites must be separated from the other well-known species (C. holothuriæ), and placed in a distinct genus. The reasons for this step are best reserved, however, until we are in a position to consider a full and revised definition of the two former species (see below, p. 58). Meanwhile, in describing the life-history, confusion will be avoided by retaining the old name of Cystobia for all of them.

I have also come across Diplocystis schneideri, Kunstler, in a new host, Periplaneta orientalis. The fortunate rediscovery of this interesting Gregarine has enabled me to compare its trophic phase with that of other known species of the genus, and also with that of Cystobia.

(1) Introduction.

For a complete account of the literature relating to Cystobia up to 1892 the reader is referred to Minchin's article (**25**) already quoted. Since that date I know of no paper dealing with this Gregarine except the systematic enumeration of Labbé (1899). This author, in his 'Sporozoa' (**17**), gave the characteristics of the genus and its known species, which may be summarised as follows:

Cystobia, Mingazzini, 1891.—A monocystid Gregarine of large size, oval or irregular in shape. The adult stage always has two nuclei, probably arising from the early association of two individuals. Spores with dissimilar poles, the epispore at one end being always turned outwards as a funnel-like projection. Eight sporozoites in the spore.

(1) C. holothuriæ (Schn.).—Cysts attached to the wall

of the blood-vessels by a delicate stalk, and also, when ripe, floating free in the body-cavity. Spores ovoid, with the epispore having the funnel at one end, and, in addition, at the other end a flat process like a lance-head. Sporozoites with rounded nucleus. Habitat: H. tubulosa, at Naples. (Also in Chiridota pellucida, according to Sars.)

(2) C. irregularis (Minchin).—Cysts always attached to the wall of the blood-vessels. Spores ovoid; epispore prolonged into a cup-like expansion, open to the exterior. The sporozoites possess an elongated nucleus. Habitat: blood-vessels of Holothuria forskåli (H. nigra).

(3) C. schneideri, Ming.—Of smaller size, and much less resistant to sea-water than the foregoing. [This is rather a slight diagnosis on which to base a new sp.] Habitat: H. polii and H. impatiens, Naples.

It will be seen, therefore, that practically nothing has been described for Cystobia with regard to such important questions as the processes of nuclear division preceding sporulation, the formation of primary sporoblasts and their conjugation, whether isogamous or anisogamous, etc. Nor was Minchin able to make out at all satisfactorily the extent and intimacy of association in C. irregularis, a point which is of great interest and importance. A knowledge of these various stages in the life-history was eminently to be desired, since during the last few years much has been learnt respecting them in the case of other Gregarines. It will be useful, first of all, to outline briefly the results of recent research in this direction.

Siedlecki (**36**) was the first to correctly work out the life-cycle, which he did for Lankesteria ascidiæ, a member of the sub-order Aseptata or Haplocyta, parasitic in the gut of Ciona intestinalis. Association occurs between two equal-sized adults. The syzygy rotates and gradually becomes spherical, and an outer and inner cyst-membrane are successively laid down. After elimination of much of the nuclear material, the remainder gives rise by successive divisions, the earlier of which exhibit well-marked mitosis, to a number of daughter- or micro-nuclei. Meanwhile the bodies of the

two Gregarines have become interlobed in a complicated manner, though each still remains quite distinct from, and not in any way united with, the other. Little uninuclear protuberances appear on the surface of both, and these are finally cut off as primary sporoblasts or gametes. According to Siedlecki, the gametes formed from one associate or parent-individual are perfectly similar to those formed from the other, and therefore isogamous. Soon after liberation the gametes throughout the whole of the cyst are seen to be in a state of rapid motion, the result of which is to get them all thoroughly mixed. They next conjugate in pairs, each member of a pair coming, in all probability, from a different half of the cyst. The definitive sporoblast becomes a spore, containing eight sporozoites, in the usual manner.[1]

On the other hand, in Stylorhynchus and Pterocephalus, two members of the sub-order Septata, Léger (**22**) and Léger and Duboscq (**23**) find a differentiation of the gametes into male and female, with, consequently, anisogamous conjugation.[2] All the elements arising from the same chamber of the syzygy are of the same sex, so that we may consider the two sporonts or associates themselves as respectively male and female. The male gametes are motile, elongated or fusiform in shape, with a minute rostrum anteriorly and a long flagellum posteriorly. Those of Pterocephalus are much smaller than the massive female elements of that parasite; while in Stylorhynchus both kinds are about

[1] Cuénot (**10**), soon after, described very similar facts for different species of Monocystis in the earthworm, and also for Diplocystis spp., cœlomic parasites of the cricket (Gryllus). As regards the latter parasites, there is no reason to doubt that conjugation is completely isogamous, as in Lankesteria. While, however, both Cecconi (**8**) and Prowazek (**31**) have confirmed, in the main, Cuénot's version of the process in Monocystis, Brasil, in a recent note (**4**) states that conjugation in this form is not completely isogamous, distinct, though slight, differences between the gametes being observable (see below, pp. 51 and 76.)

[2] It is important to notice that anisogamous conjugation is not universal in the Septata, both Berndt (**1**) and Paehler (**29**) having described isogamy in various species of Gregarina.

the same bulk, the chief differences between them being in respect of form and movement.

In other words, sexual differentiation in the latter genus is less marked. There can be little doubt, it seems to me, that this condition represents an early step in the direction of isogamy. I hold that isogamy in the Gregarines is secondary, and derived from anisogamy, being, in fact, closely correlated with the phenomenon of association; this subject, however, will be fully discussed later (see p. 73, et. seq.).

(2) Methods.

(*a*) Examination.—In examining both Holothuria and Cucumaria for the parasites, the dissection was rendered much easier by leaving them for some time previously in a jar of sea-water, to which a few crystals of menthol had been added. This stupefied the animals, and always in an expanded condition. If they were taken out after a few hours, before death had occurred, the Gregarines were in no way affected, as they do not inhabit the gut. By this precaution I avoided the disagreeable extrusion of the Cuvierian organs and the violent contractions of the body which otherwise take place.

On opening a Holothurian it is easy to ascertain, by scrutinising the vascular network and blood-vessels generally, whether any parasites are present. They show up distinctly either as little white, oval spots if in the lumen, or as spherical cysts if attached to the wall. In the former case it is a very delicate matter to obtain the Gregarines free without injuring them. I followed Minchin's procedure, snipping the vessel close to the parasite on either side and, by gently pressing the wall, causing the Gregarine to pass out. Sometimes, however careful I was, when at length I got the Cystobia safely on a slide, it would be distorted and irregular in shape, although touched as little as possible during the manipulation (for the reason, see p. 25).

In the case of C. minchinii in the Cucumariæ the

parasites are usually in the respiratory trees or else attached by a stalk to the cœlomic epithelium. Those in the latter position are always adults, and invariably more or less enveloped by a double layer of transparent epithelium. Hence they are much easier to remove and mount without damage than are the unencysted adults of C. irregularis, for the body-form is preserved unaltered, with the exception, occasionally, of the free extremity. All that has to be done is to take firm hold of the stalk of invagination with a fine pair of forceps, and carefully break it away at its point of attachment to the general epithelial layer. The parasite comes away, of course, with the stalk and need not, itself, be touched during the operation. It can thus be readily brought either into a watch-glass or on to a slide, as required for fixation.

(*b*) Fixation and staining.—Adults were usually fixed on the slide with osmic vapour by holding the slide inverted over the mouth of a bottle containing a 1 per cent. solution of osmic acid for five minutes. They were then washed well with water, stained with dilute Ranvier's picro-carmine, dehydrated, cleared, and finally mounted in balsam. This procedure gave very satisfactory results. As an alternative method, a saturated aqueous solution of corrosive sublimate was used, to which had been added 5 per cent. of glacial acetic acid. The objects were afterwards stained either with carm-alum or with alcoholic para-carmine. Both these stains differentiated the nuclei well. Some adults obtained from Cucumariæ, which had been previously preserved in 90 per cent. spirit, were rather shrunken in outline. The nuclear contents also were retracted away from the membrane, but the cytoplasm was well preserved. I sectioned a few, and stained on the slide. Thionin, followed by orange, was very successful.

For Gregarines in the blood-vessels, respiratory trees, or elsewhere, and for all cysts, either the corrosive sublimate and acetic mixture referred to, or strong Flemming, was generally best. Perenyi's fluid and picro-sulphuric were

also tried, but only with indifferent success. Material was left in sublimate and acetic twenty to forty minutes, and in Flemming two to four hours. That fixed by the former method was usually stained in bulk with borax- or para-carmine, but material by the latter generally first on the slide. If evaginated Gregarines were still in the trophic condition (see below, pp. 13 and 20) the nuclei were easily visible; if, on the other hand, the parasites had commenced to sporulate, it was usually necessary to cut the cysts before they could be examined. In sections of advanced cysts stained whole the nuclei were usually nicely differentiated; in earlier stages, however, the nuclei, for some reason or other, were indistinct and the sections had to be restained.

Sections were generally cut 3–4 μ thick, but occasionally more. In experimental cutting of the blood-vessels and of the gut, undertaken in the hope of finding very young and minute forms, the usual thickness was 10–12 μ. As a rule the cutting presented no difficulty; only when the parasites were in the retractor muscles did I find it advisable to paint the block with a solution of collodion and gum-mastic.

For staining on the slide the combination most frequently used, and the one which gave the best results, was Heidenhain's iron-hæmatoxylin method, followed by orange or eosin. Thionin and orange rendered good service, as did also Kleinenberg's hæmatoxylin, the slides being immersed in the latter for a long time, forty-eight hours or so. Safranin, although made up in two or three different ways, was rather diffuse, and never stained the nuclear reticulum, but only the karyosome. Neither Auerbach's mixture (methyl-green and acid fuchsin) nor Romanowsky's stain was of much service as a "double" stain. I find that methyl-green cannot be relied on as a chromatin stain unless used fresh on the living animal, and this was quite unsuitable for Cystobia. Romanowsky's stain also, though well adapted for blood-films and smear preparations, is most unsuitable for tissue parasites or where sections are required. Neither for Gregarines nor for Myxo-

sporidia is it of the slightest use so far as nuclear detail is concerned.

Special effects of fixatives or stains on different structures will be mentioned when dealing with them in detail.

(*c*) Attempts at artificial infection.—I endeavoured to keep the Holothuriæ alive in the aquarium, but from some cause or other they would not settle; it may have been owing to the great difference between the pressure in the tanks and that to which they are accustomed in their natural surroundings. Invariably after a short time the skin became broken and patchy, showing evident signs of maceration, and this was the sure prelude to general evisceration.[1] The animals were always kept quite separate from anything else likely to injure them, so the ill-health was not the result of being bitten. For this reason alone the prospect of successful infection and of obtaining early stages of the parasites appeared to be very doubtful. When, moreover, I came to try it, I soon found artificial infection at all to be a most difficult matter.

It was necessary to actually convey the cyst containing spores inside the Holothurian's mouth, not only to be certain that the animal really swallowed the spores, but also to know approximately the time, in order to have any chance (by killing the animals after different intervals) of subsequently observing the liberated sporozoites and their passage through the gut-wall. A Holothurian's mode of eating is to sweep up particles of sand, shell, etc., with whatever organic material may be amongst them, from the surface on which it happens to be crawling by means of its tentacles. These, of the Aspidochirote type, are furnished with an expanded, brush-like, distal end to which the particles adhere. The tentacles are in turn stuffed into the mouth and then withdrawn, the food having been sucked up into the œsophagus.

I could not trust to such a haphazard method as this with much likelihood of success, so I endeavoured to feed

[1] Ludwig (**24**) mentions this curious fact, saying that, after irritation, "die Haut sich ziemlich rasch in formlosen Schleim auflöst."

the animals directly. As soon as they were touched, however, they withdrew their tentacles into the buccal cavity, and closed this tightly up, leaving nothing visible externally. They evinced, moreover, a decided reluctance to be turned on their backs, which was necessary since the buccal cavity is slightly ventral, or at any rate normally turned ventrally. Sometimes after holding a Holothurian gently, yet firmly, with my hand, often so long that my arm was quite cramped, it would unfold its tentacles and allow me a beautiful view of its mouth. I then cautiously steered a tiny piece of blood-vessel with a cyst attached[1] to the open mouth. I have seen it pass in and apparently fall some distance down the gullet, but it was nearly always pushed up again and out into the buccal cavity, usually to be lost among the tentacles. Whether this expulsion took place actively or passively I am not quite certain, although I think probably the latter, since there generally seemed to be a current alternately of inspiration and expiration when the animal was not feeding. If the cyst stayed in as long as I retained my hold, it invariably fell out when I allowed the animal to resume its normal position. Now and again, by way of diversion, the Holothurian would suddenly eject the whole contents of its body about my hand, which I had then to extricate from the Cuvierian organs.

I also tried mixing some cysts with particles of sand and mud, making a small accumulation on the floor of a dish and placing the animal with its buccal cavity near to it. The heap would be disturbed by the tentacles, to one of which a cyst, or part of one, might even adhere, only to be brushed off by something a moment later; in short, the Holothurian would not eat to order. Once or twice I thought a few spores might possibly have succeeded in getting well down and staying there, and the animals were killed after a certain time had elapsed. I fixed and sectioned different portions of

[1] Since there is no true cyst-wall to a ripe cyst of C. irregularis (the thin cyst-membrane having before this broken down and disappeared), the spores are only held together by the delicate evaginated epithelium of the blood-vessel.

the gut, but, after laborious examination of a great many sections, no traces of Cystobia could be found.[1]

(3) Habitat and Mode of Life.

(*a*) C. irregularis.

Minchin (loc. cit.) has described the general habitat of this parasite and its relation to the vascular system. This is well shown in his figures 9, 10, and 19, and also in my figures 5, 20, and 41. Some additional observations, however, may be noted.

Trophozoites—as both young forms and adults are termed while they are still taking in nourishment—occur chiefly in the complex vascular meshwork, known as the "rete mirabile," which is attached to the second loop of the gut, the intestine proper. This is essentially the region of absorption, and the fluid circulating in these finely anastomosing vessels is doubtless very rich in nutrient solutions. Hence it is particularly suitable for the growing Gregarines, which probably pass into it directly from the intestine.

Histology of the vascular network.—The structure of this network differs considerably from that of the more anterior part of the vascular system, which runs along the first, or what may be called the stomach, loop. In the latter the longitudinal vessels are much more distinct and of larger calibre, and the vascular plexus is much simpler. The chief difference, however, is in the wall. That of a main vessel or connecting branch in this anterior region is very thin and transparent-looking, and pale or slightly reddish in colour. In section (fig. 7, pl. 1) it is seen to consist of loose, spongy cells (*sp. c.*), bordering[2] and traversing the lumen irregularly,

[1] A reason for these unsuccessful infections is perhaps to be found in the fact that, in the majority of spore-containing cysts, subsequent examination of stained material showed that the spores themselves were not ripe, having only four nuclei. This preponderance of the four-nuclear stage was noticed by Minchin (loc. cit.). Probably the enclosing epithelium often breaks down before the sporozoites are fully formed, and the spores ripen either in the body-cavity or after expulsion from the host (see below, in text).

[2] The vessels have no true epithelial lining internally.

then a delicate muscular layer (*m. l.*), and, externally, a single layer of cœlomic epithelium (*c. ep.*).

The wall of the complicated "rete mirabile," on the other hand, always appears yellow to yellowish-brown and opaque, and in section (fig. 8) is much thicker and of a firmer texture, with a corresponding reduction in the lumen. The loose, spongy tissue is less developed, and only forms a thin layer most internally (*sp. c.*). Next comes the layer of muscle-fibres (*m. l.*), and, finally, making up nearly the whole thickness of the wall, a great development of cœlomic epithelium (*c. ep.*).[1]

Relation of the parasites to the blood-vessels.—To return to our parasites. As they grow they are carried about passively (for they lack all power of movement, see below, p. 19) along with the blood-stream, and at last pass into the larger thin-walled vessels—the distributing part. Obviously it is much easier for them to evaginate the walls here than it would be if they remained in the "rete mirabile"; in fact, I never once saw a cyst attached to this latter. A curious place of occurrence of the evaginated cysts is in connection with the membrane which stretches from the ring-canals to the ossicles in the body-wall and in which run the radial canals. Lying in this membrane are numerous vascular cross-connections between the radial vessels, and to these the Gregarines are often attached in considerable numbers, having evaginated their walls.

I never noticed any cysts free in the body-cavity of the Holothurian, a point in which C. irregularis differs from C. holothuriæ, in H. tubulosa, where even sporulating cysts—not yet ripe—occur free. The fact that out of a great many cysts none were loose, although several had formed spores, and also the delicate nature of the cyst-envelope (see below, p. 36), points to the conclusion that in this species the cyst ruptures in situ, liberating the spores into the cœlome;

[1] This condition affords an interesting parallel to the layer of yellow cells around the intestine of Lumbricus, and it is not improbable that this great development of cœlomic epithelium serves a similar purpose in the two cases.

they most likely escape thence when some Cuvierian organs are extruded. Probably the stalk of attachment is here stronger, in order to prevent the Gregarines breaking loose and being carried away before spore-formation has proceeded far; in H. tubulosa, which does not possess Cuvierian organs, such a precaution is not necessary.

There is no hard and fast line to be drawn with regard to the condition the parasites are in when they evaginate the wall of the blood-vessel. They may, as sporonts, have already commenced nuclear division, or they may be quite young trophozoites. In fact, the smallest example I obtained was in the latter condition. Such cases are frequent towards autumn, when the lowering of the temperature and other factors proclaiming the end of the season may perhaps induce precocious evagination, in the endeavour to sporulate before the approach of winter. About one third of the adults met with were "out," and had become rounded off; the remainder were, of course, in the lumen, and these possessed a perfectly definite body-form.

(*b*) C. minchinii.

The habitat of C. minchinii in Cucumaria is very different from that of C. irregularis. In the first place, I never once came across the parasites in the vascular network attached to the gut, strong à priori evidence that they do not penetrate through the wall of this latter. The most general situation is inside the respiratory trees, in the wall of the branching and blindly ending diverticula. Here they occur as white, opaque little spheres. Pl. I, fig. 4, gives an idea of part of a small diverticulum, which contained seven Gregarines. When present, the parasites are sometimes very numerous and all in a practically similar stage, only varying in size. They range from 17μ to ·20 mm. in diameter, and between these limits all gradations in size are to be met with. The smallest individuals I obtained were in this situation. Figs. 31 and 32 show two of these tiny ones in section, both being

in the loose connective tissue of the wall. In each case *lum.* is the lumen of the diverticulum, *n.n.* are the nuclei in the wall, *m.f.* muscle-fibres, and *e.n.* nuclei of the cœlomic epithelium externally. Two much larger examples are shown in figs. 18, 19, pl. 2, and it will be seen that the connective tissue of the wall tends to arrange itself in layers around the parasite.

Probable mode of infection.—This situation, together with the non-occurrence in the vascular network, leads me to think that the infection is not by way of the mouth and gut, as in Holothuria, but through the cloacal aperture and into the respiratory trees. Probably the nature of the tentacles, which are here of the Dendrochirote type, and the slightly different manner of feeding account for this. Whereas a Holothuria shovels up sand and shell, together with any accompanying organic matter, into its mouth, Cucumaria spreads its branched tentacles, like a beautiful net encircling its anterior end, and waits for living organisms to be entangled in the trap. It then conveys its tentacles with their prey, one after another, to its mouth. Hence the passive spores lying about on the ground are far more likely to be sucked up with the respiratory current through the cloaca and so into the "trees." The fact, too, that the Cucumariæ are more sedentary than the Holothuriæ, not moving about so much in search of food, may explain the comparative scarcity of the parasites. This is, I believe, quite a unique instance of the "casual" or accidental method of infection, which is usually accomplished by the spores being taken in at the mouth when the host is feeding. The excretory acids known to be present in the trees probably perform in this case the function—elsewhere allotted to the gastric or pancreatic juice—of opening the spores.

Relation of the parasites to the cœlomic epithelium.—Adults of the typical gregariniform shape never occurred in the trees, but in a very curious position, trying, apparently, to penetrate the cœlomic epithelium, either of the body-wall (most frequently) or of one of the vascular strands

connecting the posterior part of the gut with the body-wall. Fig. 6*a* shows two Cystobiæ, each attached to the body-wall by an epithelial stalk, which itself is partially invaginated by the parasite. Fig. 6*b* represents one of the two drawn with a lens; the slight constriction about one third of the length from the free end marks off the portion of the Gregarine still left uncovered by the epithelium from that which is already surrounded by it. There can be no doubt that they are going in and not coming out; of this I have quite assured myself. The process is exactly the opposite to that of evagination which occurs in C. irregularis. One must perforce suppose something like the following to take place: After a successful infection some of the young parasites in the trees, instead of becoming rounded off and growing in situ, pass straight through the wall into the cœlome, assuming the typical ovoid form. (Unfortunately, I have never observed any free in the body-cavity.) Probably the parasites are not long carried about here and there by the movements of the cœlomic fluid before touching a suitable place. To this the Gregarine would at once adhere, doubtless by means of a little secretion, which at the same time starts the cœlomic epithelium at that point proliferating, with the result that a stalk is formed into which the parasite is meanwhile pushing. The process undoubtedly takes considerable time before being completed, during which the Gregarine continues growing, for I have seen parasites of very different size in this position.

A typical C. minchinii thus endeavouring to penetrate the epithelium is seen in outline and optical section in fig. 9. The stalk is, of course, broken off from its attachment to the body-wall. The dotted lines represent the limit of the epithelial investment, where it is reflexed internally. Fig. 10, drawn in surface view, shows the covering of epithelium more distinctly. The Gregarine drawn on a large scale in fig. 12 was similarly attached to one of the more or less vascular strands crossing the body-cavity. It is viewed whole, in optical section, and the lumen of the stalk (in

communication with a blood-capillary) contains some of the peculiar amœbocytes (*am.*), laden with excretory pigmented granules. The bending inwards of the wall at *b*, and the proliferation of nuclei where it is applied to the Gregarine are also well shown. The cytoplasm of the part still uncovered has mostly shrunk away from the limiting-membrane; this is probably due to the fixing. Fig. 17, Pl. 2, shows a small portion of another invaginated parasite and its covering, highly magnified. The cytoplasm is dense and granular, and closely surrounded by the inner layer of epithelium. Here, also, the lumen of the stalk (*l*) contains many amœbocytes, which are very common in the blood-capillaries of both Cucumaria and Holothuria; the peripherally-situated nucleus (*n*) of each is deeply stained.

Finally, in Pl. 2, fig. 13, we have a Cystobia, which has thrust its anterior end, as a long finger-like process, into a rather narrow vascular cord; into this the animal appears to be trying to penetrate. This parasite was found in a spirit-fixed Cucumaria, and its outer, free end is rather shrunk and irregular, as also are the outlines of the nuclei. The section along C—D (fig. 14*a*) shows the Gregarine everywhere enclosed by the double epithelial layer. *X* is a portion of the process in the strand, which is also cut through, *e* being the outer and *e'* the inner invaginated wall. *Lum.* is the lumen, more or less filled up with spongy tissue, with here and there a few amœbocytes (*am.*). Fig. 14*b* is a section drawn along the line A—B, and passing through the tip of the protuberance (*p*). This is still enveloped by a cellular layer, the cells being thickly aggregated at one side.

The above is the only instance I obtained showing such a modification of the Gregarine's shape, and I doubt whether it frequently occurs. I am more inclined to think that the parasites, after a preliminary invagination of the cœlomic epithelium, are rather enclosed and overgrown by this and the underlying connective tissue of the immediate neighbourhood than that they themselves actually penetrate deeply

when about to encyst. They are probably able to induce proliferation of the surrounding tissue to a considerable extent. Nor do I think that they usually break through the epithelial layer which they first invaginate, for in sections of encysted stages this is always present next to the body of the animal, and completely enclosing it.

Situation in which the parasites encyst.—The remaining position in which I found the parasites bears out these suppositions. Encysted Cystobiæ often occurred firmly attached to—sometimes, indeed, almost in—the strong retractor muscles which work the buccal mass. Pl. 1, fig. 11, shows three so attached, *m.* being a piece of the retractor muscle. The two larger ones are fairly separate from the muscle, connected to it by the short thick stalk (*st.*), but the smaller one is more completely imbedded. Fig. 16 is a section through a portion of the muscle with a Gregarine firmly enclosed in the proliferated epithelium and connective tissue lying external to the muscle-fibres. *A* is the originally invaginated epithelium equivalent to that seen at *b*, figs. 9, 10, and 12. *Ep.* is the normal cœlomic epithelium, which at *ep'* is flattened out and in some places wanting, evidently having been unable to keep pace with the proliferation of connective tissue. *C.*, *c.* are concentric layers of connective-tissue around the parasite, and *m.*, *m.* are the muscle-fibres. Fig. 15 shows another one so encysted in section; it is more separate from the muscle, which in this case is cut transversely; nevertheless the parasite is firmly enough attached. A thick layer of connective tissue (*c.*) surrounds it, especially fibrous nearest to the inner epithelial cells, which are here a little disorganised. The nuclei (*n.*) of the outer epithelial layer can be seen in places, extending over the surface.

Conclusions.

It will, perhaps, be suggested, may not these Cystobiæ in the connective tissue surrounding the muscle have arrived in this situation by passing along a blood-capillary as sporo-

zoites, or very young trophozoites (starting originally from the vascular system of the gut), then grow in situ and so attain to the sporont phase? I think this is conclusively negatived by the following considerations:

(*a*) I have never seen a single C. minchinii in the vascular system proper (i. e. the vessels and network attached to the gut), or in any way connected with it.

(*b*) I have never seen small forms in the connective tissue around the muscle, and indeed,

(*c*) I have never found tiny forms anywhere but in the respiratory trees.

(*d*) The distinct epithelial layer next to the parasites when in this situation. No such cellular aggregation is ever found around them while in the trees.

(*e*) The frequently seen process of attempted invagination already described represents the commencing stage of encystment. No other explanation than the one above given, namely that the animals are endeavouring to push into, and are becoming enclosed by, the host's tissue, is possible.[1]

These facts lead one inevitably, I think, to the conclusion that the parasites enter the respiratory trees directly from the outside, and that many of them pass thence into the cœlome,[2] and as soon as possible become attached to the cœlomic epithelium, either of the body-wall or of a connective-tissue or muscle strand, where, after further growth, they finally become encysted.

I never observed any sporulating stages in the respiratory trees, and so cannot say whether the Gregarines sporulate in this position or not. I do not think those which have become large and spherical in this position pass into the cœlome.

[1] Prof. Minchin kindly looked at several of my preparations, which show the process most clearly, and he is equally of the opinion that the parasites are entering and not emerging.

[2] Presumably the young forms always pass through the wall of the tree into the body-cavity, since any internal (cœlomic) openings of the former are usually denied.

(4) Form, Size, and General Appearance.

(*a*) C. irregularis.

Observed living and "free," the parasites never showed the least tendency to displace themselves; neither did I notice anything comparable to euglenoid movements causing flexion or constriction of the body,[1] such as Brasil (**2**) figures in the case of Urospora lagidis. Changes in shape, e.g. the rounding off of the Gregarines, on encysting are probably more passive than active—that is to say, impressed, as it were, upon them by their relation to the surrounding tissue; and the same applies equally to C. minchinii. In short, the Gregarines appear to be perfectly motionless;[2] this peculiarity will be readily understood when the minute structure of the peripheral region of the body has been described.

The typical form of an adult trophozoite, really of a double-adult or "couple," is that of a beautifully symmetrical ovoid. Fig. 1, pl. 1, represents a medium-sized parasite, its length being ·5 mm. and its greatest width ·25 mm. Around the middle of the body is a slight, **V**-shaped constriction, causing a break in the contour of the side. This marks the plane of junction of the two members of the couple, which is further indicated by the distinct septum running transversely across the body.[3] The two halves are always, so far as can be seen, of equal size. In each is a perfectly spherical nucleus with a single large karyosome. Another rather larger trophozoite, obtained free of the vessel and scarcely so perfect in outline, is shown in fig. 2. Here the union is complete, there being no perceptible break in the contour, and the couple might be

[1] Irregularities in contour, such as Minchin (loc. cit.) mistook for the normal appearance, are undoubtedly the result of deformation.

[2] Brasil, in a quite recent paper (**3**), characterises Gonospora varia as "completely immobile." This cœlomic parasite is, indeed, probably closely related to C. irregularis (see under Systematic Position, p. 60).

[3] The reasons why this septum is considered to represent a plane of union, and not a separating partition or plane of division, will be discussed later (see below, p. 65).

taken for one septate Gregarine, with equal protomerite and deutomerite, were it not for the important fact of there being a nucleus in each half. The two clearer spheres denote the position of the nuclei as they showed up in life. In fig. 3 is drawn a much younger stage, where the two associates, though firmly attached to one another and not separable by gentle touching, are not yet so completely united.

On the other hand, this precocious association or neogamy[1] may be still more intimate in character. In many cases, especially in trophozoites which had early evaginated the wall of the vessel, but also sometimes in adults still in the lumen, there was no septum at all, and the two nuclei were often in contact. Figs. 5 *a* and *b* show couples of this nature. In the latter *n* is part of the second nucleus, just beginning to be cut through. Here complete fusion of parasites has taken place, with the result that one appears to be looking at a single binuclear Gregarine. Such unions evidently occur extremely early in the life-history (see below, p. 64). I may here say, indeed, that I have never succeeded in finding an isolated individual, either of this species or (still less) of C. minchinii. I have searched hundreds of sections in the endeavour, but up till now have not seen a single uninuclear form. Fig. 30, pl. 3, shows the smallest specimen of C. irregularis which I obtained, and this has a diameter of barely 20 μ. Yet it has two relatively large nuclei, in this case touching each other and with no sign whatever of any dividing septum between.

(*b*) C. minchinii.

The extreme degree to which neogamy attains in C. irregularis is, so far as I am aware, the only condition to be observed in C. minchinii. The smallest examples of this

[1] I propose to use the terms neogamy and neogamous in describing this phenomenon, since, besides implying the early occurrence of the process, they also indicate its essential meaning, to which I attach great importance (see below, p. 74, et seq., where this interesting question is fully discussed).

parasite which I have seen are drawn in figs. 31 and 32. The latter individual is only 18 μ by 12 μ, and from its elongated form (not yet having become rounded off) looks as though it had but recently passed from the lumen into the wall of the respiratory tree. Its general appearance is similar to that of the C. irregularis of fig. 30. The Gregarine in fig. 31 shows only one nucleus, but the other is only one or two sections distant. Other, larger, trophozoites in the same situation are seen in figs. 18 and 19, and in neither is there any trace of a septum. The parasite in the latter figure has a diameter of 0·15 mm. and the two nuclei are far apart from each other, but in the other trophozoite, which is rather smaller, they are again in contact. This nuclear contiguity [1] is purely accidental, and does not indicate either nuclear union or separation; in all cases the nuclear membrane is quite intact. As in C. irregularis, each nucleus has a single large karyosome. The body of the parasite, when in this situation, is always practically spherical, this being, probably, because it thus offers more or less uniform resistance at all points to the surrounding tissue-layers of the host.

The true "gregariniform" shape, however, is here also that of a regular ovoid. This is exhibited by all the individuals which are endeavouring to penetrate the cœlomic epithelium. Two of these are seen in figs. 9 and 10, the larger one being 0·34 mm. in length by 0·2 mm. in width. Besides the invariable absence of any septum, another very constant feature of this species, and one which distinguishes it from C. irregularis, is the position of the nuclei. In a typical gregariniform adult the nuclei are always placed transversely (instead of longitudinally) with respect to the long axis of the body, and generally about one third of the animal's length from the anterior end, designating thus the end which is farthest in.

The transverse position of the nuclei in the gregariniform adults of C. minchinii strongly suggests that the association

[1] The same thing also occurs not infrequently in C. holothuriæ, from H. tubulosa (see Minchin, loc. cit., fig. 21).

is lateral (i. e. that the members of a pair originally join side to side after the manner in Gonospora sparsa, Léger [19]), and not terminal (end to end) as in C. irregularis.[1] This arrangement is, of course, masked while the animals are in the trees.

Trophozoites finally encysted are seen in figs. 15 and 16[2]; the former of these represents a very large parasite ·56 mm. by ·43 mm. in diameter, which should rather be termed a sporont, as it was probably ready to begin sporulation. Both individuals show only one nucleus in the section drawn, the other being some sections further on.

(c) Diplocystis schneideri.

I have been able, fortunately, to re-examine this interesting parasite, originally described by Kunstler (**16**). Although including an account of it in this paper chiefly for the purpose of comparing neogamy in this form with the same occurrence in other species of the genus and also in Cystobia, I may here indicate why I thus identify the Gregarines which have come under my notice. The parasites occurred in a single

[1] It is interesting to note, in this connection, that Sars (**32**), so long ago as 1861, figured Gregarines associated in couples and, moreover, laterally, in the Holothurian, Chiridota pellucida. The parasites apparently adhere to the outside of the blood-vessels, which have here little pear- or flask-shaped diverticula. They appear only loosely attached to each other, with, of course, a distinct wall or partition between the two members, separating the transversely-placed nuclei. Judging from the magnification given, their length was about ·125 mm. and the breadth ·06 mm. It is impossible to say whether they were adults or not, as this is all Sars describes of the parasites. In "pairing" side-to-side, and in being apparently free in the body-cavity (they do not seem to be attached by any stalk to the vessel, i. e. to have evaginated its wall), they exhibit a certain resemblance to C. minchinii; the association would appear, however, to be much less intimate in character. It would be very interesting if these Gregarines could be re-discovered in Chiridota.

[2] The irregularity in outline at times shown, unfortunately, by these encysted stages (e. g. fig. 15) is due to the difficulty I experienced in fixing them, owing to the thick connective-tissue layer through which the fixative had to penetrate. Corrosive sublimate, well sharpened with acetic acid, served best.

specimen of the common cockroach, Periplaneta orientalis, and were all in a practically similar condition, viz. that of trophozoites of varying age and size.

The animals agree, on the whole, with Kunstler's description as regards size, shape, and general appearance. I found the length of a couple to vary from 1·2 mm. up to nearly 1·7 mm., while the greatest breadth varied from ·6 mm. to 1 mm. None of those measured were quite as large as the largest Kunstler found, which attained 2 mm. in length, but since the size of these cœlomic Gregarines varies considerably, not much stress need be laid on that point. Figs. 21 and 22 show couples of large and small size respectively, that in fig. 21 being seen whole, while fig. 22 represents a section which happens to pass through the nucleus of one member of the pair. An adult syzygy is distinctly bilobed; and this was the case with all the individuals examined. Moreover, a well-marked septum is invariably present.[1] The couple is always longer than broad, more or less dumbbell-shaped in fact, and never rounded or spherical as in D. minor (vide Cuénot, loc. cit.). Further, the much larger size to which they grow and the structure of the nucleus—which is, in general, quite like Kunstler's description and figures—also tend to remove my Gregarines from this species of Cuénot's. Each couple has a general or "common" membrane completely investing it (*i.m.*, fig. 21), which is quite unrepresented in the trophozoites of Cystobia. In this respect my parasites agree equally with D. minor and D. schneideri, and differ from D. major (see below, p. 62). The above facts prove without

[1] Unfortunately, many of the specimens showed the appearance drawn in fig. 23. Whether fixed by corrosive and acetic, Flemming, or absolute alcohol, most of the protoplasm (the endoplasm) was usually found more or less retracted, leaving only the ectoplasm and delicate cuticle in close connection with the investing membrane to show the true size and shape of the animal when alive. Under a low power, of course, these together appear extremely thin, compared with the relatively huge mass of endoplasm. The apparent thickness of the septum in this fig. is due to its being viewed slightly out of the plane in which it lies; I generally found this appearance in such shrunk specimens.

doubt, I think, that these Gregarines belong to Kunstler's species.

Triple association.—Not infrequently I came across instances where the trophozoites possessed three nuclei. In none of these cases was there any septum visible; they evidently belonged to the category of very early associations. Figs. 20, 29, and 28 show examples of such, the two first being C. irregularis and the latter one C. minchinii. The C. minchinii figured is in the gregariniform stage, as usual, partly enclosed by epithelium. The middle nucleus is not quite in the transverse plane of the other two; nevertheless the three associates have, manifestly, all joined side by side. In all cases the nuclei were of equal size and quite similar in structure; there was nothing to suggest that two were formed by one of the original ones dividing.

I have also found a couple of instances of triple association in Diplocystis Schneideri (figs. 24 and 25). In fig. 25 two members of the triplet are slightly smaller than the third, but in fig. 24 all three are about the same size.[1] Similarly in C. irregularis the three members are sometimes apparently equal (fig. 20) and sometimes not (fig. 29), but the inequality in the latter case is never pronounced. In C. minchinii, on the other hand, the members of all the triplets I observed were all quite equal. This equality is, indeed, only what one might expect, since it may be said that the individuals cannot live alone long enough to become appreciably unequal in size.

(5) Minute Structure.

(*a*) Nature of the peripheral region and composition of the septal plane.

The minute structure of the peripheral part of the body

[1] Kunstler (loc. cit., fig. 3) has drawn a triple association, and rather insists upon the inequality of the members constituting it, regarding the two smaller as resulting from the division of one large one. In my fig. 24, however, scarcely any inequality is noticeable.

in Cystobia is fairly simple, and practically similar in the case of both the species I examined. The body is always limited by a very delicate membrane or cuticle, in which I could never see any striations. When, as sometimes happened, the fixative employed has caused the cytoplasm to shrink away slightly from the membrane, the latter is easily seen (fig. 12, *l.m.*).

The parasites do not show any obvious differentiation of the cytoplasm into ecto- and endoplasm. I could never assure myself, by any method of fixation, of a well-marked peripheral layer constituting a definite ectoplasm. The somewhat clearer border round the Gregarine depicted in fig. 2 is simply due to the lessening thickness of the body approaching the edge (the specimen, it should be remembered, was drawn when living). Correlated with this absence of any ectoplasmic supporting layer to the body is the extreme delicacy of the latter, and the readiness with which I found it became irregular or distorted in shape if touched or placed in an uncongenial environment. In two instances, I should add, in sections of a couple of Gregarines which were fixed with Flemming and stained with iron-hæmatoxylin and orange, a more finely granular, compacter structure of the cytoplasm can be discerned near the margin (fig. 37 *b*) denoting a slight alteration in its character, but hardly amounting to a distinct layer. I only observed it in these two examples of C. irregularis, and never in C. minchinii. A strong contrast is afforded by the distinctness of the ectoplasm in Diplocystis schneideri, where it forms a thick, well-marked layer, finely granular in character (fig. 27).

A word or two with regard to the composition of the septal plane. The septum is constituted by an extremely thin and delicate membrane, which is, however, remarkably persistent. This runs straight to the limiting membrane at the periphery (figs. 37, 39), with which, indeed, it corresponds. There is no sign of anything in the nature of ectoplasm in the dividing partition, and, moreover, where the marginal cytoplasm shows the slight alteration just mentioned, this is not continued into

the plane of junction, which would be the case were it a true ectoplasm (cf. D. schneideri).

Loss of the power of movement.—Even less is there anything corresponding to a layer of myocyte fibrillæ to be noticed in Cystobia[1]; and the same appears to be equally true of Diplocystis. The loss of mobility which is exhibited by these cœlomic parasites is undoubtedly correlated with their more confined situation, since they tend to remain in close relation with their host's tissue, instead of early becoming "free" in the lumen of the gut, as is the case with most motile Gregarines (compare, e.g., C. minchinii and a Coccidian).

Consideration of "Diplocystis schneideri."—After careful examination, I have to differ in many points from Kunstler's interpretation of the structure of the peripheral region and the nature of the septum in this parasite.[2] In fig. 27 I have drawn on a large scale a small portion of the peripheral region of two syzygies, showing the commencement of the plane of junction in each. Passing from without inwards, there is first a general investing membrane (*i.m.*) secreted by the parasite. If this represented a serous sac, it would certainly show nuclei or other traces of cellular structure, of which, however, there is not the least sign. It

[1] The fact that this lack of muscle-fibrils is accompanied by a complete absence of mobility in these Gregarines is a strong confirmation of Crawley's theory (**9**), that, "in general, throughout the Sporozoa, the possession of muscle-fibres and the power of moving from place to place go hand in hand, while those forms which are not known to move lack muscular elements," which thus attributes actual progression to the myocyte fibrillæ rather than to the extrusion of gelatinous threads posteriorly.

[2] Kunstler (loc. cit.) regarded the common or investing membrane as consisting of two layers, the outer being derived from the host, the inner corresponding to a cuticle or limiting membrane. Internal to this came the ordinary ectoplasm, which alone (in his opinion) formed the partition between the two "halves." For Kunstler considered that each couple represented a single individual in process of division, the septum being a transverse continuation, through the endoplasm, of the peripheral ectoplasm. There can be little doubt that this interpretation was entirely erroneous.

is only necessary to compare my figs. 5, 29, etc., showing a genuine peritoneal investment enclosing C. irregularis, to see the difference. The peritoneal sac by which young Diplocystis-couples appear to be first attached to the gut-wall (see Kunstler's figs. 18 and 19) undoubtedly breaks and liberates the parasites into the cœlome.

The investing membrane.—The investing membrane is homogeneous and, for the most part, fairly thin but firm and tough. Around the greater part of the body it closely surrounds the next internal layer, which is a delicate limiting membrane (*l.m.*), quite comparable to that of Cystobia. About the plane of junction, however, where the regular contour is broken by the V-shaped groove, the investing membrane or ectocyst leaves the body proper, as is shown in the figures. Its middle portion just here is invariably thickened and usually of a triangular shape. Between the ectocyst and the limiting membrane there is a space (*sp.*) of varying size. This I regard as being due to the contraction of the investing membrane. In the fresh condition the thickened ectocyst probably fills up this space and is in contact with the limiting membrane, and this part is represented in the section by the triangular tongue (*t*), the shrinkage having been doubtless caused by fixation.

In other words, the investing membrane in D. schneideri is evidently secreted at all points where the limiting-membrane and ectoplasm remain free after association of the parasites. This thickening of the ectocyst in the plane of junction must considerably strengthen the union of the couple, just at the point where it is most required, and thus minimise the risk of dissociation. So there is a slight distinction between the formation of the ectocyst here and that of the membranes surrounding an ordinary syzygy when it becomes encysted. While, in the latter case, the ectocyst is only secreted by the posterior ends of the Gregarines and forms an approximately spherical cyst (owing to the rotation of the animals), here it is secreted at all points of the body equally, and is laid down in the form of the couple. It

probably undergoes slight alterations in shape as the associates increase in age and size, but I feel certain that in life it remains contiguous to the body throughout, at any rate, the trophic period. For, although the bilobed appearance may become very slight (cf. fig. 21), yet in section the V-shaped inturning of the limiting-membrane and ectoplasm is always noticeable to a greater or less extent, and there is always this thickening of the investing membrane.

The limiting membrane cannot be traced through the septum as a distinct layer, having apparently fused with the ectoplasm to form a single homogeneous partition; it certainly, however, has entered the septal plane (compare my figures with Kunstler's fig. 8). Where it bends inwards, and also for a short distance after the junction, the ectoplasm of each associate is frequently somewhat thick and loose in character; more internally, however, throughout the greater part of the septum, it is narrow and dense.

(*b*) General cytology.

The cytoplasm.—The general nature of the cytoplasm in Cystobia is well seen in figs. 12, 18, and 19, Pl. 2, the first example being viewed whole, but more or less in optical section, and the others being actual sections. It consists of innumerable numbers of paraglycogen spherules of various sizes imbedded in a semi-fluid matrix, often with minute, highly-refringent granules of a different nature in between. In sections, which were always drawn as they appeared under a Zeiss apochromatic lens, the appearance varies rather, due chiefly to the method of fixing and staining employed. After staining with iron-hæmatoxylin and orange, one usually gets a reticular appearance, the meshes being of varying size. This is shown, for instance, in figs. 37 *a* and 52, and is due to the fact that the stain has been extracted from the paraglycogen spherules much more readily than from the ground-substance. Hence the apparent spaces of the reticulum are really occupied by the unstained grains. In fig. 37 *b* the reticulum is

very close and fairly dense, and there are besides numbers of minute highly refractile granules (*r.g.*) which have stained deeply and give the cytoplasm a granular look. On the other hand, in sections stained with Kleinenberg's hæmatoxylin, followed or not by eosin, the spherules have retained the stain and stand out distinctly from the cytoplasmic matrix, which is in these cases only faintly stained (fig. 45, Pl. 5, and fig. 36, Pl. 4).

Sometimes, however, these paraglycogen grains are very small and not prominent (figs. 38 *a* and 38 *b*, Pl. 2), but there are besides numerous larger, irregular, more flattened granules (*a.g.*); these apparently correspond to the lenticular plates, which Cuénot (loc. cit.) figures, and which constitute albuminoid reserve material. They are abundant in fig. 38 *b*, which represents part of the cytoplasm of a sporulating Cystobia, fixed with corrosive sublimate and acetic, and stained with thionin and orange. The refringent granules (*r.g.*) are also very numerous, especially in fig. 38 *a*, a similarly stained section. These granules have a purple tinge owing to their retention of the thionin, the other constituents having only kept the orange.

In Diplocystis schneideri the paraglycogen spheres attain a relatively enormous size compared with those of Cystobia (vide fig. 27 *b*, which shows a portion of the cytoplasm of the former). Between and around the spheres is seen the cytoplasmic matrix. In my sections of this Gregarine I have not observed any irregular or lenticular grains of any kind.

The nucleus.—An adequate idea of the nuclei of a Cystobia is best obtained when they have been treated with iron-hæmatoxylin. No other stain demonstrates so well the fact that there is a definite chromatic reticulum, although Kleinenberg's hæmatoxylin succeeds to a certain extent. Safranin and thionin, however, while staining the karyosomes and also the nuclei of the surrounding tissue well, come out so quickly from the remaining parts of the Cystobia nuclei, that one might imagine there was nothing more in these save

diffuse nuclear sap. Neither did picro- and para-carmine, in staining entire adults, seem sufficiently powerful chromatic stains to reveal the whole structure. They served to give a general idea of the position, etc., of the nucleus in relation to the cytoplasm, and of the structure of the karyosome, and that was all. Now, the chromatin is by no means all confined to the latter. Whether the parasites have been fixed with sublimate and acetic or with Flemming, hæmatoxylin reveals a distinct, well-marked (linin) reticulum, impregnated with chromatin,[1] the latter occurring sometimes as local thickenings of the network, and at other times as numerous, distinct, but small granules and dots. This is shown in figs. 18 and 19, Pl. 2, also on a larger scale in figs. 35 and 36, Pl. 4.

The nuclear membrane is generally well marked, and, in perfectly fixed nuclei, of evenly-rounded contour. I have never seen, in any of my preparations, the least sign of a so-called "geflammte Kern." In one or two instances, e.g. fig. 36, the membrane appears irregular and shrunk, but this is entirely due to contraction on fixation. As a rule, it stains deeply, and probably itself contains chromatin; moreover, the reticular threads often start from it.

The karyosome (always single) is more or less vacuolated in structure, and, certainly in some cases, is slung in position by very distinct threads of the reticulum (figs. 19 and 35) which appeared to end in it. In other cases this is not so marked, and the karyosome seems more suspended in the network—as if, to use a borrowed expression, "it was a football lodged in the branches of a tree." In all the trophozoites, however small, which I have examined, the nucleus has invariably a karyosome of some sort. The size and number of the vacuoles it contains is very variable, and to a certain extent dependent upon the age of the nucleus;

[1] Also in the case of Lankesteria ascidiæ, Siedlecki (loc. cit.) has pointed out that the nucleus has a distinct chromatic reticulum, in which are suspended as well some comparatively large grains of chromatin (see his figs. 2 and 3).

in very young parasites there are none, and the karyosome appears homogeneous.

The nucleus of Diplocystis schneideri differs from that of D. minor (and also of Cystobia) in possessing, usually, several karyosomes (figs. 22 and 26) of various sizes and of the usual vacuolated structure. The nucleoplasm here, as in Cystobia, is in the form of a chromatic network, not, however, so deeply staining. Sometimes it does not appear to be of the same character throughout, a certain portion, in which all the karyosomes are imbedded, being denser, more granular, and with a stronger affinity for the chromatic stain (*z*, fig. 26).[1]

(*c*) Formation and probable function of the karyosome.

In the tiny specimens seen in figs. 30–32 the karyosome is evidently in process of formation. In all three cases it is in contact at one side with the nuclear membrane, and much paler in colour than the chromatin, which is here in the form of deeply-staining grains and lumps in the nucleoplasm. Hence it is most likely that at this time it consists only of the plastinoid portion (of extra-nuclear origin ?) forming a kind of basis, to which will be added later some of the chromatin fragments, the remainder contributing to the reticulum. In fig. 30 each karyosome is drawn out and elongated in shape, and projects inwards into the midst of the cluster of chromatin grains, many of which probably come into relation with it. As the karyosome increases in size, fluid (?) vacuoles are formed in it, whose contents stain slightly, and these are, as it were, imbedded in the less fluid, darkly stained, chromatic ground-substance. Different degrees in this vacuolisation are seen in the nuclei figured in fig. 33, Pl. 2, which were drawn whole from a preparation stained with picro-carmine.

[1] A somewhat similar differentiation of the nucleoplasm, in the form of a zone or halo around the karyosome, is described by Berndt (loc. cit.) in the nucleus of Gregarina cuneata.

Expulsion of karyosomatic material into the nucleoplasm.—Some of the smaller vacuoles probably run together and unite to form larger ones, for one often gets two or three large ones—sometimes one very huge one—and a number of little ones besides (fig. 33, *b* and *c*). Fig. 34 is a section of the nucleus seen in Fig. 33 *c* (the Gregarine containing it having been unmounted and cut[1]), and it shows an occurrence by no means infrequent. In the karyosome lies a huge vacuole, which is obviously just ready to have its contents expelled into the surrounding nucleoplasm, either by diffusion through or by the actual rupture of the denser, more deeply staining, portion, which is here very thin near the surface. (I should add that, in some instances, the edge or border of the karyosome stains deeper, and appears as a thin, dark line, perhaps constituting a definite wall.) I certainly consider there is an actual discharge, in some such manner, of the contents of these huge vacuoles, for in the karyosomes of ripe trophozoites about to commence sporulation the vacuolisation is much more uniform. Fig. 36 shows the nucleus of an encysted C. minchinii (a full-grown sporont attached to the muscle), and the vacuoles in the karyosome are all comparatively small and of about equal size.

A similar elimination of karyosomatic material, though by a rather different process, is described by Cuénot (loc. cit.) in the nucleus of Diplocystis. The karyosome there buds off little portions of itself, each containing a vacuole, into the surrounding nucleoplasm,[2] where they become eventually dissolved. In Cystobia, on the other hand, the karyosome

[1] The nucleus is somewhat flattened, owing to the original preparation having been compressed by the cover-slip, and the nuclear contents are all at one side, around the karyosome; the karyosome itself is, however, perfectly normal.

[2] The same process, in one form or another, is of frequent occurrence outside Gregarines. In a Coccidian of the cuttle-fish, Eucoccidium eberthi, Siedlecki (37) describes a budding of the karyosome prior to the formation of the gametes. As many as twenty secondary or daughter-karyosomes are thus set free, many of which at length dissolve in the nuclear-sap. An analogous budding of the "nucleoli" of eggs also often takes place.

never loses its spherical contour, and the material to be expelled is either diffused out at the point where the vacuole is nearest the surface, or else squeezed out, as it were, through a minute rupture in the thin wall.

Significance of the process.—Opinions differ as to the meaning of the process. Some authors, e. g. Cuénot, attribute to it an excretory function, holding that the vacuole contains waste material ("un produit de dêchet"); others, including Siedlecki, maintain that the karyosome, which they consider to be a storehouse of reserve chromatin, is giving back by this means some of its chromatin to the nucleus in readiness for division. Bearing in mind the essential difference between true nucleoli or plasmosomes on the one hand, and karyosomes, where chromatin is intimately bound up with the ground-substance, on the other hand, it seems to me that the latter view has much in its favour.

I do not mean to imply, of course, that there is never anything in the nature of elimination from the karyosome. Speaking generally, it may be said that, where some of the contents of the karyosome are passed out into the cytoplasm and there become altered, and either expelled or re-absorbed (probably in certain cases being of use to the formative cytoplasm), we have to deal with such a removal of unrequired chromatic material. Instances of this process are seen in Monocystis and Diplocystis, described and figured by Cuénot (loc. cit.), in Lankesteria ascidiæ, according to Siedlecki (loc. cit.), and again, in the case of many eggs, where the expelled grains or spherules can be traced right to the periphery of the cytoplasm.

On the other hand, where a portion or all of the karyosomatic material becomes ultimately incorporated with the rest of the nuclear material, whether by direct dissolution or by fragmentation, it is much more probable that we have to do with a reinforcement of the chromatin of the nucleoplasm. This is almost certainly the case when the dissolution is followed by an increase in the general chromaticity of the

nucleus, as Siedlecki found in Eucoccidium,[1] and as also occurs in Cystobia. In the nucleus of the ripe sporont shown in fig. 36, for example, notwithstanding its large size, the chromatic reticulum is, if anything, denser and more marked than in the case of younger nuclei, which still have large vacuoles in their karyosomes (cf. figs. 19, 34, and 35).

In conclusion, I regard the contents of the vacuoles in the karyosome of Cystobia as also containing chromatin, but in a more liquid or "storage" form, with, at present, no affinity for chromatic stains. Only the chromatin united with the plastinoid basis stains up, and the liquid spherules are to be regarded as being imbedded in this matrix or ground-substance. There is not the least evidence in favour of the excretory nature of this vacuolar expulsion in Cystobia; in none of my sections of adult trophozoites with uniformly vacuolated karyosomes is there any sign of chromatoid grains or spherules, either in the nucleus or in the cytoplasm. Moreover, the subsequent history of the karyosome supports the view I have taken here.

(6) Commencement of Sporulation.

(*a*) Encystment.

Encystment in Cystobia is much simpler than in most Gregarines. In fact, it is often difficult to speak of any real encystment at all.

C. irregularis.—It is no unusual thing for sporulation in C. irregularis to begin before the animal has evaginated the wall of the blood-vessel, and while it still has the adult ovoid form. The Gregarine drawn in Minchin's fig. 2 (loc. cit.), which remains, unfortunately, the earliest stage with more than two nuclei that I have seen, was still in the blood-

[1] Cuénot, again, describes a sudden appearance of intensely staining chromatic grains ("chromosomes") in the nucleus of Monocystis, after a dissolution of some of the karyosomes had taken place in the nucleoplasm. Although this author does not appear to have seen anything significant therein, I should say the two facts stand in close relation to each other.

vessel and had eight nuclei. I have also obtained several examples from this situation which possessed numerous nuclei (figs. 39 and 40); in the latter case nuclear multiplication was already far advanced. In all these instances the partition separating the two associates is persistent, and the animals had not, so far, made the slightest attempt to encyst.

Taking the majority of cases, where the Gregarines have become evaginated and spherical, they are surrounded, of course, by the peritoneal epithelium, which shuts them off from the cœlome and serves, indeed, as the outer wall of the cyst. The parasites are bounded internally by a fairly thin membrane, corresponding to the originally limiting membrane, which can now be spoken of as an endocyst (*en.*, figs. 41, 44). I doubt whether any other membrane, equivalent to an ectocyst such as we find in D. schneideri, is secreted as a rule in C. irregularis; at all events, I have only rarely seen anything resembling one. A thick, protective cyst-wall is not necessary, the evaginated wall of the blood-vessel serving equally well for the purpose of enclosing the developing sporoblasts. In rare instances, however, when the parasites are in the swollen evaginations described below, and not closely surrounded by the peritoneal epithelium, the cyst-membrane does appear to consist of two parts, there being a pale, homogeneous layer outside the endocyst, which perhaps corresponds to an ectocyst (*ect.*, fig. 44*a* and *b*.)[1] It is not nearly so well marked, however, as in C. minchinii, and in typical cysts attached to the blood-vessels there is no sign of such a layer.

In sections of sporulating cysts which only contain, as yet, numbers of nuclei scattered throughout the cytoplasm (fig. 41), there is, between the endocyst and the cœlomic epithelium, a

[1] Gregarines in which the cytoplasm is becoming segregated (i. e. the outlines of the sporoblasts becoming visible) are rather liable to shrinkage, especially when in this unusual position in the membrane anteriorly. In these cases the cyst-membrane is often folded on itself in places (fig. 44*b*), the folds (*f*) appearing somewhat like strings of attachment under a low power; the membrane has been unable to shrink equally with the cytoplasm as the latter became retracted.

rather fibrous layer, staining with the plasma stain (*f. l.*, figs. 41, 44 *c*). This is not in any way comparable to an ectocyst, but is, on the contrary, a layer of the spongy tissue of the blood-vessel, somewhat altered in character, being drawn out and tending to disappear. Later on, when the cyst is full of sporoblasts and spores, it is no longer recognizable. By this time the enclosing wall is very thin; it consists only of the peritoneal covering outside, and, next internally, the endocyst, which has now become extremely delicate and difficult to make out where it is applied to the epithelial layer. Sometimes, however, the endocyst has shrunk slightly away from the latter, and it is then seen more readily (fig. 44 *d*); at other times, again, especially in ripe cysts, it has quite broken down and vanished. Indeed, in many of my sections through spore-containing cysts, however carefully cut, the delicate peritoneal wall itself is ruptured in places.

The cyst shown in fig. 42 was in an evaginated blood-capillary in the membrane already mentioned (p. 12) as running from the ring-canals to the body-wall, and conveying the radial vessels. It appears to have very thick walls, but this is due to the fact that these evaginations are swollen and expanded and partly filled with vascular fluid (*fl.*) more or less coagulated by fixation. There is no question whatever of this being a thick, gelatinous ectocyst, for scattered here and there are seen amœbocytes and blood-corpuscles (*x.*, *x.*). The wall itself (*w.*) is very faintly stained, and consists of the usual loose tissue, here excessively spongy and having an ill-defined limit internally; indeed, it is difficult to say in places where the wall ends and the cavity begins. The Gregarines in this position are much freer than those in the ordinary evaginated cysts, which are closely invested by the fibrous layer above described; and this fact probably accounts for the development of an ectocyst, albeit only slight, in such cases.

C. minchinii.—In encysted adults of C. minchinii attached to the muscles there is, as yet, no sign of any cyst-wall apart from the delicate limiting membrane. This is usually difficult to make out owing to the thick nuclear

aggregation (*a.*, fig. 16) surrounding the parasite; it is seen, however, in fig. 38 *a* (*l. m.*) between the cytoplasm and the layer of nuclei. On the other hand, when the cyst is sporulating, I find a much more distinct ectocyst than ever occurs in C. irregularis. External to the endocyst, which corresponds, in this case also, to the original limiting membrane, is a pale but firm and homogeneous-looking layer (*ect.*, fig. 44 *e*); this represents a true outer cyst-membrane. Outside, again, is the nuclear layer, which has now become rather broken down.

The spore-containing cyst, which is viewed whole in fig. 43, is attached to one of the vascular strands mentioned above (p. 14); it is surrounded by an enormous number of cells which have migrated to the locality. *Ep.* is the cœlomic epithelium attaching it to the strand (*st.*), and *c.a.* is the cellular aggregation around the parasite, the nuclei being closely packed just outside the cyst-wall (*c.w.*). Whether these are phagocytic cells or not, they do not appear to have done any injury to the Gregarine, as the contents of the cyst are quite normal.

(*b*) Fragmentation of the karyosome.

Owing to the difficulty of obtaining material at the time of the year when sporulation generally begins and the relative scarcity of ripe trophozoites (sporonts) in the material I examined, I have, unfortunately, considerably fewer stages showing the nuclear changes at the commencement of multiplication than I should have liked.[1] Still, thanks to a few fortunate preparations, which I have not the least reason to consider as otherwise than perfectly normal, and which fit in quite well with each other, I have been able to obtain a fairly

[1] Many recent writers have commented upon their inability to obtain the earliest phases in nuclear division (compare Brasil [**3**], Cuénot [**10**], Léger [**22**], and Paehler [**29**]); it will be readily understood, however, that the difficulties are enhanced in working with marine hosts, and especially where it is impossible to follow the process in vivo.

connected idea of the earlier processes in nuclear division in C. irregularis; as to those in C. minchinii, however, I am without any information.

In fig. 45 *a* is seen the earliest stage I obtained in this nuclear preparation for sporulation, and one which is most important. Only one nucleus is shown in this section, the other being further on; a section of it is drawn separately at *b*. Both nuclei are in exactly the same condition. Their outline is slightly retracted and irregular, attributable to the fact that the animal was fixed in a piece of the vascular network, being still inside the lumen of the vessel.[1] There is no septum visible in this specimen, and it is evidently an instance of precocious and complete union.

The important point to notice is that the large, uniformly vacuolated karyosome, as we saw it, for example, in fig. 36, is no longer present as such. It is represented instead by the numerous small fragments of slightly varying size seen at *f.*; they are fairly well stained, more deeply so at the periphery, and stand out distinctly from the rest of the nucleus. The karyosome has undoubtedly broken up or separated into these little, more or less spherical, pieces, each of which probably corresponds to one of the spherules of the original karyosome.[2] The nucleoplasm itself is distinctly chromatic, but not so obviously reticular, being of a more granular nature, with the granules of practically uniform size. There is not the slightest appearance of any expulsion of karyosomatic material into the surrounding cytoplasm, and the nuclear membrane is perfectly entire.

Further stages in the history of these karyosomatic fragments are shown by the nuclei drawn in fig. 46. These are daughter-nuclei of the fourth generation, all belonging to the

[1] The relatively large size of the nucleus, as compared with the apparent length of the body, is due to the fact that the sections passed obliquely through the Gregarine.

[2] Compare Gregarina blattarum, whose karyosome forms a chain ("chapelet") of numerous tiny ones by successive buddings or divisions (see Cuénot loc. cit., fig. 33).

same Gregarine. Different degrees in the process of incorporation are exhibited by different nuclei. I have arranged them in a series. Each of the daughter-nuclei seen at *a* possesses one little karyosome, corresponding, in all probability, with one of the spherules of the original karyosome, which became divided up in the parent-nucleus as seen in fig. 45. With successive divisions of the nucleus, these daughter-karyosomes have been passed on and, as it were, apportioned out among the daughter-nuclei, till by this time many of the latter have only one, or the fragments resulting from one. The periphery of each stains deeply, and the interior part also stains rather more than hitherto (compare, for example, fig. 45), as if the chromatin were becoming precipitated or re-converted into the customary staining-form.

Nuclear incorporation of the fragments.—These little karyosomes next divide up and gradually become indistinguishable from ordinary chromatic grains. In fig. 48 *b* the first stage in this division is shown; each of the daughter karyosomes has divided into two of about half the size. Further division takes place more or less irregularly, as in *c*, followed by *d* and then *e*. Sometimes the fragments may remain together after division and form a ring or chain of grains or rodlets, as in *f* and *g* (compare again the nucleus of G. blattarum). As the process goes on the grains tend to stain up more homogeneously with the chromatic stain. From either *e* or *g*—in both of which the nucleoplasm, as a whole, is becoming chromatically denser—it is but a slight step, on the one hand, to fig. 47, or on the other to fig. 46. In the former are drawn two nuclei from different multinuclear sporonts in about the same phase, *a* being taken from one still in the vessel and *b* from one that was evaginated.[1] Some of the chromatic grains, particularly in *a*, are large and prominent.

[1] It will be noticed that, although both *a* and *b* are nuclei of the fourth generation, *a* is distinctly the larger; there is, of course, a certain variation in the size of different sporulating individuals, which is manifest also in their nuclei, karyosomes, etc.

There can be little doubt that the above series represents what has been gone through in the nuclei shown in fig. 46. These are two nuclei of the eight-nuclear stage to which reference has already been made; that is to say, they are nuclei of the second generation only of daughter-nuclei. They are very similar in constitution to those of fig. 47. The chromatin grains on the reticulum are not all of equal size; some are larger than others, and many of these are probably directly derived from the fragments of the karyosome. The process in this case, therefore, has evidently been more rapid and has taken place earlier, the fragmentation of the daughter karyosomes perhaps commencing before the parent-nucleus has divided. Hence there is a certain variability in the time elapsing between the first break-up of the karyosome and its final incorporation with the rest of the nuclear material as a part of the chromatic reticulum.[1]

(7) Nuclear Multiplication.

(*a*) Nature of the early nuclear divisons.

With regard to the manner in which the first nuclear divisions take place, I have not the least doubt that they are purely direct or amitotic. Unfortunately, I have no preparations showing the earliest ones, but in a section through a sporont containing about thirty daughter-nuclei of the fourth generation there is still one of the nuclei of the third generation dividing directly into the two of the fourth, in a manner that is perfectly unmistakable. It is shown in fig. 47 *c*, and as to its absolutely amitotic character there can be no question; the nucleus has become constricted in the middle and the two halves are now being cut off from each other, half the chromatic grains going to one portion and half to the other. There is not the slightest sign of any attraction-

[1] A similar fragmentation of the karyosome with subsequent dissolution in the nucleoplasm, followed by increased chromaticity of the latter, is also described by Berndt (loc. cit.) for Gregarina cuneata, and by Caullery and Mesnil (5) for Selenidium sp.

spheres or nuclear spindle, and the former are equally absent from the resting nuclei in other parts of the preparation. Neither is there any indication of karyokinetic apparatus in the eight-nuclear stage, and as division-centres are very easy to see later on, it is not likely that I have overlooked them here. Hence, I maintain there is a very great probability that the preceding divisions are amitotic also. Another strong point in favour of this view is the fact that, when asters and nuclear spindle appear at first, and the division of the "segmentation"-nucleus [1] is mitotic, that of the resulting daughter-nuclei is so too, and the division-centres in connection with them are very apparent (Monocystis, Diplocystis, Lankesteria, see Cuénot, Siedlecki, Prowazek [loc. cit.]). I think this fact and the obviously direct division of a daughter-nucleus of the third generation (fig. 47 *c*.) are sufficient to warrant my saying that the first nuclear divisions in C. irregularis are completely amitotic.[2]

We may now take a bird's-eye view of one or two sporulating Gregarines at about this period, in order not to forget the whole in considering a part. Figs. 39 and 40 show two such couples, both from the blood-vessel, and neither in the least indicating, by cyst-like shape or the possession of cyst-membranes, any appearance of encystment. In both Gregarines the persistent septum entirely separates the nuclei derived from the parent-nucleus of each associate. Fig. 39 is a section and so only few nuclei are seen; altogether there are about thirty. Fig. 40 is drawn whole from a stained preparation, and in this the multinuclear condition is well

[1] That is, the functional portion of the original sporont-nucleus remaining after the unnecessary constituents have been expelled.

[2] It would seem, moreover, as if this fact stood in some relation with the retention of the karyosomes in the nucleus. For, in G. cuneata and in Selenidium, the two instances above referred to as resembling Cystobia in the latter respect, the first nuclear divisions are equally amitotic, although differing somewhat from those in C. irregularis in being of the "multiple" type instead of by simple binary fission.

advanced; the septum is also still present. In figs. 41 and 42, on the other hand, more of an encystment is recognisable, the exact character of which has been described above. The former is a section showing many nuclei, and no septum is visible.[1] Already it can be seen that the nuclei are of two kinds, some (*N*) being much larger than the others (*n*). In fig. 42 this difference in size has become accentuated.

(*b*) Distinction of the multiplying nuclei into two classes, somatic and germinal, and their further history.

In fig. 49 are drawn some of the nuclei from sections of another Gregarine in a condition very similar to that in fig. 41. They show the earliest stages in this differentiation of the nuclei into two kinds. A considerable change has occurred in the nuclear constitution since we last saw it in fig. 47. Some of the nuclei (the larger ones) have divided less often than others, and are of an irregularly ovoid to rounded shape; the smaller ones are more uniformly ovoid. In the large nuclei there is, in place of a dense, finely meshed, chromatic network, either a very loose reticulum (*a*) or none at all; in the smaller, the chromatin is situated peripherally, and is in the form of fairly even-sized rodlets or grains in contact with the nuclear membrane. In all cases the rest of the nucleus is filled with a non-staining nuclear sap. The chromatin in the larger nuclei is in the form of irregular, deeply-staining masses or lumps, with sometimes fine granules or chromatic dust besides, unevenly distributed throughout the extensive nuclear sap.

These two sorts of nuclei represent the division of the nuclear material into a somatic portion, which eventually breaks down and is absorbed by the cytoplasm, and a functional or germinal portion which alone will form the nuclei

[1] It may either have disappeared or never have been formed; in sections through another cyst at a similar stage there are traces of one, not so distinct, however, as that in fig. 42.

of the gametes (sporoblasts).[1] Whether there has been a qualitative as well as a quantitative separation of nuclear material, and how the selection has been brought about in the absence of karyokinesis, I am quite unable to say.

The subsequent course of events in the two kinds is very different. Fig. 49 *a* represents the least modified condition of one of the somatic nuclei; it differs chiefly from a germinal one in having its chromatin aggregated in larger masses and disposed more in the interior. At *b*, *c*, and *d*, and in the large nuclei in fig. 50, are seen further stages in the alteration. The sterile nuclei do not at present divide any more, but grow considerably, chiefly by an increase of nuclear sap; at the same time, there is an increase of the chromatoid granular matter (cf. figs. 49 *d* and 50 *b*).

Turning now to the smaller or germinal nuclei, I have no doubt that division in these is henceforth indirect or mitotic. Unfortunately, I could not see any divisions actually occurring in these two preparations, as all the nuclei were in a resting condition. Nevertheless, karyokinetic apparatus is already quite distinct in connection with many of them, and at a slightly later stage mitotic nuclear division is unmistakably in evidence. At one or both ends of certain of the smaller nuclei, and quite at the periphery,[2] is seen a little thickening which gradually assumes the form of a rounded grain. It stains deeply with iron-hæmatoxylin, and is probably of chromatic nature. From each of these bodies a few delicate rays diverge into the surrounding cytoplasm. The whole structure is better developed later on, the central grain especially becoming large and prominent (fig. 51 *a* and *b*).

Nature and origin of the division centres.—I have felt somewhat uncertain whether to regard these large con-

[1] It is interesting to note that Léger (**22**) has also described an early distinction of the multiplying nuclei in Stylorhynchus into sexual ones, ultimately forming the nuclei of the gametes, and somatic ones, which are left behind in the "somas" or cystal residue.

[2] It is difficult to say whether the thickening is at first inside or outside the membrane.

spicuous grains as centrospheres [1] or centrosomes. Notwithstanding the size to which they attain, I am now [2] inclined to consider them as more comparable to true centrosomes than to archoplasmic structures. They are entirely homogeneous in appearance, and even when by chance less intensely stained than usual, in no case can any distinct centriole or granule be perceived inside them; moreover, they have a sharply defined outline or limit. They are very different from the centrospheres in Monocystis, as figured by Cuénot and Prowazek (loc. cit.); nor do they appear to have anything in common with the acidophile structures (probably of archoplasmic nature, corresponding to attraction-spheres)[3] which Brasil (**3**) describes in connection with the nuclei of Urospora.

On the other hand, the centrosomes in my fig. 51 recall the prominent grains at the heart of the division-centres in Diplocystis (Cuénot, loc. cit., fig. 55). Again, in their deeply-staining property and practically homogeneous appearance they agree with the centrosomes figured by Léger (loc. cit.) in nuclear mitoses of Stylorhynchus, and also with the "centrioles" of Urospora (Brasil, loc. cit.), the chief point of difference being that in both these cases the size of the granules is less.[4]

With regard to the origin of the centrosomes in C. irregularis, they certainly arise in intimate connection with the germinal nuclei, and probably with these only. Each is formed just at the periphery of the nucleus, and is itself most likely of nuclear (chromatic) origin. Probably the centro-

[1] I follow Wilson (**39**) in considering as centrosphere the inner, central region of the aster (consisting of the so-called "medullary" and "cortical" zones), restricting the term "centrosome" to the grain or granule, situate, when present, actually at the centre of the astral system (Boveri's "centriole").

[2] In my preliminary note (**40**) I termed them "centrospheres."

[3] The term "attraction-sphere" may be used in a general sense, as including the whole division-centre.

[4] Hertwig (**13 a**), however, has described relatively large centrosomes in connection with certain nuclei in Actinosphærium (vide his figs. 2 and 3, pl. 5); these show a general resemblance to those of Cystobia.

some, when differentiated, causes the appearance, in the cytoplasm of the immediate neighbourhood, of the astral fibres which become united with it to form the complete karyokinetic apparatus.[1]

Indirect nuclear division.—In fig. 51 are drawn many nuclei of both kinds, taken from a section similar to that seen in fig. 42; the differences between them are now considerable. A typical sexual nucleus in the resting condition is seen at *a*; at *b* is another, probably resulting from a recent division, since it has only one attraction-sphere. Dividing stages are, here again, very rare, this being due, undoubtedly, to the relatively slow rate at which the life-history normally progresses in Cystobia. The commencement of division is shown in *c*, where the chromatin has left the periphery and occupies a central position, taking the form of grains or little masses; some of these perhaps become dissolved in the nuclear sap, for at *d* there are only two or three visible. The next stage is seen at *e*, where the chromatin is stretched out in a thick line or band of grains between the centrosomes, preparatory to breaking up and becoming divided between the two daughter-nuclei. One of the two halves resulting from division is seen at f^1. The chromatin is in the form of three or four deeply-staining grains in close association with the division-centre. The nuclear membrane next reappears, the chromatin becomes more spread out, and as the nuclear sap increases in amount, the usual vesicular appearance is regained (f^2). The daughter-nucleus is now completely reconstituted.

Mitosis itself is therefore of a comparatively simple nature in C. irregularis, but whether this condition is primitive or secondary I am unable to say. The process most resembles that described by Cuénot as occurring in the nuclei of Diplocystis. I have seen no signs of a well-developed nuclear spindle, on which are arranged beautiful V-shaped chromo-

[1] Compare Hertwig's suggestive ideas (**13**) respecting the constitution and mutual relationships of the different constituents of living protoplasm.

somes, as described in the case of Monocystis, and also by Léger for Stylorhynchus and Brasil for Urospora.

Independent division of the centrosomes.—A very interesting peculiarity of the division centres in C. irregularis must be mentioned—namely their occurrence alone and quite separate from the nuclei. For it is certain that isolated centrosomes do at this period occur, and, indeed, in many of my sections they are not at all uncommon. One of them is figured at *g*, and it will be observed that it is quite similar to those in relation with the sexual nuclei. Equally certain is it that these isolated centrosomes also divide by themselves; *h*, *i*, and *j* show this distinctly. In all cases, whether of single or dividing ones, I have carefully examined neighbouring sections of the series, but without finding any trace of a nucleus in connection with them. This independence of certain division-centres is an unusual circumstance, and, so far as I am aware, has not hitherto been described in the case of Gregarines.[1]

It may, perhaps, be suggested, Does not the appearance seen at *j* represent, rather, the final stage in nuclear division, the two halves remaining attached only by a delicate, drawn-out thread? If this were so, it would imply that practically all the chromatin of the two daughter-nuclei had become (temporarily) united with the two centrosomes. This is exactly what Cuénot describes and figures (loc. cit., fig. 55 *d*) in the mitoses of Diplocystis minor; and, as a result, the grain at the centre of each attraction-sphere (consisting of centrosome plus the chromatin of a daughter-nucleus) is very greatly enlarged as compared with the normal size of a centrosome when associated with a resting-nucleus (fig. 55 *a*—*c*). The centrosomes in my fig. 51*j*, on the contrary, exhibit no perceptible differences, whether of size or staining properties, from those above described in close connection with the sexual nuclei. Moreover, the aspect of a daughter-nucleus immediately after separation (fig. 51*f'*, already described), is quite different.

[1] It is not an uncommon feature in spermatogenesis, however, for well-differentiated kinetic centres to divide by themselves.

And, lastly, *h* and *i* represent, indubitably, earlier stages in this centrosomic division.

Disintegration of the sterile nuclei. — Turning again to the large or sterile nuclei, most of these have increased greatly in size (all the nuclei are drawn to the same magnification) and have now very large and apparent centrosomes in connection with them. Of these, there are generally three or four in relation with each nucleus, but they are not always equally closely attached. In *k*, for instance, while one is touching the nuclear membrane and appears to have pulled it out more or less, another at the left side is not actually in contact. Many of these centrosomes are about double the size of the solitary ones, as if they had not divided for some time. I am inclined to think that the attraction-spheres in connection with these large somatic nuclei do not originate from the latter, in the manner in which the others are formed at the periphery of the sexual nuclei, for I have never seen any, as it were, commencing to be developed with a small centrosomic portion (compare figs. 49 and 50). It is more likely that they correspond to some of the loose division-centres in the cytoplasm which have come into relation secondarily with the large nuclei, in order to bring about their break-up and ultimate dissemination.

For there is another variety of nuclear figure to be met with, different aspects of which are seen in fig. 52; these represent stages in the further alteration of the sterile nuclei, leading to their complete resolution into chromatoid grains and granules, which eventually become scattered throughout the cytoplasm. The granular chromatin or chromatoid matter becomes more abundant and dispersed through the nuclear sap, which is thus divided up into a number of vacuole-like meshes often containing one or more chromatic lumps. These vacuolar meshes (which somewhat resemble a number of vesicular nuclei closely aggregated) are at first variable or large in size (*a* and part of *b*), but become subdivided into smaller and more uniform ones (*c*), until they are gradually obliterated, leaving only the granular chromatic material, as in the other

part of *b*; from this to *d* is a slight transition. In all these cases there is no longer any sign of kinetic apparatus and the nuclear membrane has also quite vanished. A portion of the cytoplasm around each has been drawn as well, and, while in *b* and *c* the delimitation of the nuclear material is still fairly well marked, in *d* there is no distinct boundary between the two constituents, the granules at the periphery spreading out into the cytoplasm.[1]

This separation of the nuclei into two kinds represents the only nuclear purification, or elimination of unnecessary chromatic material, which occurs in Cystobia irregularis. The germinal nuclei, after one or two more divisions, become the nuclei of the primary sporoblasts, the formation of which we have now to consider.

(8) Formation of Gametes (Sporoblasts) and Conjugation.

Fig. 56 is a section through a cyst of C. irregularis in which the sporoblast nuclei (*n*) are all formed. The nuclei have now a certain definite arrangement, being situated at or near the periphery of irregularly shaped lobes or portions of the cytoplasm, which, indeed, they mark out and delimit. In other words, we find in Cystobia the same intertwining and interlocking of the two cytoplasms (one belonging to each half or member of the "couple") that Siedlecki describes for Lankesteria, and Cuénot for Monocystis. In Cystobia, however, especially in C. minchinii, the process is carried to a much greater extent, which is easily to be understood when the far more intimate nature of association in this genus is remembered.

Seen in section,[2] this mutual interlobing is here of such a

[1] The general cytoplasm up till now has been entirely free from chromatoid or nuclear matter, not staining with iron hæmatoxylin; in later stages, however, this is no longer the case, chromatoid granules being very much in evidence and persisting more or less conspicuously throughout sporoblast- and spore-formation.

[2] Unfortunately, nothing whatever is visible in an advanced cyst stained and mounted whole until the definitive sporoblasts (zygotes) or spores are formed.

complicated character that it is quite impossible to say to which half any given lobe or portion belongs. As a rule a very narrow space separates any two adjoining segments, but sometimes this is not easy to make out; in these cases the nuclei help to define their limits. The segments are very well marked out by this means in fig. 55, which is a section through such a stage in C. minchinii (one of the very few sporulating cysts of this species that I have come across). Here the nuclei are unusually numerous and close together, and nearly everywhere in paired rows, each row belonging to a different lobe and, in all probability, to a different associate. In fig. 44 *e* a small region at the periphery of the cyst is seen highly magnified, and shows a portion of two lobes with the narrow space (*sp.*) between. All along the margin of each segment are arranged the sexual (sporoblast-) nuclei.[1]

Segregation of the sporoblasts.—The later development of the sporoblasts follows a slightly different course from that generally described in the case of other Gregarines. When the lobes and processes are fully developed and completely intermingled the sporoblast-nuclei throughout the cyst take up a position of equal distribution, each being surrounded by about the same area of cytoplasm. In a low-power view (fig. 53 *a*) they appear, in any section, evenly arranged throughout the mass and no longer define the limits of the segments, the outlines of which have ceased to be distinct. A portion of the cytoplasm next becomes segregated around each nucleus, simply by the appearance of narrow, irregular spaces which sufficiently divide up the whole. Practically the entire cytoplasm is thus used up[2]; in other words, there is no large cystal residue or "gregarinoid soma" left over in the cyst. I can say this with absolute

[1] Neither this section nor that of C. irregularis in fig. 56, both of which were stained with thionin and orange, shows the chromatoid granules in the cytoplasm, as thionin is soon extracted from them; for this reason, in fact, I occasionally used it, since iron-hæmatoxylin stains them so intensely that the nuclei themselves tend to be obscured.

[2] See below, p. 54, for instances of what are, probably, abnormal exceptions.

certainty in the case of C. irregularis. I have examined serial sections through many cysts, but whether these contain sporoblasts just about to be liberated, gametes copulating, zygotes, or spores, they are always uniformly filled with the same (compare fig. 54). I am strongly of the opinion that C. minchinii agrees equally in this respect, judging from sections like that shown in fig. 55 and also from sections through a spore-containing cyst, in which the spores are closely packed and completely fill the cyst, there being no sign of any unused cytoplasm. I lay emphasis upon this point because of its bearing upon the actual manner in which copulation is brought about (see below, p. 52).

Fig. 53 *b* shows a small portion of a section[1] similar to that seen in fig. 53 *a*, in which this separation of the sporoblasts is taking place. At this moment the cytoplasm has a very loose, irregular, and ill-defined appearance, and it is difficult to represent exactly; in some places the splitting up is rather more pronounced than in others.[2] Besides the fact that the process of segregation is going on practically uniformly throughout the entire cyst, there is another, most important, point to be noticed. All the nuclei are identical in size and appearance. Brasil (loc. cit.) states that, in both Urospora and Gonospora, the nuclei belonging to the two halves of the cyst—to the two associates, that is—are very different in character and easily distinguishable. That is certainly not the case here. The nuclei in the rest of the section from which fig. 53 *b* is drawn, and equally in other sections of the series, appear exactly like those shown.

The gametes.—As soon as the segregated uninuclear portions are thus cut out and separated from one another they become rounded off, and each forms a primary sporoblast. The primary sporoblasts or gametes in C. irregularis are, so far as I can ascertain, quite equal and

[1] This section was stained with iron-hæmatoxylin, and the chromatoid granules are very obvious.

[2] The "cutting-out" process is, perhaps, slightly more advanced near the periphery than elsewhere.

similar; in other words, they are completely isogamous. The nuclei certainly do not exhibit the distinctions in chromatic density, intensity of staining, etc., which Brasil (**4**) finds in the case of Monocystis, and which, as he points out, are, indeed, apparent both in Cuénot's figs. 22 and 23 and in Prowazek's figs. 11 and 13 (loc. cit.). On the other hand, judging from the figures of Siedlecki, Cuénot, and Berndt (loc. cit.) respectively, the gametes in Lankesteria, Diplocystis, and Gregarina approximate very closely to those of Cystobia in their apparent similarity one with another.

This perfect agreement (morphologically speaking) between the sporoblasts is best seen in cysts which have been crushed whole on a slide after staining. In sections through a cyst at this stage it frequently happens that either a sporoblast or its nucleus is not bisected, when a casual glance would give the idea of differences in size and nuclear constitution; such an interpretation would be quite false. Primary sporoblasts are shown in fig. 57 *a*, *b*, and *c*. They are very simple in structure. Each has a definite and fairly constant shape, slightly ovoid to round. The nucleus is spherical and usually situated excentrically. There is no sign of any centrosomic granule in relation with it, such as is figured by Brasil in the case of Urospora. Practically all the chromatin lies at the periphery of the nucleus in the form of many short rodlets, leaving the nuclear sap free. The cytoplasm is full of round paraglycogen grains; other granules are not very evident in preparations stained with para-carmine but are numerous in iron-hæmatoxylin ones.

Conjugation.

As soon as the sporoblasts are definitely formed and isolated they are ready for conjugation.[1] Notwithstanding

[1] I have never observed the least appearance of anything comparable to the chromatic reduction which, according to Paehler (**29**), takes place in the primary sporoblasts of Gregarina (Clepsydrina) ovata, and which he suggests may indicate bisexual differentiation. The author could not perceive,

the apparent equality between the gametes, I regard this and other cases of complete isogamy in Gregarines as constituting, really, instances of binary sexuality, and not merely of exogamy (using this term in the sense of Hartog [**12**]); that is to say, the condition we find here is derived, in all probability, from a typical bisexual condition, although the binary sex is, in these cases, rather implied—or, more correctly, potential—than actually manifest (see also below, p. 76). It is almost certain that each gamete copulates with one developed from the other member of the couple—probably that nearest available. Although I have been unable to demonstrate this point in Cystobia, for reasons which are apparent from the context, there cannot be the least doubt that the statement is justified when the formation and constitution of the zygote in cases of anisogamous conjugation is borne in mind. The origin of the two conjugates from separate associates (parent-individuals) appears to be universal, whether the anisogamy be strongly marked (Pterocephalus) or only slight (Urospora).

Absence of any movement.—With regard to the mechanism of the process, I feel confident that in this case there is no bustle, no tumultuous "danse des sporoblasts" to be rushed through; thanks to the remarkable extent to which the intertwining is carried all is done easily and quietly. I have carefully watched live cysts for long periods, both by day and by night, but I have never seen the slightest movement taking place. Now, if there had been any animated movement I should certainly have observed it, at any rate at the periphery; and, moreover, in my sections through cysts of this period all stages in conjugation are to be met with, so that this was evidently the right time to look for the "dance." Hence I feel assured that if, at the moment of separation, each gamete is not actually contiguous to a suitable fellow-

however, any appreciable difference between either the two associates or the gametes to which they gave rise; nor does he state whether he observed this nuclear purification in elements formed from both halves of the cyst, or only in those from one, which would have helped to decide the question.

conjugant, a very slight movement or mutual attraction will bring two such, when set free, into contact.

There are, besides, very good reasons for the absence of this peculiar and characteristic phenomenon. In the first place, since practically all the cytoplasm is brought into use, there are neither lobes nor processes (as in Stylorhynchus and Lankesteria), nor a large central residuum formed by the two gregarinoid somata (as in Diplocystis and Gregarina cuneata) to be, as it were, circumnavigated, either actively or passively, before selective fusion can result. On the other hand, again, there is no apparatus for producing the movement.[1] Hence, considering all things, I no longer wonder, since examining my fixed and stained material, that I could not see any movement when observing cysts at this stage alive, and I do not believe that any such occurs.

The actual union.—In sections through a cyst in which conjugation is actually taking place, various stages are to be met with indiscriminately throughout the cyst—here the two cytoplasms commencing to fuse, there a single definitive sporoblast with the two nuclei still separate, and, lastly, a fully-formed zygote. Hence there is a slight variation in time of the process to be noticed, probably due to the primary sporoblasts not all being cut out at the same moment. The actual conjugation processes are, however, better seen in cysts which have been fixed and stained whole and then crushed on a slide. If this is done carefully, the exact stage reached in each case can be readily observed. All degrees in the union are seen in fig. 57, drawn from such a crushed preparation; *d–h* show successive stages in the fusion, and at *j* we have the definitive sporoblast, copula, or zygote formed. The

[1] Léger, in his interesting paper (**22**), shows that the chief function of the fusiform motile gametes (sterile male elements) in Stylorhynchus is to bring about, by their vigorous movements, the "mêlée sexuelle." Again, Berndt (loc. cit.) finds that, in the mealworm Gregarines, the unused cystal "reliquats" become amœboid and send out processes of various lengths, which serve to drive the peripherally situated gametes round in the cyst and mix them thoroughly together.

gametes apparently unite by the pole or point farthest away from the nucleus, and as the fusion becomes complete the two nuclei gradually approach and, at length, also unite. The nuclear union is quite simple. As in the case of other Gregarines so far described, there is no sign of a fusion-spindle such as is often met with in the zygotes of Coccidia (Coccidium, Adelea, etc.).

The copulæ in fig. 58 were all drawn from sections, in which it is easier to make out the subsequent behaviour of the fusion-nucleus. The chromatin of the two constituents becomes completely intermingled and assumes the form of fine rodlets; ultimately it appears more granular, the whole nucleus meanwhile contracting and becoming denser. While this is going on, the zygote has surrounded itself with a delicate membrane, and has begun to change its shape, taking on an ovoid form (*f*). The nucleus has also passed to one end, which we may term the basal end. A section through a cyst full of zygotes (at a rather earlier stage) is drawn in fig. 54.

Abnormalities.—Before passing on to consider spore-formation, there are one or two abnormal and degenerating appearances to be noticed in the cyst at this time. In fig. 63 *a* are seen two sporoblasts which have not succeeded in copulating. They are considerably shrunken as compared with normal ones, the cytoplasm having lost its granules and become diminished and pale. The nucleus, too, is rather smaller, and stains up almost homogeneously. Two copulæ, which for some reason or other are also unsuccessful, are seen at *b*. (Possibly, in this case, for lack of better partners, sister-sporoblasts have paired.) They exhibit practically the same structure as the uncopulated ones, only being larger; in the right-hand one a few reserve granules are still left.[1] Fig. 63 *c* shows an instance of polygamy; three sporoblasts have evidently copulated, the nuclei of two having already fused. Such cases are not rare; there are generally a few to

[1] A similar degeneration of certain sporoblasts is described by Cuénot (loc. cit.) in Monocystis.

be met with in each cyst.[1] I do not think these polygamous copulæ develop into anything; I have never come across monstrous or abnormally large sporocysts.

I cannot be quite sure whether the larger mass with many typical sporoblast nuclei seen in fig. 64*a* is an example of this on a large scale,[2] or whether, on the other hand, it represents a small portion of the cytoplasm of some lobe or other which did not entirely segregate up, but remained in this compact condition. The latter view seems to me more probable, for as many as twelve nuclei may occur in one of these masses, of which there are occasionally one or two in a cyst. While in the former case that would imply a very comprehensive polygamy, in the latter the cytoplasmic residue with all its nuclei would represent actually only a very insignificant portion of the entire sporulating cyst. However that may be, a later stage where such a mass is degenerating is seen in *b*, drawn from a spore-containing cyst. The nuclei have coalesced together, the chromatin has become granular and stains up densely, and the cytoplasm is also altered and full of chromatoid matter.

(9) Spore-Formation and Systematic Position.

(*a*) The spores.

C. irregularis.—The zygote next commences to assume the typical shape of the spore. While the basal end (where the nucleus is situated) remains round and unaltered, the other end, which is destined to form the funnel, becomes elongated and then narrowed (fig. 59 *a* and *b*). When it has thus acquired the definite shape, the clear but firm exospore or outer spore-membrane is laid down. The cytoplasm then retracts itself somewhat temporarily, leaving the funnel free.

About this time the first division of the zygote- or spore-

[1] Léger (loc. cit.) also figures exactly similar instances in Stylorhynchus.

[2] Léger mentions such cases where five or six gametes have fused.

nucleus takes place. After having become very finely granular, the chromatin is aggregated into four or five lumps or pieces (*c*). A well-marked spindle then makes its appearance, stretching transversely or slightly obliquely across the spore. Hence it is usually best seen when the spore happens to be cut transversely, as in *d*—*g*. At each end of the spindle is a distinct centrosome, which is, however, minute when compared with the large ones described above in connection with the multiplying nuclei. The chromatin fragments (or chromosomes? I have not been able to make out any definite number) are next divided and pass along the spindle-fibres to either end, where they unite again to form the daughter-nuclei, the spindle eventually disappearing (*f*—*j*). The division of the two daughter-nuclei to form four is very similar, and also takes place transversely to the length of the spore; I have not seen the division of one of these four nuclei to form one of the eight sporozoite-nuclei.

The cytoplasm possesses a marked affinity for chromatic stains owing to the chromatoid granules which it contains (see fig. 59 *c*—*j* inclusive). These are at first uniformly distributed throughout the contents of the spore; gradually, however, they become united in a mass centrally (*c*, *h*, and *j*), and form, together with a certain amount of cytoplasm not used in sporozoite-formation, the spore-residuum or "reliquat sporal." Fig. 60 shows a couple of typical spores at this binuclear stage. I think the endospore or inner spore membrane is secreted about this time, as the cytoplasm seems to fill up the funnel rather more, but at present it is very delicate and difficult to make out. Later, when the four-nuclear stage is reached (fig. 61), the cytoplasm has become finally retracted from the funnel end, leaving the endospore showing as an inner funnel (*i.f.*) inside the neck of the outer one (formed by the exospore), both being open to the exterior. The inner funnel is closed at its base by a broad, fairly thick, and deeply-staining plug or cap (*c*), which effectually shuts off the cytoplasm from the exterior. This is, doubtless, dissolved by the digestive juices of the fresh host

when the spore is swallowed, thus allowing the sporozoites to be liberated. The two membranes, which together constitute the spore-wall or sporocyst, are now easily discernible (*ex.* and *end.*). The spore-residuum (*s.r.*) is also very prominent, occupying the central part of the spore. Now and again (fig. 61*a*) the rim of the outer funnel shows a distinct tendency towards angularisation or serration.

I have nothing to add to the observations of Minchin (loc. cit.) regarding the eight-nuclear stage and the structure of the sporozoites, and will therefore refer readers to his description and figures.

C. minchinii.—After repeated and prolonged examination, I have at length succeeded in making out the general appearance of the spores of C. minchinii sufficiently well to assure myself that, as regards form, they agree fundamentally with those of C. irregularis. A ripe spore of C. minchinii (fig. 62) is, however, much smaller than one of C. irregularis. Comparing fig. 62*a* with fig. 61, which shows spores of C. irregularis drawn to the same magnification, it will be seen that the lineal measurements of a spore of C. minchinii are approximately only half those of one of the latter parasites. The shape of both is, nevertheless, strikingly similar. The only difference that need be noticed is that there appear to be two (possibly more?) delicate, curved processes in connection with the rim of the funnel. I do not feel so certain about this point as I should like to be, owing to the very few cases in which I have been able to see the entire longitudinal outline of the spore in my sections.[1]

I have been quite unable to distinguish any division of the sporocyst into exo- and endospore. In short, the delicacy of the spore-membranes in C. minchinii is much greater than

[1] The spores are so numerous, so small, and so closely packed in the cyst that it is a most difficult matter to find one cut perfectly longitudinally. Moreover, in all the material examined, only twice besides have I found spore-containing cysts. These were both crushed instead of being sectioned. Unfortunately, however, they were from spirit-fixed Cucumariæ, and the spores proved to be so crinkled and warty in outline that they were not worth drawing.

in C. irregularis, where they are firm and do not easily become distorted in appearance. Neither have I noticed any deeply-staining plug at the base of the funnel, such as is apparent in C. irregularis, although the spores of C. minchinii are similarly stained. Hence it seems to me probable that in this species the delicate sporocyst itself is dissolved. I have no doubt this difference in structure stands in relation with the different method of infection, the dissolving power of the excretory acids, etc., in the respiratory trees being, probably, much less than that of the gastric or intestinal secretions of Holothuria.

The contents of the spore, on the other hand, are readily made out. There are, as usual, eight sporozoites, in this case arranged in two distinct groups of four—one near either end of the spore; in the centre lies the sporal residuum (*s.r.*), separating the two groups. Two sporozoites are drawn separately at *b*. Each is small and spindle-like, and possesses a deeply-staining, square to rounded nucleus, situated near the centre.

(*b*) Systematic position.

We are now in a position to compare the principal species hitherto included under the generic heading of Cystobia. (For C. holothuriæ see p. 3). It will be at once apparent that C. irregularis and C. minchinii are more nearly related to each other than is C. holothuriæ to either. The two former agree as regards the shape of the spore, which is usually considered to be the principal classificatory character in a Gregarine; moreover, their distribution, which is apparently very limited, also narrowly coincides. In both these respects C. holothuriæ differs markedly. The fact that association is terminal in C. irregularis and lateral in C. minchinii is not one that can be allowed to outweigh the more fundamental points of agreement just mentioned,[1]

[1] There is the same difference in the manner of association in two closely allied species of Gonospora, namely G. varia and G. sparsa.

even though association should prove to be lateral also in C. holothuriæ.

With regard to the variation in the shape and position of the sporozoite-nucleus shown in the different cases it is a little difficult to know what value, if any, to assign to these differences, since it is somewhat uncertain whether the sporozoites, as thus compared, are equally ripe. Minchin (25) considers the sporozoites of C. holothuriæ, in the form of the definition, as not, perhaps, quite mature, and appears to think that their nucleus may ultimately become elongated. If that is so, the same may also be true of C. minchinii. Possibly, moreover, the sporozoite-nucleus in this species subsequently takes up a position near one end, but in any case the point is, at most, one of minor specific importance.[1]

It is otherwise with the difference in the form of the spore exhibited by C. holothuriæ, which is not sufficiently emphasised by its present position, especially when we bear in mind the importance attached to this character in distinguishing the different cœlomic Gregarines. C. holothuriæ has been hitherto united with C. irregularis, largely on account of the similar habitat and precocious association. Both of these features, however, may have been independently acquired by another parasite. We see the latter condition strongly developed in quite a different Gregarine—namely Diplocystis—and, granting that another form also become parasitic in the peculiar situation of C. irregularis, it would be most likely to develop the same intimacy of association. Again, the distribution of C. irregularis and C. holothuriæ is, so far as is known, quite distinct. Hence, considering everything, it seems probable that these two species are not very closely related (one being derived from or through the other, as, for instance, C. minchinii almost certainly is

[1] The position of the sporozoite-nucleus is not invariably fixed or constant even in one and the same species. In the case of the sporozoites of Urospora lagidis, Brasil (loc. cit.) finds that, while the nucleus is usually more or less central, it may be near either one end or the other (compare his figs. 79 and 80).

from C. irregularis), but ought rather to be placed in different genera. As C. holothuriæ is the type-species, this form ought to retain the generic name of Cystobia, the other two species being united in a new genus, for which I propose the name Diplodina.

Relationships.—Diplodina irregularis and D. minchinii are, undoubtedly, closely allied to Gonospora, G. varia, in particular, having a fundamentally similar spore. On the other hand, the tailed spore of Cystobia holothuriæ resembles that of one or two genera placed by Léger (**19**), on account of that very character, in a family distinct from the Gonosporidæ—namely, the Urosporidæ. The affinities of C. holothuriæ seem to be, in fact, with Lithocytis schneideri, and, in a slightly less degree perhaps, with Urospora (cf. lagidis).

Definition.—We may therefore summarise the systematic position of the parasites above discussed as follows:

Family, Gonosporidæ; genus, Diplodina (nom. nov.).

A neogamous Gregarine of large size and regular shape, parasitic in Holothurians. When in the "free" or gregariniform condition the adult couple is perfectly oval in outline, when encysted it is spherical. Conjugation is completely isogamous. The ovoid spores have dissimilar poles, the basal end being rounded, while the exospore at the opposite end is prolonged as a funnel- or cup-like expansion open to the exterior.

(1) D. irregularis (Minch.). Association terminal (end-to-end). Septum between the two members either present or absent. The cysts remain attached to the wall of the blood-vessel. The size of the spore averages 26 μ × 12 μ. The endospore, also, is turned outwards, as an inner funnel open to the exterior, its base being closed by a cap or plug. The sporozoites possess an elongated nucleus near one end. Habitat: blood-vessels of H. forskåli.

(2) D. minchinii, Woodc. Association lateral. Septum never present. Sporulating cysts usually occur in the connective tissue of the body-wall or retractor muscles. The

size of the spore averages 14 μ × 6 μ. There is probably no inner (endospore) funnel open to the exterior; moreover, no sign of a plug is visible. Sporozoites possess (in all instances observed) a central nucleus, square or rounded. Habitat: respiratory trees and body-cavity of Cucumaria pentactes and C. planci.

Family, Urosporidæ; genus, Cystobia, Ming., 1891.

The exospore at the basal end of the spore is prolonged into a flat process, like a lance-head.

C. holothuriæ (Schn). Not having had an opportunity of personally studying this parasite, I am unable to add to the definition above given (p. 3).

The consideration of these neogamous Gregarines affords an excellent opportunity for a general discussion of the phenomenon of association, as it is known to occur in the order.

(10) Precocious Association or Neogamy.

(*a*) Comparative account of the principal variations in the manner and time of association.

We may commence by following the gradual increase in the precocity and intimacy of the "pairing" as exemplified by different forms. Leaving out of account, for the present, the "chains" formed by certain Gregarines, which are probably often broken and differently reconstituted, and whose significance is not fully understood, we have, in the simplest cases, a union of two adult trophozoites immediately prior to encystment and the commencement of sporulation (Lankesteria, Monocystis, Stylorhynchus, etc.). The dividing partition separating the two associates does not usually break down until nuclear division is well advanced. Next, we get the binary syzygies of most adult Gregarines, which proceed to encystment after remaining attached to each other for some time, as in Gregarina cuneata and other meal-worm

Gregarines (Berndt [1]), etc. The first indication of precocity is perhaps seen in Zygocystis, where the individuals always associate as soon as they become adult trophozoites.

Diplocystis.—The development of neogamy to an advanced degree, along parallel but quite independent lines, is well shown by Diplocystis on the one hand and Diplodina and Cystobia on the other. In the case of Diplocystis major, a cœlomic parasite of the cricket (Gryllus), Cuénot (loc. cit.) found that the Gregarines can grow to a certain size alone (up to ·2 mm. in diameter) but not to their full extent, i. e. to become sporonts; couples, however, attain to a diameter of 1·3 mm. Still, the mutual adherence is very feeble—the two individuals being easily separated—and, moreover, often indiscriminate, several Gregarines of different ages being at times grouped together (see Cuénot's fig. 40, Pl. 20), some of which have to be got rid of as de trop. A further advance is seen in D. minor, in which the longest diameter of a couple is, at most, ·6 mm. Here "pairing" takes place much earlier in life. Solitary individuals larger than 30 μ are never met with, and, indeed, association is sometimes accomplished before the parasites have passed from the gut-wall into the cœlom. Moreover, the union is closer and more definite, the two individuals becoming enclosed in a common membrane, which may be looked upon as an early-formed ectocyst. Nevertheless, inside this, the double nature of the syzygy is distinctly to be seen, there being an obvious V-shaped constriction all round the middle, marking the plane of junction, and only slightly less prominent than in my fig. 3 (see Cuénot, fig. 41, or Minchin [26], fig. 22).

Next comes D. schneideri, the trophic phase of which I have above re-described. While the association in this parasite may be, apparently, sometimes more precocious and intimate than in D. minor,[1] an advance upon the condition

[1] In none of the individuals of D. schneideri which I examined was the septum absent. Kunstler, however, in his fig. 16 (loc. cit.), has drawn a small example, still attached to the intestinal wall by the peritoneal epithelium, which appears to lack a septum. Cuénot (loc. cit.) is of the opinion that

found in that species is chiefly noticeable in the modification of the encystment-process (see above, p. 27). Nothing of this kind is mentioned by Cuénot in his description of D. minor. Judging from this author's figures, the formation of the ectocyst there resembles the manner in which it occurs in the ordinary process of encystment, as the spherical shape of the couple is much more cyst-like and the ectocyst does not appear to enter at all into the plane of junction. D. minor represents, therefore, a condition intermediate between ordinary encystment and the secreting of an early ectocyst at all points of the body, closely following the adult form, which is thereafter retained, as in D. schneideri.

Thus we can conveniently arrange the different species of Diplocystis in a series, as follows:

(1) D. major, in which precocious association is already necessary if the trophozoites are to become full grown, but its occurrence is haphazard and promiscuous, and the actual attachment only feeble.

(2) D. minor, in which neogamy occurs early in life, and its permanence is assured by precocious encystment.

(3) D. schneideri, in which the ectocyst-formation is modified and distinctly adapted to the trophic phase of the life-history.

Diplodina and Cystobia.—In these two genera we find the most advanced condition of neogamy known among Gregarines.

In D. irregularis this extremely early and intimate joining appears to be in process of development. Many individuals—probably the majority—are content to unite so as to acquire at length the appearance of one septate but

Kunstler must have overlooked one in this case. The septum when present is, however, so obvious, and the structure of the particular specimen was, for the rest, so clearly seen, that I do not think this view altogether certain. It is not necessary, moreover, to suppose that the septum is invariably present. Quite possibly in certain, perhaps exceptional, instances neogamy in D. schneideri may be so precocious and intimate that no partition separates the two associates, just as is often the case in Diplodina irregularis (see next page).

binuclear Gregarine. Fig. 3 represents the least intimately joined couple that I have observed. In this case association had probably taken place but a short time previously. With growth, however, the "double" nature of the parasite (as indicated by the V-shaped constriction) would, doubtless, have become less and less obvious, until the typical adult condition was reached (figs. 1, 2). On the other hand, many individuals unite at the earliest opportunity and very intimately;[1] in such cases the actual cytoplasm of the two associates becomes joined,[2] and there is no indication whatever of the plane of union.[3]

In D. minchinii complete union of the two associates is found in all cases (cf. figs. 9, 10, 12, 18, and 19). This species, so far as the cytoplasm is concerned, is absolutely indistinguishable from an ordinary Monocystid Gregarine. The only clue, indeed, to the plane of junction is the invariably transverse arrangement of the nuclei (see above, p. 21).

There can be no doubt that the presence or absence of a partition between the two associates depends upon how soon neogamy occurs. Where we find an intimate cytoplasmic union it is almost certain that the association has taken place between naked sporozoites, when there would be no limiting

[1] Associations belonging to this category appear to be more numerous as the season advances. Probably such factors as temperature, change of season, and the supply of nutriment available in the host at the time, exert an influence on the parasites, and induce them to anticipate, and prepare for, the ultimate phase of their existence—sporulation.

[2] This fusion is not to be considered comparable to the complete and homogeneous union which occurs in actual conjugation (see below, p. 68).

[3] The position of Cystobia holothuriæ with regard to this biological feature is somewhat doubtful. Mingazzini (**27**) says that a septum divides the Gregarine while it is attached to the blood-vessel, but Schneider (**35**) only saw one in cysts which had fallen off into the body-cavity. Minchin (**25**), again, did not see a septum in any case. Probably the explanation of these conflicting observations is that this parasite exhibits the same variation in this respect that D. irregularis does. Further study of the earlier phases of the life-history, while the trophozoites are still in the vessels, is necessary to decide the point.

membrane to divide the two halves. For I have never seen the least indication of absorption of the septum, during the trophic phase, when it is present (see above, p. 25). Though I have not had an ocular demonstration of the actual joining so early, my fig. 32, showing in the wall of the respiratory tree a D. minchinii only 18 μ by 12 μ, not yet rounded off since its entry, is strong evidence in support of this view. The fact that I have never observed a uninuclear form, however small, in the wall of the tree, where the parasites are readily distinguishable, also points to the same conclusion. In addition, the universal occurrence of cytoplasmic fusion in this species seems to imply that any unpaired individuals very quickly die off, being probably quite unable to become even young trophozoites alone.

The union itself, in D. minchinii, most likely occurs in the lumen before the parasites have penetrated into the wall.[1] In D. irregularis, in those cases where the association is between young trophozoites, it probably takes place in the lumen of the blood-vessel; where neogamy is most intimate it doubtless occurs when the individuals are little more than sporozoites, but in what situation I am unable to say with certainty. The passage of the parasites to their ultimate situation may be very rapid, for I have often found intimately associated trophozoites which have evaginated the wall of the vessel while very young (see above, p. 13). Probably in such cases the sporozoites have associated at once, before passing through the gut-wall and into the lumen of the vessel.

The morphological condition found in Diplodina is to be interpreted as one of union, and not of imperfect division.—I am thus unable, it will be seen,

[1] Unfortunately, searching for very minute forms in this situation is most difficult work, owing to the numbers of loose cells of various kinds, which constitute in places a spongy tissue, tending to block up the lumen. Moreover, since the solitary sporozoites would not remain long in the lumen before associating and passing into the tissue, only by very good fortune would one come across them in a section.

to bring forward any absolute proof that in Diplodina we have to do with union at all, and not, on the contrary, with division of varying incompleteness. It may be worth while, therefore, to say briefly why the trophozoites of this genus are to be regarded as couples, and not as individuals which have undergone imperfect division.

The case of Diplocystis schneideri is of much importance in this connection. Here, also, is an instance where neither a uninuclear individual nor the actual formation of a pair has yet been observed. However, in D. major and D. minor, two closely allied species, we have certainly to do with the union of distinct individuals. Now, the condition found in D. schneideri differs only very slightly from that of the adult trophozoites of D. minor, and, as explained above, is easily derivable therefrom. Hence it is hardly open to doubt that D. schneideri is also an example of precocious association, and not of early partial division. We have seen, moreover, that in very young forms the septum between the two halves may, apparently, be absent.

The genus Diplocystis affords, perhaps, the strongest argument in favour of a similar interpretation of the morphology of Diplodina. One or two other corroborative points may also be mentioned. Comparing trophozoites of D. irregularis of different age, it is at once seen that, as growth proceeds, the separation between the two halves of a syzygy invariably becomes less instead of more pronounced. It can confidently be said that the two members of a "couple" never separate, which is the reverse of what one would expect if they resulted from the division of a single individual. If that were so, moreover, we should have conjugation taking place between the progeny (gametes) of the two halves of what was originally one individual (derived from one sporozoite), an occurrence for which there is no precedent or analogy among the whole of the Telosporidia. For these reasons, therefore, I think it most likely that the trophozoites of Diplodina—and equally, of course, those of Cystobia—are correctly regarded as neogamous.

(*b*) Biological Considerations.

The reason for neogamy.—The reason for the gradually increasing precocity and intimacy of association, as we can trace it in different spp. of Diplocystis and Diplodina, is undoubtedly to be found in the absence of any power of movement in these cœlomic parasites. The whole process, in short, is the result of an endeavour to insure a suitable and durable association, since the animals are not able to move about themselves in search of a partner when ready to commence sporulation. The fact that neogamy is only known to occur in these non-motile forms bears out this view.

Let us consider D. major, for example. It is purely a matter of chance whether two individuals of the right age are brought near enough—as they are passively washed about in the body-cavity—to attract each other reciprocally and associate. Besides this, there is the danger of unsuitable and indiscriminate grouping, and also of a separation of two partners (if not ripe for encystment) when violently disturbed; in such cases a second association might be impossible. Probably, therefore, a considerable percentage of individuals thus isolated from one reason or another is doomed to phagocytic destruction. A method so haphazard must prove very expensive, and in the other two species association is modified as above described. Thus unequal and multiple associations are, as a rule (though not entirely) prevented, but the membrane probably interferes, to a certain extent, with the absorption of nourishment by the trophozoites.

At all events, this plan is not followed in Diplodina and Cystobia. In these parasites the difficulties in the way of association between adult trophozoites would be even greater, because of the more confined situation. We find, correspondingly, in these genera no stage equivalent to that seen in D. major. Those races of individuals which did not adopt early and permanent association appear to have all died off.

Effect on the individuals.—Notwithstanding the appa-

rent intimacy of the two members of the couple in those cases where the septum is absent, it may safely be said that there is nothing approaching a true conjugation as yet between the two halves. Though I have many a time found the two nuclei in contact, I do not believe there is any nuclear fusion or interchange of material; for I have never seen the slightest sign of a break-down in the nuclear wall or of alteration in the nuclear constituents.

It is more difficult to be certain with regard to the cytoplasm. Personally, I think the two cytoplasms remain (until sporulation) as individually distinct in the cases where there is no septum as in those where it is present. The fact that in D. irregularis the septum may persist until nuclear multiplication is considerably advanced, when the two cell territories remain for the time being quite separate, renders it highly probable that this is true when the septum is absent, although the delimiting plane is not distinguishable. The reason for the inability to see the plane of junction in such a case is not, I consider, because it is no longer there, but because it is not constituted by a layer (ectoplasm, or limiting membrane) different from the general cytoplasm.

In all the other Gregarines whose life-history has been investigated the complete morphological "separateness" of the cytoplasm of the two associates is a recognised feature (see Introduction, p. 5), and there is no reason to suppose it is otherwise in Diplodina. That there is any complete and homogeneous intermingling of the cytoplasm of the two individuals, resulting in one binuclear organism, I do not for a moment believe; such an actual merging of two distinct entities into one takes place first later, when the gametes copulate. Association certainly gives a stimulus to further development, and, as we have seen, neither growth nor life can go on in Diplodina without neogamy. Nevertheless, I think that such stimulus is principally cytotactic (see below, p. 73), and of a radically different character from that which is imparted by true conjugation.[1]

[1] See Hertwig's suggestive essay (**14**) on the significance of fertilisation.

I do not even think that the intertwining lobes and processes above described are developed in D. minchinii until nuclear division is well advanced. The very definite and constant position of the nuclei in the gregariniform adults of this species (see figs. 9, 10, and 12) is strong evidence that the "body" of each associate remains (except in the plane of junction) quite as distinct morphologically as it does, for instance, in Gonospora sparsa (see Léger's figs. 3 and 4, Pl. 20 [**19**], or Minchin's fig. 20 [**26**]).

Is triple association successful?—The Diplodina triplets drawn in figs. 20 and 28 seemed perfectly healthy, and possessed typical nuclei; the one drawn in fig. 29, however, has not the customary appearance and looks rather abnormal. This is a large specimen, being ·6 mm. by ·4 mm., and its nuclei have stained up almost homogeneously, the karyosomes being small and indistinct. The cytoplasm, too, has an altered look, with deeply staining lumps and grains (*g*), probably of an albuminoid nature, unevenly scattered about; in addition, a few vacuoles (*vac.*) are present near the margin. This syzygy, in short, appears as if it were already commencing to degenerate. Opinion differs as to whether these triplets are capable of successful sporulation or not; the weight of evidence, where their further development has been followed, seems to point to their subsequent degeneration.[1] The reason for the binary nature of a typical syzygy will be fully understood when the essential significance of association has been discussed.

Probably, in any case, the associates require to be of about equal age and size if the union is to be successful. Berndt (loc. cit.) mentions that if the members of an ordinary syzygy are of considerable difference in size, no true cyst-

[1] Berndt (**1**) states that in Gregarina cuneata associations of more than two individuals invariably come to nothing. Cuénot (loc. cit.) instances only very rare cases of triple association in Diplocystis, one of which has apparently produced sporoblasts. Judging from his fig. 47, however, the sporulation would not seem to have been successful, the sporoblasts being extremely minute and scarcely visible, very different from the well-developed layer in the normal cysts figured.

wall is formed, and the animals soon die off. Siedlecki (loc. cit.), Léger [**19**] and Brasil [**3**] give examples where one member of a couple has produced primary sporoblasts in a normal manner, while the other, most likely because the above condition was not fulfilled, has remained stationary and, indeed, succumbed. One Gregarine of a couple can in certain cases, apparently, exert sufficient influence upon its associate to induce it to commence sporulation, although it itself may not be ripe enough to do so, and as a result not only does it not benefit by the stimulus or "Reiz" of the other, but this, on the contrary, appears to harm it and it succumbs instead.

(11) General Significance of Association.

Descent of the Telosporidia.—We are thus led to a consideration of the origin and significance of association in general. We may commence by endeavouring to trace the descent of the Telosporidia—that sub-class of the Sporozoa which includes the Gregarines—from a Flagellate stock. For Schaudinn (**33**) has recently demonstrated that a certain malarial parasite of birds, namely Halteridium noctuæ, is, in reality, only a phase in the life-cycle of a Trypanosome, and it is becoming increasingly probable that the Hæmosporidia as a whole are derivable from, and in many cases still closely connected with, Hæmoflagellates. There can be little doubt that the Coccidia and Gregarines have an origin similar to that of the Hæmosporidia, for the first-named are closely allied to this order, and the differences exhibited by the Gregarines are easily understood when their somewhat different relation to the host is borne in mind. Hence it appears most likely that the whole of the Telosporidia have originated from a Flagellate ancestor.[1]

In the case of the Hæmosporidia the original parasitic

[1] This ancestor may be assumed to have been of a fairly generalised type, such, for instance, as that whose life-history is outlined by Doflein (**11**), p. 53.

Flagellate became specially adapted for life in the blood. With the acquirement of an alteration of hosts the life-history has become extremely complicated, but the details do not concern us here. It is probably in this order, however, that the Flagellate phase is retained to the greatest extent.

The Coccidia are to be regarded as having resulted from the original parasite becoming entirely intra-cellular. With this is correlated their non-motility and the great specialisation which we find in the sexual process. The numerous male gametes are highly differentiated and constitute, so far as is known at present, the only phase in the life-cycle where the Flagellate ancestry becomes manifest. The female individual, or megagametocyte,[1] no longer divides up to form many gametes, but itself becomes one directly after nuclear reduction. In Cyclospora, however, Schaudinn has recorded (**34**) certain abnormal cases of the persistence and further multiplication of the "reduction nuclei" of the female element, followed by multiple fertilisation. This occurrence points very strongly to the conclusion that there were originally many female gametes also, a condition which would agree with that found in Gregarines.

Lastly, we come to the Gregarines, which are by far the most successful group. I do not agree with Minchin (**26**, pp. 272, 273) in considering this order (any more, indeed, than the other Telosporidia) as derived from an originally intra-cellular form, which re-acquired, secondarily, an intercellular habitat. It is much more likely that the Gregarines have never been entirely intra-cellular, since, in that case, it is almost certain they would have completely lost their mobility and any cytoplasmic differentiation they possessed. Moreover, the female individuals would also have tended to reduce the number of gametes, of which we find no trace.

Whereas, in the evolution of the Coccidia, the young parasite at length penetrated completely into the host-cell and stayed there, in no instance do we find such an advanced

[1] In many Coccidia bisexual differentiation becomes marked in the adult trophozoites, or even earlier.

condition in Gregarines. Speaking broadly, further development in this order can be regarded as having taken place along two distinct lines. In the one case, characteristic of the great majority of Septate forms, the young growing parasite penetrates only partially into the host-cell after attachment,[1] and remains in that position only during its early growth.[2] For the rest of its trophic (and, of course, its sporogonic) life the Gregarine is free in the gut. Concurrently with the loss of the flagellum the parasite has developed the peculiar gregarinoid movement (easily derivable from one of a euglenoid nature) and, correlated with this, we find a marked differentiation of the peripheral cytoplasm.

The other line, that of the Monocystids, branched off early, before the septum characterising most intestinal or Polycystid Gregarines became developed. The young parasite, at the beginning of its life, followed one of two courses. Either it penetrated entirely into the host-cell and remained there for a long time before falling into the gut (e. g. Lankesteria ascidiæ) or, on the other hand, it passed inwards between the cells of the mucous membrane and came into intimate relation with the connective tissue, etc., of the sub-mucosa, being, as a rule, intercellular. In this latter manner have originated the cœlomic forms. In many cases (above described) these have now, for the first time, lost their motility; in other words, these cœlomic Gregarines have developed a trophic condition similar to that prevailing in the Coccidia, but quite apart and independent from them. Neither on account of their habitat nor because of the apparently simple nature of the sexual process (see below, p. 79, et seq.) are the Monocystids to be

[1] This question of attachment is very interesting as regards its bearing upon the Flagellate ancestry of the order. Léger (**21**) has recently described various parasitic Flagellates (Herpetomonas, Crithidia), characterised by the possession of a gregariniform phase, in which the flagellum either becomes greatly shortened and reduced, serving as an organ of attachment to the epithelial cell of the host, or else disappears.

[2] In one or two instances (e. g. Stenophora) the sporozoite penetrates completely into the cell, and the parasite remains intra-cellular until growth is finished. This is, undoubtedly, a secondary and not a primitive condition.

regarded as more closely representing the ancestral Gregarine than the Polycystids. One primitive character which has been preserved, however, is the unseptate condition of the body.

Origin of association.—With regard to its sexual reproduction[1] our ancestral Gregarine, doubtless, greatly resembled at first the parasitic Flagellate from which it was derived, numerous gametes being developed inside a membrane (probably little more than the unused parental residue), and sexuality being by this time marked; at any rate, we may assume that the male elements were smaller and more active than the females. It was at this stage that association made its first appearance, and to this adaptation one cannot doubt that the highly successful evolution of the order is chiefly due. Association, to commence with, would be quite accidental and indiscriminate, at first only occurring to any purpose between individuals which were dividing up to form gametes, when we may suppose them mutually capable of exerting an attractive or cytotactic influence. Such a coming and remaining together would obviously allow of a far greater percentage of the gametes copulating than if the male elements had to wander about trying to find the female ones. In other words, successful reproduction—ample provision for which is especially necessary in a parasite—would be much facilitated and increased. The advantage thus gained would lead to the development of this beneficial modification, and reciprocal attraction would not only be strengthened, but would tend to be manifested earlier, before the gametes were formed. In this way we get the cytotactic (chemiotactic?) influence thrown back to, and becoming apparent in, the parent individuals—that is, the adult Gregarines themselves. It is but a slight step in advance to suppose that this gradually led to the formation of the gametes being regulated by, and dependent on, the

[1] Asexual reproduction, it may be mentioned, has become completely lost in most Gregarines.

occurrence of such association;[1] in other words, the process acquired a distinct physiological import, in that by it a developmental stimulus was imparted to each associate. We thus arrive at the present-day condition in the group.

Essential importance of the process.—Association is necessary in the great majority, if not in all, Gregarines for sporulation to take place; in other words, we may say that an interacting stimulus such as is mutually exerted by the two associates is requisite to produce the formation of gametes or primary sporoblasts, and without it the animals are unable to fulfil their complete life-cycle.[2] A very interesting parallel case, proving that association may undoubtedly become necessary for the production of gametes, is seen in the Coccidia. Here, with the exceptions to be mentioned, the phenomenon is unknown, the gametocytes giving rise to the gametes apart and quite independently. In Adelea mesnili, however, we generally find association of the male and female gametocytes either in the same or in neighbouring cells. The male individual (microgametocyte) may, however, still develop microgametes apart from the female individual (megagametocyte), but in that case they come to nothing (see Perez [**30**]). In Adelea ovata and Klossia helicina, on the other hand, unless the male gametocytes become associated with the female ones, neither can they form male elements nor can the female cells maturate and become ready for fertilisation (see Siedlecki [**38**]). That is to say, we have here an attempt on the part of certain Coccidia to imitate the highly successful expedient of the Gregarines; and just as in the above-mentioned parasites association is obviously necessary to induce the formation of gametes (equivalent to sporoblasts), so it has undoubtedly become in the vast majority of Gregarines.

[1] There are many examples of ripe solitary sporonts dying off instead of producing sporoblasts, and this is, in all likelihood, because they have been unable to associate.

[2] Siedlecki (loc. cit.) has already expressed a similar opinion with regard to the stimulating effect of association, in the case of Lankesteria ascidiæ.

As the life-cycle of any Gregarine becomes completely known, it will almost certainly be found that each individual is, at some time or other prior to the commencement of sporulation, associated with a fellow one, such association being in nearly all cases permanent, and followed by encystment and true conjugation. I have not been able to find a single instance where it has been definitely proved that a single Gregarine, which has never associated, can sporulate, and the more life-histories that are thoroughly investigated the more unlikely does such an occurrence become. Siedlecki, Cuénot, Berndt, and Léger, in their recent researches, respectively emphasise the fact that they never observed single encystment at all. Brasil observed solitary encystment occasionally in Gonospora and Urospora, but it was unsuccessful; the nuclear multiplication was abnormal and the cyst fell a prey to the phagocytes of the host. Still, one or two authors have described the occurrence of normal solitary encystment, and appear to consider that successful sporulation without conjugation (i.e. parthenogenesis) results. These instances are considered below. What I maintain, however, is that, granting such cases do occur, in all probability there has been a temporary association previously, when the developmental stimulus was imparted; and, indeed, where solitary sporulation is described, temporary association is usually mentioned as also taking place.

Relation between association and conjugation.—We may now compare the varying relations between association and conjugation which we find in the group. The least modified condition is seen in the Septate Gregarine Pterocephalus, where there is markedly anisogamous conjugation, after association and common encystment of the couple (see Léger and Duboscq [**23**]). Now, it is obvious, when the formation of gametes became delayed until after association, and their successful copulation was thus practically insured, there was no longer the same necessity for the gametes to be differentiated. Hence, as might be expected, we find a reduction to isogamy taking place, of which Stylorhynchus

already shows the commencement (see above, p. 5). A most interesting stage in this reduction is exemplified by Urospora lagidis (and also by Gonospora varia), recently described by Brasil (loc. cit.). Both kinds of gamete are here of about equal size and alike in shape. There is, however, a sharp nuclear distinction apparent. The nuclei of the elements proceeding from one half of the cyst are smaller and chromatically denser than those belonging to the gametes from the other half.[1] The former are presumably male elements and the latter female, though this is not explicitly stated by Brasil.

The final stage is that of complete isogamy, where there is no discernible evidence of sexual differentiation whatever, at any rate in the gametes themselves. Typical instances are those of Gregarina, Lankesteria, and Diplodina, all of which have been discussed above. Nevertheless one may, I think, regard binary association as itself, in a measure, physiological evidence of sexuality. And, indeed, in a few instances (e.g. Aggregata vagans, Stenophora varians, Pterocephalus, and Diplocystis clerci) there are morphological differences between the two associates which Léger (**22**) is inclined to consider as indications of sexual differentiation. If, then, one may ascribe binary sex to the parent individuals (associates), even where the gametes are completely isogamous, this would explain why, in most cases, association is binary, and why sporulation is unsuccessful in the triple syzygies commented upon above.

Unusual and specially modified forms.—While in most of the cœlomic Gregarines actual copulation is evidently still necessary, since the trend of development has been to insure a lasting union, there are, on the other hand, one or two forms known in which conjugation of the gametes—i. e. the true sexual process—can be apparently dispensed with. The starting-point of parthenogenesis in the order is perhaps seen in instances where we find both conditions, copulation

[1] An almost similar state of things is really presented also by Monocystis, which has been lately re-examined by this same worker (**4**).

and independent development of the sporoblasts, co-existent (e. g. Ophryocystis, Léger [**20**]). Again, in Ceratospora mirabilis from the body-cavity of Glycera, Léger (**19**) describes and figures the formation of (many) sporoblasts and spores separately in the two associates, the septum apparently remaining unabsorbed and preventing copulation. If this can be relied upon as being a normal mode of spore-formation, it would seem that we have here, also, the beginning of this independent development of the sporoblasts.[1]

We have next to consider the case of two or three forms which are said to be capable of encysting and successfully sporulating alone, in addition to following the customary course. The evidence brought forward so far is, however, by no means conclusive. Léger has described (loc. cit.) solitary encystment and sporulation in Gonospora varia,[1] as well as the permanent encystment of a couple. Brasil, however, in his paper on the same species (**3**) says nothing about the independent (complete) sporulation of each member of a couple.[2]

On the other hand, Brasil draws attention to the work of Caullery and Mesnil on Gonospora longissima and Selenidium, spp. Unfortunately, the observations of these authors are too scanty to help much in deciding this question. Concerning the former parasite they remark (**7**) that the cysts seem formed either of one or two individuals, but nowhere do they definitely state that the cases of solitary encystment were successful. In addition the occurrence, now and again,

[1] It must be confessed that this observation (and also that of Gonospora below) stands greatly in need of corroboration. Léger's early work was all done, it must be remembered, before true conjugation was known. Probably sometimes, at any rate, the dividing septum breaks down, allowing conjugation to take place, for in some cysts of Ceratospora active movement of the gametes (Schneider's "danse des sporoblasts") was observed.

[2] Brasil notes an instance of a cyst of Urospora in which one individual had died off and was degenerating, while the other, to judge from its healthy appearance, seemed to be pursuing its normal course, with what ultimate success, however, we are not informed.

of very intimate association[1] in this species, renders it not unlikely that such an encysted couple, when sporulating, may have been mistaken for a single individual. Again, a species of Selenidium from the gut of Spio martinensis appears to encyst as often singly as in couples, nuclear changes taking place in both cases (see C. and M. [5]). Actual spore-formation, however, was never observed, since sporulation is not completed while the parasites remain inside the host. S. echinatum, from Dodeceria, exhibits the same latitude in the matter of encystment. Nevertheless, C. and M. state (6) that in three hosts in which spore-containing cysts were found they were undoubtedly normal, constituted in all cases by associated couples.

To sum up, we may say that while, except in Ophryocystis, successful independent sporulation cannot be regarded as proved, it is not improbable that such a modification of the typical life-history may be met with, in special circumstances. Consider Gonospora varia, for example. Here we have a cœlomic parasite which is non-motile, but which has not developed neogamy. Hence association, just when desired, must be more or less precarious. It would not be surprising, therefore, to find that this form had acquired the ability to develop also parthenogenetically. There can be, however, little doubt, I think, when the whole line of argument of this and the preceding section is considered, that if such independent sporulation occurs, it is only after prior temporary association.[2] Such association, although the trophic

[1] In certain cases, Caullery and Mesnil observed that the partition dividing the two associates disappeared. Brasil (loc. cit.) points out the analogy between this occurrence and the neogamy of Cystobia [Diplodina]. It probably also represents a step taken to bring about permanent association; it will be seen, however, that this parasite is proceeding upon a slightly different line from that taken by Diplodina, where the septum, once formed, never breaks down (in trophic life).

[2] Commenting upon association in the Selenidium from Spio, Caullery and Mesnil (loc. cit.) say that the adherence of the two members is very feeble, and they are easily and often separated. Hence, it is extremely probable that the individuals which sporulate alone have at one time or another been associated.

zoites are not ready to encyst permanently, may well suffice, under certain modified conditions, to impart the essential developmental stimulus.

Gregarine "chains."—A word or two, finally, with regard to the curious "chains" or aggregations of Gregarines, such as are known to occur, for example, in Eirmocystis, Clepsydrina, etc. Their significance is very problematical. It is possible that the cytotactic attraction has become modified in function. Its primary object, to induce the formation of gametes, being, as in Diplodina, delayed or in abeyance, it may perhaps serve a subsidiary purpose in trophic life.

Isogamy, in the Gregarines, is to be regarded as the more modified, and not as the more primitive, condition.

In conclusion, it may not be superfluous to point out some of the reasons why the general course of evolution above outlined seems to me to have much more in its favour than what is practically the opposite view. I should, indeed, hardly consider it necessary to do so were it not that this contrary view has been recently supported by Brasil (**3**), and Nusbaum (**28**). Both these authors consider isogamous copulation to represent the more primitive condition in the order. Brasil regards the case of Urospora and Gonospora as marking an early stage in the evolution of anisogamy, the highest development in this direction being attained by Pterocephalus. Nusbaum thinks that association at first was well developed, and served as a stimulus to the two members, which gave rise to isogamous gametes (example, Lankesteria). Subsequently, the process became less important, the two associates not entering into such intimate contact; correlated with this we have the formation of male and female elements (example, Stylorhynchus). Lastly, the association has a quite transitory significance, and is, in short, a reduced phenomenon; the associates themselves

are not necessarily of opposite sex, and the encystment is only temporary, the gametocytes becoming free later and giving rise to markedly anisogamous gametes (example, Schaudinnella).[1]

Now, the great success of the Gregarines is undoubtedly due to their power of closely and permanently associating during the period of formation of the gametes. Granting, therefore, the possession by the parasites (in the first place) of such a useful property, it seems most unlikely that they would dispense with the same (as, according to Nusbaum, Schaudinnella has done) without very good reason. For in that case we should have a tendency exactly the reverse of that to which the prosperous development of the order has above been attributed. Moreover, the primitive forms in the gut (supposedly isogamous), being capable of movement, could have no difficulty in associating; it is not easy to see, therefore, why they should adopt a less certain mode of sexual reproduction.

The cœlomic Gregarines are very interesting in this connection. Here, if anywhere, association is difficult of attainment (cf. above, p. 67). Hence, if anisogamy in this order has been developed from an isogamous condition, we might reasonably expect to meet with it here, since by this means the necessity for permanent association would be obviated. We find, on the contrary, however, that, speaking generally, it is these cœlomic forms which are most nearly or entirely isogamous. So far from acquiring anisogamous gametes (comparable to those of Pterocephalus or Stylorhynchus), they have, in many cases, developed the condition of neogamy—that is to say, they have been at pains to insure association. And in the few cases where, if anywhere, permanent association can apparently be dispensed with this is certainly not by the development of anisogamy, but by the very unusual modifi-

[1] Nusbaum (loc. cit.) does not endeavour to explain why, as is apparently the case, the male elements of this remarkable form are non-motile, which is hardly what one would expect according to his view of the course of evolution.

cation of parthenogenetic reproduction. It would seem rather as if these isogamous forms could not become (again) anisogamous.

The Coccidia are very interesting in this connection. From what has been said above, it follows that, if permanent association together with isogamy is to be regarded as the more primitive condition in the Gregarines, it was probably also a feature of the ancestral Coccidian. We might, therefore, reasonably expect to find association persistent, in some cases at any rate, and—equally probably—precociously developed. Now, in no instance is this occurrence known in the Coccidia as a primitive condition. But we do find it now and then (see above, p. 74) as a quite secondary condition,[1] acquired later and independently. That is to say, in spite of the extreme specialisation of the sexual process, which (as Minchin, loc. cit., points out) is entirely to facilitate sexual reproduction, rendered difficult by the intra-cellular habitat and non-motile condition of the parasites, certain forms have found it profitable to associate precociously, thus insuring fertilisation and allowing of an economy to be effected in the number of (male) gametes.

We see, therefore that in this closely allied order no support is to be found for the theory that the separate formation of anisogamous gametes is derived from a condition of association and the formation of isogamous gametes.

One or two other points may be briefly noted. Anisogamy is only known to a marked extent in intestinal forms. The ancestral Gregarine was, in all probability, an intestinal form, from which the cœlomic parasites have been derived. As we have seen, the latter are modified by the greater

[1] In the few cases where association is met with there is only one megagamete formed as in the other Coccidia; in these forms, moreover, and only in these, we find few (often four) microgametes developed. Schaudinn's work on Cyclospora (see p. 71) tends to show, however, that the original condition in this order also was the production of many female gametes. If, therefore, association in Adelea mesnili, etc., was a primitive character, there would be, in all probability, many female (and, of course, male) gametes.

degree of parasitism attained. Brasil (loc. cit.) admits this influence upon their trophic organisation, but thinks, apparently, that the exact nature of the reproductive process is uninfluenced by this feature. I do not think this view can be maintained. If not directly, the influence can certainly be traced indirectly. There is abundant evidence that modifications in trophic life themselves influence the mode of reproduction; examples of this have been numerous in the preceding pages. Hence for this reason alone we ought to be very cautious in assuming that the manner of reproduction of these cœlomic Gregarines is primitive. Lastly, when we remember that the Flagellate origin of the Gregarines is almost certain, it seems most natural to regard a condition where we have motile anisogametes as being more primitive than the one we find in the Monocystids, which has most likely resulted in the way I have described above.

Tabular comparison.—It may be useful to summarise, in tabular form, the principal stages in this line of development, as illustrated by known forms. Schaudinnella itself is best left aside. This is, undoubtedly, a very exceptional parasite, primitive in some respects (e.g. in the character of its association), but specialised in others. As Brasil says, it cannot be very well compared with any known Gregarine. In my opinion it represents a primitive Telosporidian parasite which has endeavoured, as it were, to go in more than one direction at once, combining, to a certain extent, both Gregarine and Coccidian features.

(A) Intestinal forms.

(1) The gametes are highly differentiated. Example: Pterocephalus.

(2) The conjugating elements, though readily distinguishable into male and female, are not so markedly differentiated: certain very motile and spermatozoon-like male ones are sterile, and have acquired a subsidiary function. Example: Stylorhynchus.

(3) The gametes are, to all appearance, perfectly similar (isogamous). Examples: Gregarina, Lankesteria.

In all these cases there is permanent association between ripe sporonts, followed by common encystment.

(4) The isogamous gametes (greatly reduced in number) may develop parthenogenetically, the septum between the two associates not breaking down. Example: Ophryocystis.

(? 5) The gametes may develop parthenogenetically, the association being only temporary (Selenidium ?).

(B) Cœlomic forms.

(1) The gametes are only very slightly differentiated, and chiefly distinguishable by their nuclei. Permanent association and common encystment are usual. Examples: Urospora, Gonospora, and Monocystis. (This stage is easily derivable from A 2.)

(2) The gametes are quite isogamous. Precocious association occurs. Diplocystis, Diplodina (probably also Cystobia).

(? 3) The gametes may develop parthenogenetically, the association being either permanent, in which case the septum does not break down (Ceratospora ?), or else temporary, the two associates afterwards sporulating separately (Gonospora varia ?).

Detailed Summary.

My investigations have related chiefly to Diplodina (Cystobia) irregularis (Minch.), parasitic in Holothuria forskåli, and to D. (C.) minchinii Woodcock, from Cucumaria pentactes and C. planci. All the material was collected near Plymouth, in which neighbourhood these Holothurians have a very localised distribution. The Cucumariæ are scarce, and the percentage of infected individuals is very small.

I have also examined the trophozoites of Diplocystis schneideri, from a new host, Periplaneta orientalis.

(3)[1] Diplodina irregularis lives either in the lumen of, or attached to, the blood-vessels. There is no definite relation between the growth, or period in the life-history, of the animals and the time of their evagination of the wall of the vessel. They may either come out when quite minute, or, on the other hand, they may commence sporulation while still in the lumen. The parasites, whether as trophozoites or as cysts, are never free in the cœlome. The cysts are most probably ruptured in situ, the liberated spores escaping when some Cuverian organs are extruded.

The habitat of D. minchinii is very varied. The parasites are most numerous in the wall of the respiratory trees. They also occur attached to the cœlomic epithelium of the body-wall, of the retractor muscles, and of various more or less vascular strands which cross the body-cavity, chiefly in the hinder part. They are never in, or in any way related to, the vascular system proper in connection with the gut, and obviously do not reach the site of infection by way of the mouth and intestine, as does the other species. All the evidence points to the conclusion that the spores enter the host through the cloacal aperture, being sucked up by the inhalant current into the trees.

The parasites in the cœlome are always partly covered by a double layer of epithelium, the inner one being really an invagination of the outer one. There is not the slightest doubt that the animals are passing in and not emerging. The process is the reverse of the evagination process met with in D. irregularis. The later stages constitute more an overgrowing and surrounding of the parasite by the epithelium and connective tissue of the host than an actual inpushing on the part of the Gregarine itself, which only rarely occurs to any extent. The parasite becomes at length completely encysted and ready for sporulation.

(4) The gregariniform adults of both species are perfectly regular in form and typically ovoid. They are quite motionless. Each adult is really a "couple," Diplodina being a

[1] The numbers refer to the corresponding sections.

neogamous Gregarine, or one in which precocious association occurs. In D. irregularis the two associates are sometimes separated by a distinct septum and sometimes not, this being dependent upon the time of union. In either case, however, the adult couple—when the union is completed—presents superficially almost the aspect of a Monocystid Gregarine. In D. minchinii there is never any septum, the union of the two cytoplasms being intimate. In this species the association is lateral, while in D. irregularis it is terminal.

The general appearance of Diplocystis schneideri agrees with Kunstler's description.

I have occasionally met with instances of triple association in both species of Diplodina, and also in Diplocystis.

(5) There is a marked absence of differentiation in the peripheral region of the body of Diplodina. The general cytoplasm is limited by a delicate membrane, but there is no appearance either of ectoplasm or of myocyte-fibrillæ. In strong contrast is the firm and definite ectoplasmic layer in Diplocystis schneideri. Moreover, in this parasite the couple is always enclosed in an investing membrane, comparable to an early-formed ectocyst, which is laid down in the form of the couple and specially thickened around the plane of junction.

The cytoplasm in Diplodina has a quite typical gregarinoid structure. The nucleus possesses a distinct chromatic reticulum, in which is slung or suspended a single karyosome. With growth, the karyosome becomes very vacuolated, the smaller vacuoles running together to form two or three large ones, the contents of which are manifestly passed out into the nucleoplasm. I regard this process, not as one of excretion, but as a reinforcement of the chromatin of the nucleoplasm.

(6) The process of encystment in D. irregularis is very slight. In the majority of cases there is no ectocyst-formation whatever, and the limiting membrane serves as a delicate endocyst. The most obvious cyst membrane, indeed, is the peritoneal epithelium enclosing the cyst, which persists after the endocyst has broken down and disappeared. In

D. minchinii the encystment process is more typical, and there is a well-marked ectocyst.

The nuclear changes at the commencement of sporulation in D. irregularis are very important. The karyosome becomes divided up in the nucleoplasm, and with successive divisions of the sporont-nucleus the resulting fragments (daughter-karyosomes) become apportioned out among the daughter-nuclei. The karyosomatic fragments break down still further, and ultimately become incorporated with the chromatin of the nucleoplasm. I do not believe any nuclear material is, at this period, eliminated.

(7) My observations tend to prove that the earliest nuclear divisions are completely amitotic. The multiplying nuclei become distinguishable into two kinds, germinal ones, which subsequently form the sporoblast-nuclei, and large somatic or sterile ones, which eventually become dissipated in the surrounding cytoplasm. This process represents the nuclear purification of D. irregularis. The germinal nuclei divide by mitosis, and the attraction-spheres have very large and apparent centrosomes. These attraction-spheres can also exist and divide independently, and are to be met with scattered about in the cytoplasm. Some appear to come into relation with the large sterile nuclei, and probably help to bring about their disintegration.

(8) In both D. irregularis and D. minchinii, and especially in the latter, the process of sporoblast-formation is characterised by the remarkable extent to which the intertwining of the lobes and processes of the two associates is carried. As a result of this practically all the cytoplasm is utilised to form the gametes. There is no cystal residue ("gregarinoid soma") left over. The primary sporoblasts or gametes are quite simple and all alike morphologically, and conjugation is, therefore, completely isogamous. I have never observed the least movement in live cysts at this period, and do not believe the so-called "danse des sporoblasts" occurs.

(9) Spore- and sporozoite-formation in D. irregularis

follows the usual plan. To Minchin's description of the spore it may be added that there is a deeply-staining plug or cap closing the mouth of the inner spore membrane at the funnel end; this is doubtless dissolved by the digestive juices of the fresh host, thus allowing the sporozoites to pass out.

The spore of D. minchinii is very similar in form to that of D. irregularis, but only about half the size. The sporocyst is very delicate, and is probably itself dissolved; the fact that I have not observed any plug at the base of the funnel also points to this conclusion.

These two parasites are much more nearly related to each other than is either to Cystobia holothuriæ, and for this reason have been placed in a distinct genus. Diplodina is undoubtedly closely allied to Gonospora; the affinities of Cystobia holothuriæ, on the other hand, are rather with Lithocytis and Urospora.

(10) The consideration of these neogamous Gregarines has afforded a useful opportunity of discussing the phenomenon of association as a whole.

Considering first the variations in time and manner of the process, we see that precocious and intimate association has been especially developed in the three genera Diplocystis, Diplodina, and Cystobia. These have proceeded along rather different and independent lines, which reach their culminating points in D. schneideri and Diplodina minchinii respectively. In the former genus the desired result is attained by successive (phylogenetic) modifications of the encystment process; in the latter (and also in Cystobia) the tendency is towards intrinsic cytoplasmic union between the two associates.

Precocity of association is undoubtedly correlated with the absence of movement in these cœlomic forms. It is simply an endeavour to insure a suitable and durable association. There is certainly nothing approaching true sexual conjugation, as yet, between the two members. Even where neogamy is most intimate the nuclei remain, manifestly, quite distinct, and I think the cytoplasm of each associate also retains its

morphological separateness and individuality excepting in the plane of union, although, from the nature of the case, this is not apparent.

(11) Seeing the efforts made by these non-motile cœlomic parasites to insure association, we are naturally led to try and account for its obvious importance.

The result of recent research points strongly to the conclusion that the Telosporidia, as a whole, are descended from a Flagellate ancestor. This ancestral Flagellate would possess motile gametes, which were, probably, more or less anisogamous. Such an ancestor we may regard as being common to the three Telosporidian orders, namely the Gregarines, the Coccidia, and the Hæmosporidia, the differences exhibited by them being easily understood when their different habitat and degree of parasitism are borne in mind.

Of these orders the Gregarines are by far the most successful, this being undoubtedly due to their possession of the power of association. Hence, if this were a primitive condition, it would almost certainly be apparent (as such) in the Coccidia, since by it sexual conjugation is rendered practically certain. The state of affairs in that order seems to show conclusively that association is not a primitive, but rather an acquired, condition, one which has been acquired to any extent only by the Gregarines.

I regard the power of cytotactic attraction as having become so developed and specialised in this order that the formation of gametes is now entirely regulated by and dependent on such cytotactic influence; in other words, association is most probably necessary for sporulation to take place. There is abundant evidence to show that, in most forms at any rate, this is undoubtedly the case. Correlated with this, differentiation of the gametes is no longer necessary, and a beautiful series of stages exemplifying the gradual transition or reduction from anisogamy to isogamy is now known.

The above view seems to me much more probable than the opposite one, namely that association is a primitive condition tending to become less important with increasing anisogamy.

In this connection it is, I think, a significant fact that, speaking generally, isogamy is most prevalent in the cœlomic forms, which are the most modified and which in certain instances have developed neogamy.

General Summary.

In conclusion, it may be said that Diplodina is a very advanced and specialised Gregarine. Its principal modifications are those of non-motility, absence of cytoplasmic differentiation, neogamy, and complete isogamy; these are closely correlated with one another, and are for the most part ultimately traceable to the degree of parasitism attained by the genus.

Diplodina and (also) Diplocystis are to be regarded as forms which, following slightly different but parallel lines, have pursued to the farthest extent what may be described as the main or typical course of evolution of a cœlomic Gregarine.

University College, London,
July, 1905.

Since the above was written only two or three papers dealing with Gregarines have come under my observation. There is only opportunity here to add a word or two with reference to these, in so far as they bear upon the more important points considered in my work.

Reference has been made to Brasil's preliminary note on the sexual reproduction of Monocystis. In 'Arch. Zool. exp.' (4), vol. iv, p. 69, 1905, he describes the process in detail, and it is apparent from his figures that the gametes resemble those of Urospora and Gonospora. Besides the nuclear differences between the two kinds of element, however, there is also a slight inequality in size, one set (microgametes?) being rather smaller than the other (megagametes?).

Schnitzler ('Arch. Protistenk.', vol. vi, p. 309, 1905), in describing the reproduction of Clepsydinia (Gregarina)

ovalà, confirms Paehler's account with regard to the absolute agreement in appearance between the conjugating elements. He finds, moreover, that the process of nuclear maturation described by that author (see footnote, p. 51) occurs equally in both kinds. Hence in this form there is complete isogamy. It is interesting to note that in Monocystis this nuclear reduction is apparently delayed, Brasil having observed it to take place in the zygotes. Another point noticed by Schnitzler is the occurrence of two kinds of cyst, containing respectively small and large spores (micro- and mega-spores). As the author surmises, this fact may perhaps stand in relation with the occurrence of solitary as well as common encystment and sporulation.

Lastly, Crawley ('Amer. Nat.,' September, 1905, p. 607) derives the Telosporidia (i.e. Coccidia, Hæmosporidia, and Monocystid Gregarines) from a Polycystid Gregarine. As will be seen on referring to page 71 et seq. above, I do not concur with this view. The ancestral Gregarine (derived from a "gregariniform" Flagellate) was probably not so highly differentiated and specialised morphologically as the Polycystids now are. These are rather to be looked upon as constituting one line of descent, the Monocystids another. The Hæmosporidia and Coccidia, again, have branched off in other directions from the ancestral Flagellate.

January, 1906.

Analysis of Contents.

Bibliography.

1. Berndt, A.—"Beitrag zur Kenntniss der im Darme der Larve von Tenebrio molitor lebenden Gregarinen," 'Arch. Protistenk.,' vol. i, pp. 375–420, Pls. XI–XIII, 1902.

2. Brasil, L.—"Contribution à la connaisance de l'appareil digestive des Annélides Polychètes; L'épithélium intestinal de la Pectinaire," 'Arch. Zool. exp.' (4), vol. ii, pp. 91–255, Pls. IV–VIII, 1904.

3. Brasil, L.—"Recherches sur la reproduction des Grégarines Monocystidées," op. cit., N. et R. (4), vol. iii, pp. 17–38, Pl. II, 1905.

4. ——— "La genèse des gamètes et l'anisogamie chez les Monocystis du Lombric," 'C. R. Ac. Sci.,' vol. cxl, March 13th, 1905.

5. Caullery, M., and Mesnil, F.—"Sur une mode particulière de division nucléaire chex les Grégarines," 'Arch. Anat. Microsc.,' vol. iii, pp. 146–167, one plate, 1900.

6. ——— ——— "Sur quelques parasites internes des Annélides," 'Misc. Biol.' ('Trav. Stat. Zool. Wim.'), vol. ix, pp. 80–99, Pl. IX, 1899.

7. ——— ——— "Sur une Gregarine . . . presentant . . . une phase de multiplication asporulée," 'C. R. Ac. Sci.,' vol. cxxvi, pp. 262–264, 1896.

8. Cecconi, G.—"De la sporulation de la Monocystis agilis, St." 'Arch. Anat. Microsc.,' vol. v, pp. 122–140, Pl. V, 1902.

9. Crawley, H.—"Progressive movement of Gregarines," 'P. Ac. Philad.,' vol. liv, pp. 4–20, Pls. I–II, 1902.

10. Cuénot, L.—"Recherches sur l'évolution et la conjugaison des Grégarines," 'Arch. Biol.,' vol. xvii, pp. 581–652, Pls. XVIII–XXI, 1902.

11. Doflein, F.—"Die Protozoen als Parasiten und Krankheitserreger," (G. Fischer, Jena.). 1901.

12. Hartog, M.—"Some Problems of Reproduction—II," 'Quart. Journ. Micr. Sci.,' vol. xlvii, pp. 583–608, 1903.

13. Hertwig, R.—"Die Protozoen und die Zelltheorie," 'Arch. Protistenk.,' vol. i, pp. 1–40, 1902.

13a. ——— "Ueber Kerntheilung, Richtungskörperbildung und Befruchtung von Actinosphaerium eichhorni," 'Abh. bayer. Ak.' (2), vol. xix, pp. 1–104, 1898. Eight plates.

14. ——— "Ueber Wesen und Bedeutung der Befruchtung," 'S. B. Ak. Munch.,' pp. 57–73, 1902.

15. Koehler, —.—[On synonymy of different species of Holothuria], 'Zool. Anz.' vol. xx, pp. 507–509, 1897.

16. Kunstler, J.—"Diplocystis schneideri," n.g., n.sp., 'Tabl. Zool,' vol. ii, pp. 42, Pl. IX, 1892.

17. Labbé, A.—Article on "Sporozoa" in 'Das Thierreich,' 5te Lief, Berlin, 1899.

18. Laveran, A., and Mesnil, F.—"Sur quelques particularités de l'évolution d'une Grégarine et la reaction de la cellule-hôte," 'C. R. Soc. Biol.,' vol. lii, pp. 554–557, 1900. Nine figs.

19. Léger, L.—"Recherches sur les Grégarines," 'Tabl. Zool.,' vol. iii, pp. 1–182, 1892. Twenty-two plates.

20. Léger, L.—"La reproduction sexuée chez les Ophryocystis," 'C. R. Soc. Biol.,' vol. lii, pp. 927–930, 1900.

21. ——— "Sur quelques Cercomonadines nouvelles ou peu connues parasites de l'intestin des Insectes," 'Arch. Protistenk.,' vol. ii, pp. 180–189, 1903. Four figs.

22. ——— "La reproduction sexuée chez les Stylorhynchus," op. cit., vol. iii, pp. 304–367, Pls. XIII and XIV, 1904.

23. ——— and Duboscq, O.—"La reproduction sexuée chez Pterocephalus," 'Arch. Zool. exp.,' N. et R. (4), vol. i, pp. 141–151 1903. Eleven figs.

24. Ludwig, H.—Article on "Echinodermata" in Bronn's 'Klassen und Ordnungen des Thierreichs' ('Holothuroidea,' vol. ii, pt. 3.)

25. Minchin, E. A.—"Observations on the Gregarines of Holothurians," 'Quart. Journ. Micr. Sci.,' vol. xxxiv, pp. 279–310, Pls. XXVII and XXVIII, 1893.

26. Minchin, E. A.—Article on "Sporozoa" in Lankester's 'Treatise on Zoology,' vol. i, pt. 2, pp. 150–360, 127 figs., 1903.

27. Mingazzini, P.—"Le Gregarine delle Oloturie," 'Rend. Acc. Linc.' (4), vol. vii, pp, 312–319, 1891.

28. Nusbaum, J.—"Ueber die geschlechtliche heterogame Fortpflanzung einer im Darmkanale von Henlea leptodera Vejd. schmarotzenden Gregarine—Schaudinnella henleæ, mihi," 'Zeitsch. wiss. Zool.,' vol. lxxv, pp. 281–307, Pl. XXII, 1903.

29. Paehler, F.—"Ueber die Morphologie, Fortpflanzung und Entwickelung von Gregarina ovata," 'Arch Protistenk.' vol. iv, pp. 64–87, Pls. V and VI, 1904.

30. Perez, C.—"Le cycle évolutif de l'Adelea mesnili, coccidie cœlomique parasite d'un Lepidoptère," op. cit., vol. ii, pp. 1–12, Pl. I, 1903.

31. Prowazek, S.—"Zur Entwickelung der Gregarinen," op. cit., vol. i, pp. 297–305, Pl. IX, 1902.

32. Sars, M.—"Oversigt af Norges Echinodermer," Christiania, 1861.

33. Schaudinn, F.—"Generations- und Wirthswechsel bei Trypanosoma und Spirochæte," 'Arb. a. d. kaiserl. Gesundheitsamte,' vol. xx, pp. 387–439, 20 figs., 1904.

34. Schaudinn, F.—"Studien ueber krankheitserregende Protozoen. I. Cyclospora caryolytica, Schaud," op. cit. vol. xviii, pp. 378–416, Pls. XII–XIII, 1902.

35. Schneider, A.—(Various memoirs on "Gregarines nouvelles ou peu connues"), 'Tabl. Zool.,' vols. 1 and 2, Poictiers, 1886—1892.

36. Siedlecki, M.—"Ueber die geschlechtliche Vermehrung der Monocystis ascidiæ," 'Bull. Ac. Cracovie,' 1900, pp. 515–537, Pls. I and II.

37. ——— "Étude cytologique de la Coccidie de la Seiche," 'Ann. Inst. Past.,' vol. xii, pp. 799–836, three plates, 1898.

38. ——— ''Étude cytologique de Adelea ovata, Schn.," op. cit., vol. xiii, pp. 169–192, Pls. I—III, 1899.

39. Wilson, E.—"The Cell in development and inheritance," 'Columb. Univ. Biol. Ser.,' vol. iv, 1900.

40. Woodcock, H. M.—"On Cystobia irregularis (Minch.) and allied, 'neogamous' Gregarines" (preliminary note), 'Arch. Zool. exp.,' N. et. R. (4) vol. ii, pp. 125–128, 1904.

DESCRIPTION OF PLATES 1–6,

Illustrating Dr. H. M. Woodcock's paper on "The Life-Cycle of 'Cystobia' irregularis (Minch.), together with Observations on other 'Neogamous' Gregarines."

All figures with a high magnification were drawn as the sections appeared under either the Zeiss apochrom. 1·40 N.A. 3 mm. lens, or the similar 2 mm. one, with different compensating eyepieces. All figures, excepting fig. 6, were drawn with the aid of the camera lucida.

Reference Letters.

a. or *am.* Loose wandering cells, amœbocytes, etc. *a. g.* Albuminoid granules (lenticular grains). *bl.* or *bl. v.* Blood-vessel. *c.*, c^1. Layers of connective tissue. *c. a.* Nuclei of cellular aggregation. *c. e.* or *c. ep.* Cœlomic epithelium. *c. n.* Nuclei of same. *c. w.* Cyst wall. *cypl.* Cytoplasm. *e.* Outer epithelial layer. e^1. Inner invaginated one. *ect.* Ectocyst. *en.* Endocyst (or endospore). *ep.* Attaching epithelium. *ex.* Exospore. *f.* Fragments of karyosome. *fl.* Fluid contents of lumen. *f. l.* Fibrous layer of tissue. *G.* Gregarine. *g.* Albuminoid grains or lumps. *i. m.* Common or investing membrane. *k.* Karyosome. *l.* l^1. Limits of cup-like investment. *lum.* (or *l.*). Lumen or cavity. *L. M.* Longitudinal muscle. *l. m.* Limiting membrane. *m.* or m. *l.* Muscle layers. *mes.* Mesentery attaching gut. *N.* Nucleus (of parasite). *n.* n^1. Nuclei of epithelial layer. *n.* of *c. w.* Nuclei of cyst-wall (vascular epithelium). *p.* Tip of protrusion. *p. g.* Paraglycogen grains. *r. g.* Minute refractile granules. *ret.* Reticulum. *s.* Septum. *s. r.* Sporal residuum. *sp.* Spores. *sp. c.* Spongy cells (loose tissue). *sp. n.* Sporoblast nuclei. *st.* or *A. st.* Stalk of

attachment (epithelial). *t.* Tongue of investing membrane. *T. M.* Transverse muscles. *vac.* Vacuoles. *v. s.* Vascular strands. *z.* Special zone of the nucleoplasm.

PLATE 1.

Mode of life and general appearance, "Cystobia" irregularis and "C." minchinii.

Fig. 1.—C. irregularis in the blood-vessel of Holothuria forskali; outline drawn alive, the details filled in after mounting and staining. (The nuclei of the wall rather diagrammatic). Osmic, picro-carmine. × 80.

Fig. 2.—Ditto, obtained free of the blood-vessel; drawn alive. × 100.

Fig. 3.—Ditto, younger stage. Mounted whole. Osmic, picro-carmine. × 150.

Fig. 4.—Portion of diverticulum of respiratory tree of Cucumaria pentactes, showing seven C. minchinii *in situ*; viewed whole. (In two cases two of the parasites overlap, but they are not actually in contact.) Flemming, para-carmine. × 80.

Fig. 5.—Two young evaginated couples of C. irregularis (*n.* is the second nucleus beginning to be cut through, *ep.* epithelial sac in which the parasite lies). (A) Seen whole; corr. subl. + acetic, para-carmine × 80; (B) in section; corr. subl. + acetic, iron-hæm. + orange. × 250.

Fig. 6.—(A) Portion of body-wall of Cucumaria pentactes, showing two C. minchinii attached. × 3. (B) The upper parasite of (a). × 12.

Fig. 7.—Portions of the wall (a) of a large distributing vessel, and (b) of a small cross-connection in H. forskali. From a section. Corr. subl. + acetic, iron-hæm. + orange. × 400.

Fig. 8.—Portion of a section through the wall of a vessel in the "rete mirabile." The actual diameter of the lumen is much less, but it is less obstructed by spongy tissue. Corr. subl. + acetic, iron-hæm. + orange. + 400.

Figs. 9 and 10.—Adults of C. minchinii attached to the cœlomic epithelium, which they have invaginated; (*b*) shows where this is reflexed internally, (*a*) being the part of the parasite still uncovered. Fig. 9 seen in optical section; corr. subl. + ac., carm.-alum; Fig. 10, as a transparency; Flemming, para-carmine. Both × 150.

Fig. 11.—Portion of retractor muscle, showing three C. minchinii completely encysted. The small one at (*a*) is most imbedded. × 80.

PLATE 2.

Mode of life and general appearance (continued). C. minchinii. (All figures except fig. 20 relate to this parasite.)

FIG. 12.—Inpushing adult, to show especially the bending inwards of the invaginated epithelium. (The cytoplasm has shrunk away a little from the limiting membrane.) Flemming, picro-carmine. × 250.

FIG. 13.—Ditto, with a protrusion (*x*) penetrating into a vascular strand. (Nuclei of parasite are rather shrunk.) 90 % alc., para-carmine. × 150.

FIG. 14.—Two sections of preceding. (a) Along line C—D; (b) along A—B. In the former the parasite is everywhere enveloped by the double layer of epithelium. Thionin + eosin. × 150.

FIG. 15.—Section of parasite encysted in connective tissue around retractor muscle. Corr. subl. + acetic, Klein. hæm. × 80.

FIG. 16.—Section of another one, more imbedded. The inner layer of epithelial nuclei distinctly seen at *a*. Flemming, iron-hæm. × 120.

FIG. 17.—Section through part of an invaginated parasite (*p.*), showing the peculiar amœbocytes with peripherally situated nucleus (*n.*) in the lumen of the stalk. Iron-hæm. + orange. × 700.

FIGS. 18 and 19.—Sections through parasites in the wall of the respiratory tree. Flemming, iron hæm. + orange. × 325.

FIG. 20.—Evaginated C. irregularis; mounted whole. Triple association. (The cytoplasm is rather retracted at one side.) Osmic + picro-carmine. × 50.

PLATE 3.

Structure of Diplocystis schneideri. Triple association in Diplocystis and "Cystobia"; precocious association.

FIG. 21.—Diplocystis schneideri, viewed whole. Flemming. × 50.

FIG. 22.—A section through a couple. Corr. subl. + acetic, iron-hæm. + orange. × 50.

FIG. 23.—Another whole specimen. The endoplasm has shrunk away from the outer layers (see text). Corr. subl. + acetic. × 50.

FIG. 24.—Ditto, triple association. The septal partitions are seen in two places. Flemming. × 50.

FIG. 25.—Another triplet, the members of which are not quite so intimately joined. Corr. subl. + acetic. × 50.

FIG. 26.—Nucleus of D. schneideri (from a section). *m.* Nuclear membrane. Corr. subl. + acetic, Klein. hæm. × 200.

FIG. 27.—Portion of the periphery of two couples of D. schneideri, showing the plane of junction (see text). *sp.* Space. (a) Klein. hæm., (b) para-carmine, after corr. subl. + acetic. × 600.

FIG. 28.—C. minchinii. Triple association. (The outer, free part of the parasite is rather irregular.) Flemming, para-carmine. × 120.

Fig. 29.—C. irregularis. An evaginated triplet. This parasite has a somewhat altered, unhealthy look. Osmic, picro-carmine. × 150.

Fig. 30.—The smallest example of C. irregularis observed; already evaginated. The cytoplasm is scanty and without any granules. Corr. subl. + acetic, iron-hæm. + orange. × 1000.

Figs. 31 and 32.—Ditto, of C. minchinii. Both in the wall of the respiratory tree. *n.* Nuclei of tissue-cells. Flemming, iron-hæm. + orange. × 1000.

PLATE 4.

Minute structure. Encystment and sporulation.

Fig. 33.—Different nuclei of C. irregularis to show the different degrees in the vacuolisation of the karyosome (seen whole). Osmic, picro-carmine. × 400.

Fig. 34.—Section through the nucleus of *c.* in the last figure (for description see text). Iron-hæm. + eosin. × 700.

Fig. 35.—Section through a typical nucleus (C. minchinii). Flemming iron-hæm. + orange. × 500.

Fig. 36.—Large nucleus and portion of surrounding cytoplasm of an encysted C. minchinii. Klein. hæm. + orange. × 400.

Fig. 37.—Portions of the periphery of two couples of C. irregularis, to show the plane of junction (see text). Corr. subl. + acetic, iron hæm. + orange. × 600.

Fig. 38.—(a) Periphery of C. minchinii to show cytoplasmic detail. (b) Portion of cytoplasm of C. irregularis. (a) 90 % alc., (b) corr. subl. + acetic; thionin + orange. × 600.

Fig. 39.—Section through sporulating C. irregularis from the lumen of the blood-vessel. Corr. subl. + acetic, iron-hæm. + orange. × 80.

Fig. 40.—Sporulating C. irregularis from same situation (seen whole). More advanced stage. (The nuclei of this specimen were better stained than usual, probably because it was not an "encysted" parasite.) Corr. subl. + acetic, para-carmine. × 100.

Fig. 41.—Section through encysted sporulating C. irregularis. *N.* and *n.* Large and small (sterile and germinal) nuclei. Corr. subl. + acetic, iron-hæm. + eosin. × 250.

Fig. 42. Ditto. This parasite was in a swollen, "spongy" evagination (see text), of which *w* is the wall. *x.* Amœbocytes and blood-cells. Flemming, iron-hæm. + orange. × 120.

Fig. 43.—Entire sporulating cyst of C. minchinii, attached to a fibrous vascular strand. The cyst (seen in optical section) is full of ripe unstained spores. Corr. subl. + acetic, para-carmine. × 85.

FIG. 44.—Varying character of the encystment process in "Cystobia"; *e* is a section of C. minchinii, the rest being of C. irregularis (for description see text). (*N*. Nuclei of inner epithelial layer and of connective-tissue layers.) Iron.-hæm. + orange or eosin (except *e*, which is thionin + orange). All × 850.

PLATE 5.

Nuclear changes and multiplication. (All figures are from sections, and all relate to C. irregularis, except fig. 55, which is of C. minchinii.)

FIG. 45.—Commencing nuclear changes: *b*. is the other nucleus of the parasite. Corr. subl. + acetic, Klein. hæm. + eosin. × 300.

FIG. 46.—Two nuclei of the 8-nuclear stage. Iron-hæm. + orange. × 1150.

FIG. 47.—(*a*) and (*b*) Daughter nuclei of the 4th generation; (*c*) one of the third generation dividing. Corr. subl. + acetic, iron-hæm. + orange. × 850.

FIG. 48.—Stages in fragmentation of the daughter-karyosomes. All nuclei of the fourth generation. Corr. subl. + acetic, iron hæm. + orange. × 700.

FIGS. 49 and 50.—Commencing distinction of the multiplying nuclei into large sterile ones and small germinal ones.

(49) Thionin + orange
(50) Iron-hæm. + eosin } after corr. subl. + acetic. × 850.

FIG. 51.—Later stage. *a*. *b*. Resting sexual nuclei. *c*.–*f*. Dividing ones. *g*.—*j*. Centrosomic division. *k*. Sterile nucleus. (The arrows point to figures constructed from two or three sections.) × 850.

FIG. 52.—Stages in the disintegration of the large sterile nuclei. Flemming, iron-hæm. + orange. × 850.

FIG. 53.—(*a*) Equal distribution of the nuclei and commencing segregation of the cytoplasm into sporoblasts. × 175. (*b*) Part of periphery of similar cyst. Cytoplasm loose and full of chromatoid granules. Both corr. subl. + acetic, iron-hæm. + orange. × 850.

FIG. 54.—Cyst containing zygotes or copulæ, about stage *c*–*d*, fig. 58. No cystal residue. Corr. subl. + acetic, para-carmine. × 175.

FIG. 55.—Earlier stage in C. minchinii, showing the intertwining processes. (The connective tissue, etc., surrounding the cyst is not shown [see fig. 44 *e*.]). Corr. subl. + acetic, thionin + orange. × 175.

FIG. 56.—Corresponding stage in C. irregularis. Owing to the sporoblast nuclei (*n*) being less numerous and less closely arranged, the outlines of the segments are not quite so sharply defined. Corr. subl. + acetic, thionin + orange. × 175.

PLATE 6.

Conjugation and spore-formation. (All figs. except fig. 62 relate to C. irregularis.)

Fig. 57.—The isogametes (primary sporoblasts) and stages in their conjugation. From a crushed preparation. Flemming, para-carmine. × 600.

Fig. 58.—Fusion of the sexual nuclei in the zygote; condensation of the definitive nucleus and change of shape of the copula. From sections. Corr. subl. + acetic, para-carmine. × 600.

Fig. 59.—*a*, *b*. Formation of outer cyst-membrane (exospore); *d–j*. first division of zygote-nucleus. *a*, *b*. Whole from crushed preparations; corr. subl. + acetic, para-carm.; *c–j*. from sections (spore seen obliquely), corr. subl. + acetic, iron-hæm. + orange. × 600.

(The remaining figs. are from sections.)

Fig. 60.—Spores with two nuclei; in *b* only one is entirely in plane of section. Corr. subl. + acetic, iron-hæm. + orange. × 600.

Fig. 61.—Fully formed spores with four nuclei. (*c*. Cup or plug, closing mouth of inner funnel.) Corr. subl. + acetic, iron-hæm. + orange. × 850.

Fig. 62.—(*a*) Ripe spores of C. minchinii, with eight sporozoites. (*b*) Two sporozoites. Corr. subl. + acetic, iron-hæm. + orange. × 850.

Fig. 63.—(*a*) Degenerating primary sporoblasts; (*b*) degenerating copulæ; (*c*) polygamous copula. Flemming, para-carm. × 600.

Fig. 64.—Polynuclear masses from sporulating cysts. (*a*) About time of conjugation; (*b*) during spore-formation. Corr. subl. + acetic, iron-hæm. + orange. × 600.

The Anatomy of Oncholaimus vulgaris, Bast., with Notes on two Parasitic Nematodes.

By

F. H. Stewart, M.A., B.Sc., M.B.,
Lieut. Indian Medical Service.

With Plates 7—9.

I BEGAN this work with the intention of trying to ascertain if any light could be thrown on the comparative morphology of the cœlom and Nephridia by the study of Nematodes. The uncertainty as to the true nature of the body-spaces in this class is well known. No one has been sufficiently daring to identify any space in Nematodes with the cœlom. The space between the body wall and the gut is generally admitted to be a schizœle.

Ray Lankester (**11**, p. 9) writes: "In some few groups of Cœlomocœla the cœloms have remained small, and limited to the character of simple gonocœls. This seems to be the case in the Nemertina, the Planarians, and other Platyhelmia." The Nematodes are not mentioned, since nothing is definitely known in regard to them.

Thanks largely to the work of Jägerskiöld (**9**), we know that the excretory organs of Nematodes are unicellular, with intra-cellular canals, and, physiologically at least, interchangeable with skin glands, but they have not yet been homologised with Nephridia. Ray Lankester, in the same article (**11**, p. 14), writes: "True Nephridia are only found in the

Platyhelmia, Nemertines, Rotifers, Chætopods, and embryonic Mollusca."

In all text-books the Nematodes are placed in a very isolated position, and no attempt is made to compare them with other groups. This, I believe, is largely due to the fact that most work has been done on parasitic forms, and that these have been held up as the types of the group. But even although the great majority of a group were parasitic, it is to the free-living minority we should go for the type, and not to the secondarily modified majority.

I therefore selected for study a free form Oncholaimus vulgaris (Bast.), a parasitic form Ascaris clavata (Rud.) to compare with it, and an embryo of a parasite to connect the two, if possible.

No member of the genus Oncholaimus has been investigated by modern methods. I therefore have gone thoroughly into its anatomy.

In regard to A. clavata the alimentary and excretory systems have been fully described by Jägerskiöld (**9**). I have, therefore, limited my work to the reproductive system, while in the embryo I have confined myself to the excretory organs.

The specimens were all obtained at St. Andrews, and a large part of the practical work was done at the Gatty Marine Laboratory there. My thanks are due to Professor McIntosh for the use of the laboratory and for his kind assistance in many other respects.

Oncholaimus Vulgaris (Bast.)[1].

Measurements:

Male	.	Length	. 10—15 mm.
,,	.	Breadth (max.)	·135—·221 mm.
Female	.	Length	. 12—15 mm.
,,	.	Breadth	. ·18—·225 mm.

The body is elongated and cylindrical, tapering very slightly and slowly to the anterior extremity (Pl. 7, figs. 1

[1] Bastian (**2**).

and 2), which is square in profile, rapidly to the posterior, which forms a rounded and rather blunt cone (Pl. 7, fig. 5).

The animal is quite translucent; the only pigment present is in the intestinal wall, golden-brown granules arranged in patterns, which produce a tesselated appearance, and are sometimes sufficiently abundant to cause the animal to appear of a brown colour, even to the naked eye.

The mouth is terminal, and is surrounded by three very flat papillæ (Pl. 7, fig. 2, *a*).

Immediately behind the latter is a ring of short setæ (ibid., *b*), while smaller hairs are scattered over the anterior portion of the body, and arranged along the dorsal (*c*) and ventral lines.

The anus (Pl. 7, fig. 5, *cl. ap.*) is subterminal, ·153 mm. from the tail, in the midventral line.

The cellular character of the longitudinal lines (Pl. 7, fig. 1, MDL, LC, MVL) and the striation of the muscle fields (Pl. 7, fig. 5) are obvious. Prominent objects also are the cup-shaped pharynx, with its three teeth (Pl. 7, fig. 2); the œsophagus (Pl. 7, fig. 1); surrounded by the œsophageal ring and collar of ganglion-cells (Pl. 7, fig. 1); the pigmented intestine; the hyaline ducts of the tail glands filling up the post-anal region (Pl. 7, fig. 5); the ventral gland in the male, opening ·112 mm. in front of the nerve-ring; and the large cells of the body space which generally lie in the submedian lines (Pl. 7, fig. 1, *a*).

The gonads occupy a large part of the body in either sex, but are more conspicuous in the female; the large chalk-white ova (text-fig. 2, *ov.*, p. 124) in the uterus can be distinguished even with the naked eye. Under a low power the central vulva(*vu.*) surrounded by the cellular mass of vulvar and vaginal glands and the elongated ovaries (*ovr.*) running alongside the uteri can be seen.

In the male the gonads appear as a fairly uniform cellular cylinder opening posteriorly in common with the gut. Here also are to be found the sabre-shaped spicules (Pl. 7, fig. 5, *sp.*) with their central accessory piece.

It is convenient in describing the animal to divide the body according to the regions of the alimentary canal. I shall, therefore, frequently refer to pharyngeal, œsophageal, intestinal, rectal, and post-anal regions. The œsophageal region it is convenient to divide into anterior œsophageal in front of the nerve-ring, posterior œsophageal behind it.

Habitat.—Oncholaimus vulgaris is very common under stones between tide-marks. It is essentially a sociable animal, twenty to thirty being often found together under one stone; it is not usual to find individuals isolated.

The Cuticle.

The cuticle forms a continuous layer over the whole body, as in all Nematodes, and passes in through all the apertures, mouth, anus, cloacal opening, and vulva to become continuous with the cuticular linings of the cavities into which these apertures open.

It is a structureless membrane ·00215 mm. thick, and with certain stains shows a division into two layers (Pl. 7, fig. 15). Hairs occur in certain localities: (1) in a circle round the head at the base of the lips; (2) scattered over the anterior œsophageal region, and (3) arranged along the mid-dorsal and mid-ventral lines (Pl. 7, figs. 1 and 2). They are more abundant in the dorsal than in the ventral line, and in the œsophageal than in other regions of the body. The numbers vary considerably in different individuals, but an average would give about forty for the dorsal and twenty for the ventral line. In the female several occur in front of and behind the vulva.

Each hair is formed by a projection of cuticle, while at the base the cuticle is perforated by a minute canal containing a core of protoplasm passing out from the epidermis. The hairs along the median lines spring from the centre of shallow depressions in the cuticle.

The canals at the roots are, as pointed out by Jägerskiöld (**10**), identical with the integumental pores of Bastian (**2**).

In prepared specimens the hairs are very frequently broken off.

The cuticle is also perforated by the openings of the ventral and tail glands (Pl. 7, fig. 5, *tg.ap.*) and of the glands of the lateral lines.

From the base of the lips four flattened pouches (Pl. 7, fig. 2, *e*) extend outward and backward in the substance of the cuticle as far as the oral circlet of hairs. They are semicircular in shape, the base directed toward the mouth, the arc away from it. At the base of the lips the pouches are continuous with each other, and here the four pore-like openings to the exterior are situated.

The pouches contain a number of coarse granules with amphophil staining reaction. I have not been able to trace any connection between these granules and the epidermal protoplasm.

From the nature of the contents I believe the function of these structures to be glandular. They appear to correspond, not with the "Seitenorgane" of other forms, but with the circumoral "patterns" and glands described by De Man in O. fuscus (**12**).

The Epidermis and Nervous System.

It is hardly possible to separate these two. The only structure which is definitely specialised as nervous is the circumœsophageal ring; other structures which are nervous in function, such as the ganglionic mass which surrounds the ring and the anal ganglion, shade off into the general epidermis. I shall, however, describe the latter first.

The epidermis consists of four lines of cells—the longitudinal lines (Pl. 7, fig. 3, MDL, MVL, LL), which run from one end of the body to the other, and which project into and divide the muscular layer of the body wall, and of a thin layer of protoplasm—the subcuticula or hypodermis (Pl. 7, fig. 3, *sc.*), which connects these four lines, and lies between the cuticle and the muscular layer. This consists merely of

an outgrowth of protoplasm from the cells of the longitudinal lines and contains no nuclei. The longitudinal lines are, in fact, situations where the epidermal nuclei are aggregated, and where the nutrition and general government of the entire epidermis is carried on.

I have described the lines as cellular, but although cell-walls do occur, they are often not complete, the protoplasm of one cell being at one point continuous with that of another, or several nuclei may appear in one mass of protoplasm, and as no cell-walls are to be found in the subcuticula, all the cells whose protoplasm is continuous therewith are presumably in this way continuous with one another, although this layer is so thin that divisions might exist in it but be undemonstrable.

The four longitudinal lines lie, as usual, one mid-dorsal, one mid-ventral, and two lateral, dividing a transverse section of the body-wall into four quadrants. They extend, as I have stated, from one end of the body to the other. Their absolute and relative breadth varies in the different regions. In the œsophageal regions all four lines are of fairly equal breadth, and occupy each about one fourteenth of the circumference, each showing about four cells in transverse section.

In the intestinal region (Pl. 8, fig. 24) the median lines decrease in size, showing only one or two cells in section; the lateral lines, on the other hand, are considerably broader and occupy each about one eighth of the circumference. Opposite the gonads the lines are very much flattened from pressure. Behind the anus they are of approximately equal breadth, and each occupies one eighth of the circumference.

Two types of cells occur—viz. (1) cells whose shape varies from square to oval, and whose greatest diameter varies from 0·0086 to 0·0107 mm. The protoplasm is fairly abundant and is non-granular. The nucleus, spherical or oval, measures from 0·0043 to 0·00538 mm., and has a well-marked nuclear membrane containing numerous minute chromatin granules and a nucleolus (Pl. 7, fig. 9). (2) Rather smaller cells,

0·0043 mm., varying in shape, but more generally circular in sections, the protoplasm scanty, nucleus spherical or oval, 0·0032 mm., and staining diffusely with basic dyes (Pl. 7, fig. 8, T_2). In addition the lateral lines contain gland-cells, but these will be described separately. Type 1 is most common.

Where there is not much pressure from the bulk of internal organs, as in the œsophageal and post-anal regions, the cells project into the space between the body-wall and gut. Opposite the gonads, however, they are very much flattened. In the posterior œsophageal region Type 1 cells often show vacuolation; while in the median lines in the intestinal region they are generally triangular on section, the apex directed to the cuticle, and the protoplasm shows fibrillæ passing in from the subcuticula to surround the nucleus.

The submedian lines (Pl. 7, fig. 13, *s.m.l.*) are not epidermal, but are merely mesodermal partitions between groups of muscle-cells.

Jägerskiöld (**10**) describes epidermal sub-median lines in neighbourhood of the nerve-ring in Cylicolaimus magnus; nothing corresponding is present in our animal. In Thoracostoma acuticaudatum he mentions sub-median lines, but does not make it clear whether they are cellular. The subcuticula is an excessively fine sheet of protoplasm; in fact, in places it is so fine that it is impossible to demonstrate it. It is continuous with protoplasm of the cells at the margin of the longitudinal lines.

De Man (**12**) in reference to the genus Oncholaimus and more particularly to the species O. fuscus, describes the subcuticula as richly cellular and granular. Neither of these statements applies to O. vulgaris.

I have used the term "epidermis" freely. I see no reason why this structure in Nematodes should be veiled under terms such as "hypodermis," etc., Wandolleck (**15**), Jammes (**7**), and Hamann (**4**), are agreed as to its ectodermal origin. The only authority who maintains its meso-dermal nature is Zur Strassen (**14**).

The nervous system consists of the circumœsophageal ring and ganglionic collar and of the ganglion in the wall of the rectum or cloaca. These are the only structures which are in any way separated from the general epidermis.

The nerve-ring (Pl. 7, fig. 4, *nr*) lies at the junction of the first and second thirds of the œsophagus, and consists of fine fibrillæ united into a bundle. It contains a few nuclei, 4 to 6, which resemble those of the Type 1 cells of the epiderm, but are somewhat smaller. A sheath of fine connective tissue derived from the fibrillar stroma which here fills the space between the body-wall and the gut encloses the ring. The sheath stains more deeply than the nerve-fibrils. Processes which in places can be shown to be hollow pass off from the sheath, and join the connective tissue surrounding the cells of the collar and the muscle-cells. I have in a few instances found nerve-fibrils in these hollow processes, but they are so fine that it is not possible to follow them for any distance. They probably connect the ring with the collar-cells and the longitudinal lines.

The circumœsophageal collar (Pl. 7, figs. 1 and 3) extends from nearly the commencement of the œsophagus to a short distance behind the nerve-ring, and is composed of cells growing in from the longitudinal lines. It completely fills the space between the body-wall and œsophagus. In preparations of the entire animal it can be clearly seen as a compact cylinder of cells.

The cells are of three types, viz. cells of the same character as Types 1 and 2 of the longitudinal lines, and in addition large oval cells (Pl. 7, fig. 3, bc^1) ·016 mm. in diameter, with abundant protoplasm which may show very faint, irregular, basophile markings, the nucleus ·0064 mm. with a definite nuclear membrane, finely granular acidophil contents, the chromatin aggregated into a large spherical pseudo nucleolus. These cells are, I believe, identical with the basophil cells which occur in the space between the body-wall and the gut in other regions of the body; they differ from them, however, in some points, and as I can only speculate in regard to their

functions, I shall discuss them under the section dealing with this space and its contents, rather than include them in the nervous system, to which they may not belong.

Yet another type of cell, the coarsely granular acidophil, occurs among the collar-cells as well as elsewhere, but is certainly not nervous.

The fibrillar groundwork binds together all these various units.

De Man (**12**) for the genus Oncholaimus describes numerous cells lying in the body cavity in front of and behind the nerve-ring.

Bastian (**2**) also mentions their presence.

Jägerskiöld (**10**) for Cylicolaimus magnus and Thoracostoma acuticaudatum describes and figures what is evidently an œsophageal collar; the cells shown in his drawings are, however, not so numerous as those in our subject. He frankly states that he has not examined the matter fully, and he does not connect them in any way with the nervous system, but considers them to be the same as the phagocytic "büschelförmige organ" described by himself, Nassonow (**13**), and others in parasitic forms. This opinion, which I believe to be an error, accounts for the fact that he also considers them to be identical with the "floating gland-cells" of Bastian and the "fat cells" of De Man. These cells occur isolated in the "body cavity" through all regions of the body, and are entirely different from this localised compact structure.

In parasitic Nematodes also a cellular investment to the nerve-ring is found; *e. g.* Hamann describes and figures it in Lecanocephalus (**5**) as connected with all four longitudinal lines, and the same holds good for the embryo (A. capsularia) which I shall describe later.

The anal and cloacal ganglia (Pl. 7, fig. 6, *ag.*) are also formed by ingrowths of cells from all four longitudinal lines. Shortly before reaching the level of the anus in the female, or of the cloacal opening in the male, cells begin to project inwards from the dorsal and the two lateral lines, passing

towards the dorsal wall of the anal canal or cloaca. Here they form a layer one cell deep, lying on the cuticular lining of the space, and continuous at the sides with the protoplasmic wall. Since the latter is continuous with the cells of the ventral line, this line may also be presumed to take part in the formation of the ganglion. The cells are of Type 1.

The only sensory organs to be found are the hairs above described. They are, as above noted, specially aggregated on the head and in front of and behind the genital apertures.

To sum up, the nervous system is very imperfectly differentiated. The circumœsophageal ring and collar form the brain of the animal; the longitudinal lines, and possibly the subcuticula as well, form the conducting paths, both motor and sensory, in the latter capacity receiving stimuli from the sensory hairs. I have not found processes from the muscle-cells to the lines such as occur in other forms; the motor mechanism is, therefore, obscure. The anal and cloacal ganglia doubtless control defæcation and copulation.

The Excretory and Glandular Apparatus.

Three sets of glands are included in this system:

(1) The single excretory ventral gland.

(2) The series of glands of the lateral lines.

(3) The three tail glands.

These glands resemble each other in being all unicellular. The ventral and tail glands have ducts of considerable length, formed by outgrowths of the protoplasm of the cells; the glands of the lateral lines have no ducts but open directly through the cuticle.

The ventral gland[1] occurs in males and immature females; it is absent in mature members of the latter sex. It is composed of one large cell, which lies in the body cavity immediately ventral to one of the lateral lines, in males on

[1] Golowin (3) has described this gland in O. vulgaris. I have not been able to procure a copy of his paper, which is, I presume, in Russian. He regards the three "Keimdrüsen" of the tail as of the same nature as this gland.

the right side, in immature females on the left. The cell body, which forms the secreting portion of the gland, is found at a level a little behind the commencement of the intestine; from it a duct runs forward to the level of the nerve-ring, and bending towards the midline, opens by a minute pore in the median ventral line, ·112 mm. in front of the ring.

The cell (Pl. 7, fig. 12) is of a flattened oval shape. The nucleus is central, and contains one large pseudo-nucleolus. The protoplasm is hollowed out by large vacuoles, which in some of the specimens contain numerous large basophil granules arranged round their periphery.

The duct (Pl. 7, fig. 4, *vgd.*; Pl. 7, fig. 13) is narrow and cylindrical. It has a fine protoplasmic wall, which is apparently an outgrowth from the gland-cell. The contents are homogeneous, and stain with basic dyes. Near its termination the wall of the duct becomes continuous with the cells of the median ventral line, which separate, continuing the lumen to the pore in the cuticle.

The glands of the lateral lines lie in series at the margins of the lateral lines. They are found as far forward as the posterior limit of the œsophageal collar and as far backward as the commencement of the rectum.

They consist of large, pear-shaped cells (Pl. 7, fig. 7), with the pointed ends directed to the cuticle. The outlines are sharply marked off from the other cells of the lateral lines.

Each cell is filled with large rounded granules, with amphophil staining reaction. The nucleus lies toward the rounded extremity, has a nuclear membrane, chromatin granules, and true nucleolus. From the pointed end a minute duct leads through the cuticle, filled with a hyaline substance which frequently projects beyond the surface as a small spike. The glands are identical with those described by Jägerskiöld (**10**) in Cylicolaimus magnus.

The tail glands are three in number. In preparations of the entire animal they are exceedingly conspicuous as hyaline, club-shaped masses, extending from the extremity to some distance in front of the anus (Pl. 7, fig. 5, *tgd.*;

Pl. 7, fig. 6), the middle gland extending further forward than the other two.

In the posterior intestinal region they lie ventral to the intestine, in males in the grooves between the intestine and the ductus. They pass on either side of the rectum, and behind the level of the anus occupy almost the entire space within the muscular wall. They open by a single pore on the tip of the tail. On section, the protoplasm shows a highly vacuolated appearance, the contents of the vacuoles staining very uniformly, suggesting a colloid. The nucleus resembles that of the ventral gland.

The duct has a very fine membranous wall; the contents are basophil, sometimes acidophil. The three ducts remain separate until a point immediately before the external pore. There is, however, only a single such pore.

Muscle of the Body Wall.

The muscular layer is the thickest part of the body wall. The cells which compose it are arranged in eight longitudinal fields, four on each side, dorsal, dorsolateral, ventrolateral, and ventral (Pl. 7, fig. 3, DM, DLM, VLM, VM), separated from each other by the four epidermal longitudinal lines and by the four submedian lines. The latter, as I have stated above, are not epidermal in O. vulgaris, but are merely mesodermal partitions.

The entire eight fields extend forward to the anterior extremity; only four, the two dorsolateral and the two ventrolateral, reach the posterior, the two dorsal and the two ventral ending at the level of the anus. In transverse section the fields at the different levels show on the average the following number of cells:

	Buccal Region.	Œsophageal.	Intestinal.	Post Anal.
Dorsal	1	2	1–2	0
Dorsolateral	4	7	13	7
Ventrolateral	6	7	14	6
Ventral	2–4	6	6	0

The muscle-cells are of the usual Nematode type. I have

not been able to detect any of those fibre-like projections from the undifferentiated portions of the cells to the longitudinal lines which occur in other forms, and no doubt act as nerves.

The Body Space.

The interval between the body-wall and the gut is, in sections, found to be occupied by a substance the characters of which vary in different regions. The gonads, glands, etc., are imbedded in it.

As the subject is a somewhat contentious one, I shall first describe the substance in question and then discuss its nature.

It extends through almost the entire length of the body; the only regions in which it is impossible to prove its presence are at the level of the pharynx and behind the anus. It naturally varies in development according to the space to be filled, is most abundant around the termination of the œsophagus (Pl. 7, fig. 13) and around the rectum, where a considerable interval occurs between the alimentary tract and the body wall. It is also fairly abundant where a large organ such as the ovarian cæcum or testis ends.

In that part of the œsophageal region which lies behind the nerve-collar it forms a fibrillar network (Pl. 7, figs. 8, 10, and 13 *m.*) The fibrillæ are tortuous, but their general direction is from the muscular layer inward to the œsophagus. In places the meshes between the fibrils are circular, as if they had been occupied by globules of some substance. Over the outer surface of the œsophagus the fibrils interlace, forming an irregular membrane, while the same occurs over the internal surface of the cells of the body-wall, muscular and epidermal, the interwoven fibrils applying themselves closely to these cells, or passing in between the muscle fields at the submedian lines, and to a lesser extent between individual muscle-cells. At the submedian lines, indeed, they reach the epidermis.

They stain intensely with nigrosin, and also take up eosin, but with less avidity.

Nuclei occur in the tissue (Pl. 7, fig. 8, *mn.*), but are not

common. They generally lie opposite the longitudinal lines, but also occur opposite the muscle fields. They resemble the nuclei of the Type 2 epidermal cells, staining diffusely with basic dyes, measure ·00215 to ·00322 mm. in diameter—that is, rather smaller than the nuclei of Type 2. I have not been able to find any protoplasm surrounding. They are completely isolated from the epidermis by the fibrils.

In the region of the collar, owing to the presence of the ganglionic cells, the tissue is not so much developed (Pl. 7, figs. 3 and 4). Its characteristics are the same as already described, but as it forms a stroma for the collar cells, the general direction of the fibrils is rather parallel with the body-surface than radial, since these cells are growing in from the epidermal lines across the muscle fields.

In front of the collar the tissue is still less developed, but other tissues, such as the body-wall, are also somewhat meagre as they approach the pharyngeal region.

I believe that this fibrillar network has a very definite function—viz. that of connecting the outer surface of the œsophagus with the body-wall and affording a surface of origin for the œsophageal muscle, so that when this contracts the entire value of the contraction is devoted to widening the lumen. In parasitic forms a similar surface is provided by the thick cuticle which surrounds the œsophagus.

Throughout the greater part of the intestinal region, owing to the close approximation of the gut and body-wall, and to the presence of the gonads, the space is narrow. The substance filling it is best studied in the interval, triangular in cross section, between the gut, body-wall, and gonad tube, or where the gonad tubes end, but it must not be imagined that it is confined to these regions, since it forms a complete, although narrow, cylindrical covering for the gut.

In sections through this region stained with thionin and eosin (Pl. 7, figs. 14, 15) a dull-pink hyaline ground-work occurs with a very fine, more intense pink, granulation. The very fine, obscure fibrillation which is found in the protoplasm of the epidermis or of the gonad tube-wall is not present.

In sections stained with nigrosin and in some stained with eosin a few fibrillæ can be detected. They are, however, much finer than those of the œsophageal region, and strongly resemble fibrin filaments.

Nuclei (ibid., *mn.*) occur in this matrix identical with the nuclei in the œsophageal region—·00215 to ·00322 mm. in diameter. Around them is sometimes a thin film of protoplasm which stains more distinctly than the matrix, but which has not a sharp line of demarcation from it. It can be demonstrated that these nuclei have no connection with either epidermal or muscular cells, with gut or gonads, or with either of the types of cell which will be described later which lie in the body-space. They are often to be found lying completely isolated and free in the matrix.

At the commencement of the intestine the œsophageal fibrillar network does not end abruptly, but the fibrillæ become gradually more and more scanty.

In this situation, and near the termination of the intestine, muscular fibres traverse the space, running almost longitudinally. They are passing very obliquely from the body-wall to the gut.

The interval between the narrow rectum and the body-wall is considerable, but here the connective tissue is largely replaced by muscular fibres and by the ingrowth of epidermal cells to form the anal ganglion. There is, however, a basis of fine fibrils.

In the post-anal region also the epidermal cells project inward to such an extent that it is difficult to say how much of the fine tissue surrounding the duct of the tail glands is epidermal and how much, if any, is mesodermal.

Two types of cell occur imbedded in this substance:

(1) The coarsely granular acidophil cell (Pl. 7, fig. 3), oval in outline, maximum diameter ·01076 mm. with a distinct cellular membrane. The protoplasm does not stain, but contains numerous large spherical acidophil granules. The nucleus is central, and stains rather diffusely, although it shows some granulation (chromatin). These cells occur most

frequently opposite the epidermal and submedian lines, but also opposite the muscle fields. They are to be found throughout the entire length of the body with the exception of the pharyngeal region.

(2) Rather flattened cells (Pl. 7, fig. 10, bc^2) generally crescent-shaped in transverse sections of the animal, owing to compression between the gut and the body-wall. Maximum diameter, ·02152 mm. The protoplasm contains a basophil substance. The nucleus is more or less spherical, has a nuclear membrane, and finely granular acidophil contents. The chromatin is aggregated into a large spherical pseudo-nucleolus.

These are the cells referred to above as possibly identical with the third type of cell in the collar. They differ from this type, however, in the fact that the basophil markings in the protoplasm are much more extensive and constant, and that the material filling the nucleus is definitely acidophil, not amphophil.

They occur most commonly opposite the submedian lines, but also opposite the muscle fields. Commencing at the posterior limit of the collar, they are found as far back as the rectum. As to function, they do not appear to be phagocytic; they are possibly nervous.

The only previous reference to an organised tissue filling the body space in Nematodes is on the part of von Linstow (**11** *a*), who describes a "plasma cylinder" surrounding the gut in Thoracostoma denticaudatum (Schn.) and Enoplus edentatus (v. Linst.) "von dem dorsal und ventral je zwei Leisten ausstrahlen." His figures, however, show no nuclei in this "cylinder." Jägerskiöld (**10**) for Cylicolaimus magnus writes: "Hie und da findet sich zwischen dem Darme und der Muskulatur eine ganz homogene, bisweilen sich stärkfärbende Schicht. Ich kann sie nur als eine Art coagulierter Flussigkeit deuten, denn alle Kerne fehlen. Vielleicht ist es sogar nicht allzu gewagt zu vermuten dass diese Flüssigkeit, die, wie angedeutet wurde, nicht überall oder gar immer vorhanden ist, nur als folge kräftigen Ergreifens mit der Pincette oder dergleichen kleinerer Verletzungen exsudiert worden. Denn dass die Thiere normalerweise

eine freiströmende Leibes flüssigkeit zeigen, habe ich, wie schon erwähnt, nie bemerkt." He suggests that v. Linstow's "plasma cylinder" is such a coagulated fluid, the "leisten" the submedian lines. As mentioned above, however, he describes the fibrillar stroma supporting the collar, but he apparently has not observed that it extends beyond the limits of the cellular collar—if it does so in Cylicolaimus magnus, or that there are any nuclei present apart from those of the collar cells. He does not attach any morphological importance to it.

To commence, then, I may repeat that there is no doubt that the body cavity in the œsophageal region is occupied by a nucleated fibrillar stroma.

The nature of the substance filling the rest of the body cavity is debatable.

There are three views which may be taken in regard to it—

(1) That it is a pathological exudate coagulated by the fixing fluid.

(2) That it is a physiological body cavity fluid, coagulated in the same way, and containing cells.

(3) That it is a mesenchyme tissue of rather low organisation.

(1) This is the view taken by Jägerskiöld, and his reasons for it have been quoted above. But in dealing with specimens preparing for sectioning I have never, until they were securely fixed, used any coarser instrument than a camel-hair brush, so that I can see no reason why a pathological exudate should be present.

It is, of course, not at all necessary to suppose that C. magnus and O. vulgaris are identical in this respect; but as they resemble each other very markedly in other points, it would be natural to expect that they should also resemble each other in this highly important morphological point.

Jägerskiöld states that the substance in question is only present in certain localities, and that it is non-nucleated. I have found it present in almost every region of the body and continuous, and it contains nuclei proper to itself.

(2 and 3) The substance when stained with eosin has a

certain resemblance to a coagulated albuminous fluid, but it should be contrasted with, e. g., the cœlomic fluid of annelids, as seen in section. The latter, if stained with nigrosin, appears as an exceedingly loose reticulum of very fine fibrils, which do not stain very intensely; the former, in some places, shows a very fine fibrillation, with a homogeneous background, in others stains intensely and evenly. I would not, however, lay much stress on this, since I know of no test which would enable us with the microscope to differentiate a coagulum from a lowly organised jelly. On the other hand, a coagulum formed in a fluid ought to contract, and not completely fill the space occupied by the fluid; the substance in question completely fills the space, while the thickness of the walls lining the space forbids the idea that they might have contracted on a loose coagulum.

The presence of nuclei and their character is the strongest argument in favour of the third view. Any nuclei occurring in a fluid must belong to floating cells. Such cells would probably be amœboid, and would almost certainly have a reasonable amount of protoplasm and definite cell outlines. The nuclei which I have found, on the contrary, are either entirely naked or have a very fine pellicle of protoplasm, which shades off into the surrounding matrix. This, I think, suggests that they are connective-tissue nuclei rather than the nuclei of wandering cells. They are also identical with the nuclei of the stroma in the œsophageal region.

For these reasons I would put forward the view that the body cavity is filled by a mesenchyne, which in the œsophageal region takes the form of a nucleated fibrillar network in the regions behind the œsophagus of a jelly-like substance, also nucleated. The reason for the difference in character of the tissue in the œsophageal and other regions is, that in the former it has a special function, which I have explained above. At the same time, I put this view forward only tentatively. It would be rash to speak dogmatically on so important a point without having thoroughly investigated a number of allied forms.

Alimentary System.

The alimentary system is divided into pharynx or buccal cavity, œsophagus, intestine, rectum, and cloaca in the male, anal canal in the female.

The pharynx (Pl. 7 , fig. 2, *ph*.) is cup-shaped, narrowing at its hinder extremity ·085 mm. long, ·034 mm. in diameter.

The mouth is surrounded by the diaphragm-like ring of the lips. In life the lips are in constant motion.

The cavity of the pharynx is shamrock-shaped in transverse section. It has a cuticular lining, and from this lining there project into it three large teeth (ibid., *d*.) composed of the same substance, one lying in the dorsal line, the other two subventral. At the tips of these teeth are situated the openings of the œsophageal glands. Outside the cuticle is a fine membrane connected with the cells of the longitudinal lines.

The œsophagus (Pl. 7, fig. 1, *oes*.) is 1·3 mm. in length. Its general shape is that of an heraldic club. In diameter it measures, at its commencement, ·0525 mm., at the level of the nerve-ring the same, and at its broadest part posteriorly ·0862 mm.

The walls are thick and muscular, the direction of the fibres radial. When at rest the internal surfaces are in apposition and the lumen appears in cross section tri-radiate—one radius in the midventral line, the other two subdorsal. There is a cuticular lining considerably finer than that of the pharynx.

The organ is essentially a strong suction pump, since the radial muscle-fibres in contracting must open out the lumen with considerable force. This appears a somewhat anomalous organ for a free-living form.

The œsophageal glands occur in the posterior quarter of the organ, as canals ramifying in the muscular substance. They unite to form the ducts, which run forward and open into the pharynx at the tips of the teeth.

The only evidence of a secreting epithelium is to be found in the presence of cells with finely granular protoplasm in the lumen of the ramifying canals. The lumen also contains numerous sharp spherical granules which stain intensely with acid dyes.

The ducts (Pl. 7, figs. 3–4, *oes. d.*) also lie in the muscular wall, alternating with the radii of the œsophageal lumen, one dorsal, two sub-ventral. They are elliptical in cross section, and have a very fine protoplasmic lining. They also contain the same granular material.

On leaving the œsophagus, the ducts lie along the pharynx, external to the cuticle, between two layers of the membrane referred to above. This membrane also supplies them with a lining as they pass up the centre of the teeth to their openings.

The intestine is about 11·5 mm. in length, cylindrical, except where it is compressed by the reproductive organs. In the living animal it has a tesselated appearance from the patterns formed by numerous golden-brown globules contained in its wall.

The wall is formed of columnar epithelium, from twenty-four to thirty cells occurring in a transverse section. The cells vary in depth from 0·015 mm. near the commencement, to 0·008 mm. near the termination. Their protoplasm does not stain, and contains the golden-brown globules referred to above. In certain of the cells numerous coarse acidophil granules occur, strongly resembling those of the coarsely granular acidophil cells of the body space, but, on the average, slightly smaller. The cells containing these granules are distributed in what appears to be an entirely capricious manner, generally wedged in between cells entirely free from granules.

I attempted to discover if there was any relation between these cells and the granular cells of the body space, but was unable to find any. The two kinds of cells do not occur in apposition, or even, as a rule, in close proximity, and there is no correspondence between the frequency of occurrence of the two in different animals or in different regions of the same

animal. As to the nature of the granules there is no clear evidence. If they represented digested food in the process of absorption, a more uniform distribution should occur. They are probably either a digestive secretion produced in cells which, although specialised, show no other signs of specialisation, or excretory. There is no sign of a basement membrane external to the epithelium.

The contents are very meagre, apparently portions of small Algæ, but one fact is very striking—viz. the presence in the adult female of large masses of spermatozoa, which have found their way in through the gonenteric canals.

The rectum is a short tube leading from the intestine to the anal canal or cloaca. Its width, including walls, is 0·016 mm. The wall has an outer layer of circular muscle-fibre and an internal epithelium, while ganglion-cells which have grown in from the longitudinal lines are to be found on the dorsal surface. It terminates abruptly on joining the cloaca or anal canal. The former I shall describe with the male reproductive organs; the latter is a very short invagination of epidermis with cuticle, in the dorsal wall of which the anal ganglion lies.

Male Reproductive Organs.

The male reproductive organs (text-fig. 1) consist of two testes (*at.*, *pt.*), an anterior and a posterior, lying in two cavities, the testicular regions of the reproductive tubes (*atr.*, *ptr.*). The testicular regions open into a single ductus ejaculatorius (*de.*), which unites with the rectum to form the cloaca (*cl.*). The anterior testicular region runs in a straight line backward into the ductus; the posterior, on the other hand, lies along the side of this structure, and it is at its anterior extremity that it opens into it.

The symmetry, it may be assumed, was originally bilateral, the reproductive tract consisting of a pair of cavities with a common duct, and that, as the result of the narrow form of the body, one of the cavities has been turned through an angle

of 180°. The entire system lies ventral to the alimentary canal.

Each testicular region with its contents forms an elongated mass, tapering at either extremity. In cross sections the outline varies from semicircular to triangular in adaptation to the other internal organs. The anterior mass is the larger; where it attains its greatest breadth it occupies as much as two thirds of the body cavity.

In preparations of the entire animal the richly cellular character of the testis and the mass of developing sperms can be readily distinguished.

On examination by serial sections, the testicular region is found to consist of a tube with epithelial walls, the commencement occupied and closed by the germinal syncytium, the termination becoming continuous with the epithelial lining of the ductus.

The epithelium of the wall is, throughout the greater part of the length, very fine and flattened (Pl. 7, fig. 17, *gw.*). It is only possible to demonstrate the protoplasm in places where the contained sperms have been artificially separated, but the flattened nuclei (i b., *ngw.*) are always readily detected. Toward the junction with the ductus the epithelium increases in depth, forming a well-marked layer still without cell limits, but with fairly large oval nuclei. A fine layer of muscular fibre appears on the outside. This portion is analogous or, possibly, homologous with the vas deferens described by Jägerskiöld (**10**) in Cylicolaimus magnus and Thoracostoma acuticaudatum.

The germinal syncytium (Pl. 7, fig. 16) which occupies the fundus consists of a mass of nucleated protoplasm, which is in continuity with the protoplasm of the wall: in other words, it is a specialised portion of the epithelium lining the gonocœl. From this syncytium the sperm mother-cells are developed, growing down and filling the lumen of the tube, multiplying and undergoing development as they pass down.

The nuclei of the syncytium at the commencement are spherical, or oval, ·0043 mm. in greatest diameter, have clearly

marked chromatin granules, and one or two pseudo-nucleoli. On passing further from the fundus, the nuclei increase in size, the protoplasm becomes relatively less, then cell outlines appear; the nuclei have increased to ·00645–·00753 mm., and the chromatin takes the form of a network (Pl. 7, fig. 17, *sg.*). About the commencement of the last quarter of the region the sperm mother-cells begin to divide, the chromatin is aggregated in larger granules, the nuclear membrane disappears; still further on the chromatin takes on a star-shape. The cells (mature spermatozoa) are here more numerous and smaller, ·0043 mm. in diameter, are not so closely packed, and are surrounded by some residual protoplasm. Spermatozoa are found in the anterior portion of the ductus. The rest of this organ is generally found to be empty.

The ductus ejaculatorius is a straight cylindrical tube, although at its commencement slightly flattened. The posterior testicular region opens into it a short distance behind its origin from the anterior.

Its wall consists of two layers—internal epithelial, external muscular (Pl. 7, fig. 17, DE.). The epithelium is cubical, becoming columnar towards the termination of the tube. The cells are highly vacuolated, and the free surfaces present a frayed-out appearance, as if a secretion had been discharged. In places this secretion can be made out as numerous acidophil granules.

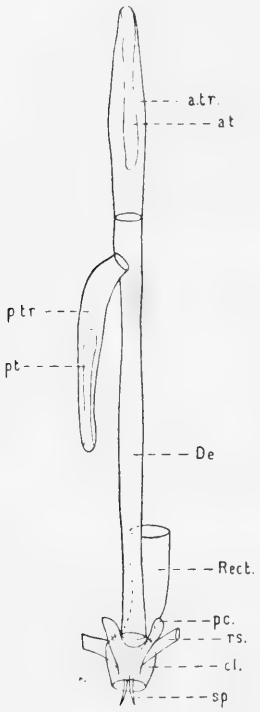
The muscular wall consists at first only of a layer of circular fibres. Flattened nuclei with a fine film of protoplasm can be made out on the outer surface, no doubt the nuclei of this layer. In the posterior quarter there is also an external layer of longitudinal fibres.

At its termination the ductus unites with the rectum to form the cloaca (text-fig. 1, *cl.*). As this latter structure is intimately connected with the copulatory apparatus, I shall describe it here rather than under "The Alimentary System."

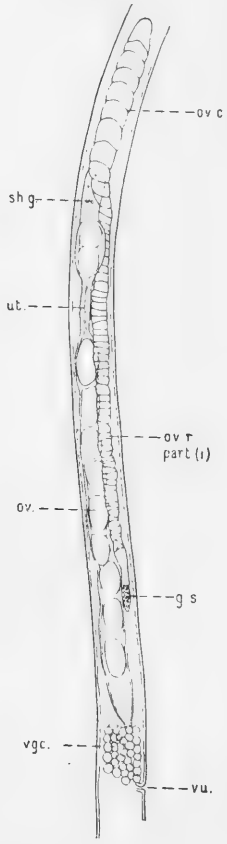
It is a short chamber, at its commencement roughly cylindrical in section, but becoming flattened towards its external opening, which is slit-like.

The wall (Pl. 7, fig. 6, *cl.*) is continuous with the epithelium of the ductus and rectum, and with the epidermis in the ventral line. It consists of protoplasm, which shows a circular striation as if it might fulfil a muscular function. Nuclei are

Text-fig. 1.



Text-fig. 2.



fairly numerous. Cells from the dorsal and the two lateral lines extend inward through the body-space towards the cloaca and form a layer of cubical epithelium on its dorsal wall, the cloacal ganglion (ibid., *cl.*, *ag.*). There is a cuticular lining continuous with the external cuticle.

The accessory sexual apparatus consists of the two spicules

(Pl. 7, fig. 5, *sp.*) with their accessory piece, with the musculature which governs them and assists in expelling the sperm. Lying dorsal to the cloaca a muscular mass (Pl. 7, fig. 6, *ps.*) is to be found, imbedded in which are the spicules and accessory piece. The fibres run in the same direction as the long axis of the body and parallel with the spicules. In front they envelop the anterior ends of the spicules, behind they are attached to the wall of the cloaca. This muscle obviously acts as protractor of the spicules.

A second set of muscles (ibid., RS) passes from the body-wall to the posterior portion of the ductus, to the cloaca, and to the protractor muscle. It consists of a series of muscular bundles lying in the coronal plane of the animal and on the side of the body-wall attached mainly to the lateral lines. By enclosing the posterior portion of the ductus it assists in expelling the sperm; by the attachment to the protractor it is enabled to act as retractor of the spicules, and by its attachment to the cloacal wall as dilator of the cloaca.

The spicules are curved, pointed at their free ends, ·13 mm. long. They consist apparently of a chitinous material, are hollow at their upper portions, containing protoplasm. The accessory piece is triangular, grooved at the sides, the spicules fitting into the grooves.

Female Reproductive System.

The female reproductive organs (text-fig. 2) consist, as is usual in Nematodes of two tubes, uniting before reaching the external aperture. The tubes do not lie side by side, but one is found in front of, the other behind, the external aperture. This aperture is situated in the middle of the mid-ventral line of the body, the entire gonads being included in the middle two quarters of the body.

Each tube consists of ovarian region (text-fig. 2, *ov. r.*), including an ovarian cæcum (*ov. c.*), uterus (*ut.*), and vagina. The ovarian cæcum lies furthest from the external aperture, the rest of the ovarian region being bent back on the oviduct.

The first part of the ovarian region (*ov. r.*)—that is, that part which is not ovarian cæcum—has the form of an elongated cone, the base being continuous with the ovarian cæcum. It measures 1·87 mm. in length. It is completely filled by the ovary and by the mass of developing ova.

The ovarian cæcum, or second part of the ovarian region, is a blind prolongation of the first part. The openings into it of the first part and of the uterus lie side by side. It is oval in shape, with a truncated end at its junction with the first part and the uterus. It measures ·85 mm. in length, ·221 mm. in breadth.

The ovarian cæcum is the original gonad cavity, as will be shown when describing the immature female organs. The first part is a secondary outgrowth from it.

The wall of the ovarian region (Pl. 8, figs. 19 and 20) is formed of excessively fine flat epithelium. Indeed, so fine is this epithelium, that except near the termination of the organ, the only evidence of its existence consists in the presence of very much flattened nuclei (*ngw.*) closely apposed to the sides of the ovary and of the column of ova. The protoplasm of this epithelium cannot be distinguished even with a magnification of 1000 diameters. The state of matters in the immature female which I shall describe later, and the fact that this layer of flattened nuclei can be traced into the epithelium lining the ovarian cæcum, leave no doubt, however, as to the real existence of a wall.

The wall of the ovarian cæcum (Pl. 8, fig. 18, *gw.*) is composed of flattened epithelium, in which no cell outlines are discernible. The nuclei are flattened oval. The transition from the first part to the cæcum is, of course, gradual, not abrupt.

The germinal syncytium (Pl. 8, fig. 20, *gs.*) occupies the fundus of the tube, and doubtless springs from the epithelial lining. It consists of a protoplasmic mass, which shows an affinity for basic stains. Nuclei are imbedded irregularly in it, at the commencement 4 to 8 in a transverse section, increasing up to 12, and then again decreasing until at the end of the syncytium not more than two occur in any section. The

nuclei are oval, have a nuclear membrane, distinct chromatin granules, and nucleolus. At the commencement of the syncytium they measure ·0136 mm. in their greatest diameter, at the termination ·0477 mm. There is a narrow, more condensed, ring of protoplasm round each nucleus.

The syncytium occupies about one eighth of the length of the ovarian region. It is followed by a column of disc-shaped ova (Pl. 8, fig. 19, *ov.*), which have become separated from it. These ova increase in size as they pass toward the ovarian cæcum, and become cylindrical rather than disc-like in shape. About the middle of the column the ova measures ·0484 mm. in diameter.

The ovarian cæcum contains about eight ova. A space is left between the ova and the dorsal wall, through which the ovum which occupies the fundus, and which is the most mature, can pass to reach the oviduct. This mature ovum is richly supplied with yolk-granules (Pl. 8, fig. 18, *ov.*).

The next division of the gonad tube is, in O. vulgaris, physiologically merely an oviduct, but morphologically it is identical with the subdivision which functions as an uterus and receptaculum seminis in other free Nematodes—e. g. Cylicolaimus magnus and Thoracostoma acuticaudatum. As this division has been described as an uterus by Jägerskiöld, I shall retain the name for the sake of uniformity. The uterus measures 2·5 mm. in length. Its calibre and shape vary according to its contents. It may be distended by a series of large ova or entirely empty. The latter condition is the exception. Generally from four to twelve opaque white ova can be seen in each uterus (text-fig. 2, *ov.*) lying end to end like a short chain of beads. The uterus then adapts itself, of course, to the shape of the ova. When empty it is collapsed and flat.

At its commencement a glandular mass, ·204 mm. in length, projects into and fills the lumen. This is the shell-gland (text-fig. 2, *shg.*, Pl. 8, fig. 19, *shg.*). Ova, after passing it, are found to have acquired their shells. Its shape is very much that of the ovarian cæcum, resembling a conical bullet, the

pointed end directed towards the cæcum. The base is concave, and receives into its concavity one end of the first uterine ovum. The gland is attached to the right wall of the uterus, except at the base; here it projects freely into the lumen.

The gland is interesting histologically. It is formed of protoplasm continuous with the wall of the uterus. Cell outlines are not present. At the apex the nuclei are large and spherical, have a very prominent pseudo-nucleolus and a nuclear membrane; at the base, however, the nuclear membrane has disappeared, and the nuclear substance is diffused into the protoplasm. Contiguous nuclei thus become continuous, and, as the nuclei are arranged at the periphery of the mass, a basophil circle, in transverse sections, results. In this basophil circle the pseudo-nucleoli stand out, and might easily be mistaken for the nuclei themselves.

The wall of the uterus (Pl. 8, fig. 20, *ut.*) for the greater part of its length is composed of a cubical epithelium. This is, of course, considerably flattened where the uterus is distended by the ova. Near the junction with the ovarian cæcum the outlines of the cells become indistinct. When, as sometimes happens, there is no ovum in contact with the base of the shell-gland, the wall is here thick and the epithelium has a glandular appearance.

The ova in the uterus (i b., *ov.*) are oval in shape and measure ·27 mm. in length. The protoplasm is obscured by the mass of yolk-granules. A single nucleus occurs in each ovum, and this is invariably undergoing karyokinetic division, presumably in preparation for the extrusion of the polar body, although I have not observed this body. The shell is ·001 mm. thick and is sculptured, narrow ridges running over its outer surface.

Spermatozoa do not occur in the uterus.

The vagina (Pl. 8, figs. 22, 23, 24, *vag.*) is a glandular and muscular tube ·595 mm. in length. The glands surrounding it (*vgc.*), with those which lie around the vulva, give it a richly cellular appearance in preparations of the entire animal. At its origin from the uterus it is tightly constricted, but it soon

opens out although its lumen is not so wide as that of the uterus. Shortly after its commencement, where the lumen begins again to widen out, a narrow canal passes through its dorsal wall and opens into the intestine. This is the gonenteric canal.

The wall of the vagina consists of three layers—an internal epithelium, a middle muscular, and an outer glandular layer.

The epithelium is composed of cubical cells, the walls of which are thick and consist of specially condensed protoplasm. Within these walls the protoplasm does not stain, so that the nucleus appears to lie in a vacuole. The muscular layer is thick, the fibres circular. Where the two vaginæ meet (Pl. 8, fig. 24) the fibres pass outwards to the body-wall, forming a longitudinal layer around the short common terminal portion of the tube. It is difficult to distinguish any definite epithelial lining in this portion, but a fine film of cuticle is invaginated through the external aperture. The aperture is surrounded by a sphincter internal to the longitudinal fibres.

The vaginal and vulvar glands consist of cells lying in the body space. Around the first half of the vagina they form a single layer, and as they are pear-shaped give the appearance of a rosette in transverse section. I have not been able to demonstrate any openings from these cells into the lumen of the vagina, but from the definiteness of their arrangement it seems natural to suppose that their secretion is discharged into the vagina. Beyond the middle of the vagina the cells begin to arrange themselves around the vulva, and processes pass from them to a circle of minute pores surrounding this aperture (Pl. 8, fig. 24, *vug. ap.*).

The vagina always contains masses of spermatozoa (ibid., *s.*). Near the vulva these are spherical, with stellate nuclei, but higher up at the uterine end they become elongated and the nucleus almost thread-like.

Fertilisation must presumably take place in the vagina during the passage of the ova. It is somewhat peculiar that spermatozoa do not find their way up to a level at which the ova are without their shell, as occurs in the forms described

by Jägerskiöld. I have not detected any opening through the shell, but such an opening would no doubt be very minute, and it is difficult to obtain a complete series of sections of the ova without some slight tearing of the tough shell.

The vulvar aperture is slit-like, the greatest diameter transverse to the long axis of the body.

The gonenteric canal.—This is a minute duct which, as stated above, opens into the vagina close to its commencement (Pl. 8, fig. 23, *gec.*)—in fact, just below the sphincter at the junction of uterus and vagina. It traverses the dorsal wall, passing vertically through the muscular layer. In this part of its course a few minute nuclei indicate the presence of an exceedingly fine lining of epithelial nature. Emerging from the vaginal wall, it lies in the body-space (Pl. 8, fig. 22, *gec.*) with nucleated protoplasmic walls which conduct the canal to the gut in the midventral line (Pl. 8, fig. 21, *gec.*) and becomes continuous with the alimentary epithelium. Passing from the vagina to the gut, the canal inclines slightly away from the mid-point of the body—*i.e.* the anterior canal inclines slightly towards the head, the posterior slightly towards the tail.

The function of this canal is to carry off superfluous spermatozoa. Spermatozoa are found in it and one of my sections shows a sperm passing from it into the gut. The gut in the adult female contains large masses of sperms.

No such canal has been described previously in any nematode. Its comparative morphology will be discussed later.

Reproductive Organs in the Immature Female.

I was fortunate enough to secure a specimen which was full grown, but in which the reproductive organs were undeveloped. In this specimen the gonads occur in from one fifth to one fourth of the length of the body. The vulva lies somewhat behind the centre.

They consist, as in the adult, of two tubes, an interior

and a posterior, uniting to open by a single aperture to the outside. The tubes are straight, not bent back upon themselves as in the adult, but, as will be explained further on, the fundus does not correspond with the commencement of the first part of the ovarian region in the adult, but with the fundus of the ovarium cæcum.

The tubes are only potentially tubes; towards the fundus, where the oogonia occur, these cells completely fill the lumen; in that part destined to become vagina the lumen is occupied by a solid core of cuticle, and between these two regions the "tubes" consist of solid rods of protoplasm, which show their tubular nature only by the arrangement of the nuclei in a single layer around the periphery.

If each tube were divided into five parts, the first part, counting from the fundus, would contain the oogonia (Pl. 8, fig. 25). Here the wall (*gw*) is of flattened epithelium, not showing cell outlines, the nuclei flattened oval, with chromatin granules and nucleolus.

At the junction of the first and second fifths in the ventral wall the germinal portion of the epithelium is situated. Here the protoplasm of the wall grows into the lumen, the nuclei, still retaining the same characters, become larger and more spherical. The oogonia (*og*) as they develop from this protoplasmic projection pass, not towards the external aperture, but towards the fundus. The youngest nuclei which can be definitely recognised as oogonial resemble the nuclei of the germinal projection, but are more oval, and are larger, measuring 0·0016 mm. in diameter, and the chromatin granules are smaller and fewer in number. A transverse section at this point shows about eight such nuclei. The protoplasm is scanty, shows no cell outlines, and stains with basic dyes.

In the later stage of development the nuclei have become still larger, 0·0161 mm., and consequently there are fewer in a transverse section. The chromatin granules have disappeared, the nuclear vesicle being filled by a finely granular material which does not stain, the nucleolus is large. The protoplasm is relatively greater in amount. The immature

ovum which occupies the fundus has a nucleus of the same character, but still larger, 0·0172 mm.

Comparing this with the adult, it is obvious that the projection of germinal epithelium corresponds with the commencement of the ovary, and the fundus of the tube with the ovarian cæcum. In development to maturity the germinal syncytium and the oogonial mass grow in the direction of the external aperture, evaginating the epithelial wall, and forming the reflected portion of the adult gonads.

Of the remaining four fifths of the tube, three fifths consist of a solid protoplasmic rod, with nuclei arranged around the periphery. In the last fifth the epithelium becomes columnar (Pl. 8, fig. 25). The cuticle of the body-wall passes into the lumen of the gonads as a solid core, but extends only a very short distance beyond the union of the two tubes. Gonenteric canals do not occur.

The last quarter of each tube and the united portion are surrounded by gland-cells (*vgc.*) lying in the body space. Processes pass in the direction of the vulva, but I have not been able to detect any openings through the cuticle.

Ascaris clavata (Rud.).

Female Reproductive Organs.

The female reproductive organs of A. clavata occupy one half of the length of the body. If the body is divided into ten parts, they extend from the end of the third to the end of the eighth part. The external aperture lies at the end of the third part.

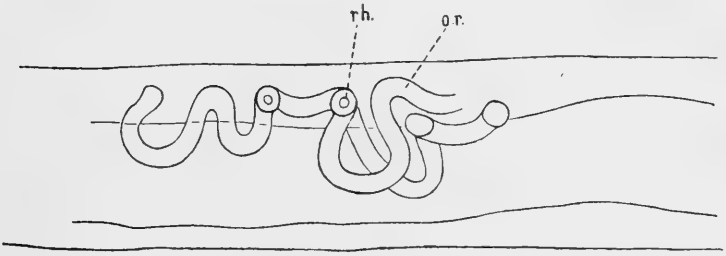
They conform to the general Nematode type, consisting of two germinal masses lying at the fundi of two tubes, the two tubes uniting before reaching the external aperture.

Physiologically each tube may be divided into two regions—viz. one in which the ova develop from the germinal syncytium, and undergo maturation, the other strictly a passage

to the exterior, but in which fertilisation and a certain degree of development also take place.

The first portion is, as usual, termed "ovary," although this term should strictly be applied only to the germinal syncytium, and to the rhachis with the oogonia attached to it.

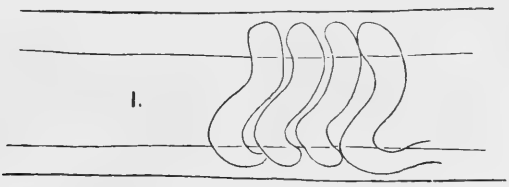
Text-fig. 3.



The second part is divided into oviduct, receptaculum seminis, and uterus. The common portion is formed by the union of the two tubes in the vagina.

The ovarian region (text-figs. 3, 4, 5, 6, *ov. r.*) is by far the largest; it extends from the end of the eighth to the tenth part

Text-fig. 4.



of the body forward to the end of the fourth—i.e. it extends through almost the entire reproductive region. It is, in addition, highly convoluted; in a specimen rendered transparent with, e. g., cedar-wood oil the tightly-packed coils form a most conspicuous object. The commencement is slightly conical, the diameter increasing as we pass down, but after a comparatively short distance the diameter ceases to increase, the tube being for the greatest portion of its length perfectly cylindrical.

The wall (Pl. 8, fig. 26 A, *gw.*) is excessively thin, consisting

of a layer of flattened epithelial cells. At the fundus this layer becomes continuous with the germinal syncytium; in other words, the germinal syncytium is a specialised portion of the epithelium lining the gonocœl.

The germinal syncytium and the ova developing from it extend from the fundus down the tube as an unbroken column, completely filling the lumen, and this mass is, of course, the true ovary. In the germinal syncytium numerous nuclei occur, imbedded rather irregularly in the relatively scanty protoplasm. As we pass down the tube the nuclei begin to arrange themselves in a single layer at the periphery of the column; the central, non-nucleated protoplasm is the rhachis. Next, cell outlines appear around the nuclei, and the rhachis becomes smaller with the increasing size of the developing ova. Finally the rhachis ceases, the ova are arranged in successive tiers, closely packed against each other (Pl. 8, fig. 26 A, *ov.*) The protoplasm of the ova at this point consists of a meshwork. Before the ova becomes separated from this continuous column and pass into the oviduct eosinophil granules (yolk) make their appearance.

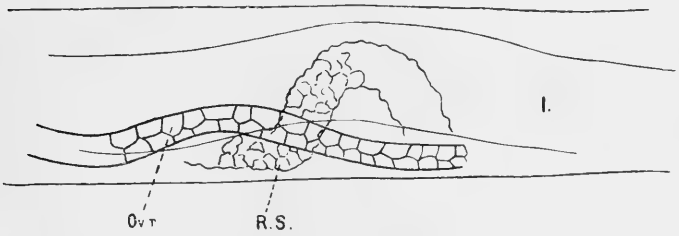
The division of the remaining portion of the genital tract into regions is necessarily somewhat arbitrary. Each region shades off into the one preceding and the one following it, and in different individuals the appearances at the same level vary. The same portion morphologically may be empty and constricted, or distended with ova or spermatozoa. In the following description I have divided it, according to the characters of the wall, into oviduct, receptaculum seminis, and uterus; the vagina is the single terminal portion.

The oviduct and receptaculum (text-fig. 5, R.S.) follow the ovarian region. They extend from about the middle of the body backward to the end of the sixth to tenth, then turn forward again for a short distance, and become continuous with the uterus. Their course is slightly tortuous, only slightly when compared with the ovarian region. The appearance of the tube varies with the contents; at its commencement it contains at most a few single ova and spermatozoa, and here it

is fairly narrow, viz. ·102 mm. This portion is the oviduct (Pl. 8, fig. 27). Further on it widens out somewhat, to become the receptaculum, measuring ·255 mm. in diameter.

The wall of both oviduct and receptaculum consists of an epithelium with an external basement membrane (ibid., *bm.*). In the oviduct the epithelial cells are rounded, the lamina

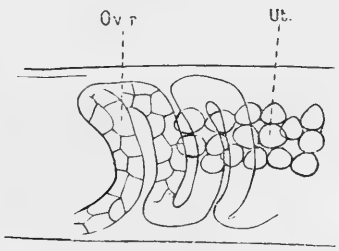
Text-fig. 5.



poorly marked. The outer portions of the epithelial cells show indistinct circular fibrillation, as if they might act as a sphincter.

On passing to the receptaculum the lumen widens out, and

Text-fig. 6.



the epithelia cells become less spherical, although they still project into the lumen. Their protoplasm is reticular. The basement membrane becomes thick and distinct. The contents consist of spermatozoa, which frequently form a single layer on the surface of the epithelium, and of ova in small groups. Fertilisation takes place here.

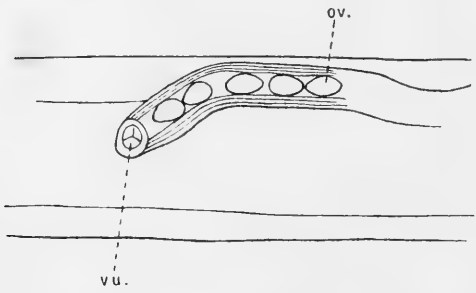
The uterus (text-fig. 6, Ut.) extends from about the end of the sixth tenth forward to the middle of the body, where it

unites with its fellow to form the vagina. It is the widest and most distended portion of the tract. In a transparent specimen the mass of ova which fills it is very prominent. Its course is fairly straight, with the exception of an S-shaped bend at one point, where it is constricted. Its wall (Pl. 8, fig. 28) is three-layered, consisting of epithelium and basement membrane as in the oviduct and receptaculum, with the addition on the outside of a layer of flattened epithelioid cells.

The epithelium is more flat than in the oviduct. On the internal surface the protoplasm is differentiated into a more dense, almost cuticular, layer.

The outer epithelioid layer (*ml.*) is exceedingly fine, and it

Text-fig. 7.



is difficult to demonstrate it except where the nuclei cause a slight bulging. Its function I believe to be muscular. It is continuous with the muscular layer of the vagina. Hamann describes a similar layer in Lecanocephalus as muscular.

The uterus is always distended with masses of spermatozoa and segmenting ova. Spermatozoa predominate towards the commencement, ova toward the termination. Toward the commencement the spermatozoa produce a remarkable appearance, arranging themselves on the surface of the epithelium in a closely-packed single layer, the nuclei lying at the free ends (ibid., *s.*). It is very easy to mistake them for a tall columnar epithelium.

The vagina (text-fig. 7) extends from the junction of the two uteri at the level of the middle of the body to the external

aperture which lies in the mid-ventral line at the end of the third tenth of the body.

In preparations of the entire animal the appearance of this portion varies according to whether it is distended or not. At its commencement it contains large quantities of ova, and is not to be distinguished from the uterus. Further on, however, it contracts, and forms a fairly thick walled muscular tube, with here and there a single ovum in its lumen.

In sections this division is also clear, the upper portion thin walled, the lower thick and muscular. Histologically, indeed, for about the first quarter the wall is identical with that of the uterus. Then it begins to change, the epithelium becomes more and more columnar, the basement membrane more and more indistinct, merging on the one hand with the outer wall of the epithelial cells and with the partitions between them and on the other with the outer muscular layer. This outer muscular layer is continuous with the outer epithelioid layer of the uterus; it consists of circular muscular fibres.

Towards the termination there is also an internal cuticular lining, continnous with the cuticle of the body wall.

The external aperture is puckered, and, if open, would be circular. Around the aperture the epithelium of the vagina becomes continuous with the subcuticular layer of epidermis.

Male Reproductive Organs.

The male reproductive organs consist of a single tube, in contrast to the double female tubes. In other respects analogies, if not homologies, are easily demonstrable. The germinal mass again is attached to the fundus, and projects down the lumen. The tube is divided into a portion in which development and maturation of germs take place and into a portion serving merely as a passage to the exterior, the former consisting of a region containing the testis corresponding with the ovarian region, the latter of a vas deferens corresponding with the oviduct, a seminal vessel corresponding

with the receptaculum and uterus, and a ductus ejaculatorius corresponding with the vagina.

The testicular region occupies two thirds of the reproductive division of the body. It is, in addition, highly tortuous. The wall is identical with that of the ovarian region, consisting of an excessively flat epithelium (Pl. 8, fig. 29, *gw.*). The germinal syncytium again arises from this epithelium at the fundus. The vesicular nuclei of the syncytium are at first scattered through the protoplasm. They soon arrange themselves around the rhachis, however, measuring ·003 mm. in diameter.

Further down, they invade the rhachis (Pl. 8, fig. 29), running in lines through it (·0043 mm.). Cell outlines begin to appear in the protoplasm, leaving some residual protoplasm between the cells. The rhachis ceases, and the cells lie free in the lumen, packed against each other. The nuclei continue to increase in size (·0064 mm.), the protoplasm is scanty. In the lower reaches the protoplasm again increases. The spermatogonia divide by karyokinesis and form the spermatozoa, spherical bodies, ·0053 mm. in diameter, showing fine amœboid processes, the nucleus represented by a single chromatin granule (Pl. 8, fig. 30, *s.*).

The vas deferens (Pl. 8, fig. 30, v.*d.*) is a short muscular passage from the foregoing division to the seminal vesicle. Its length is about ·34 mm. It is exceedingly narrow, ·068 mm. compared with ·41 mm. of the vesicle. The wall consists of a cubical epithelium with an outer muscular layer.

The seminal vesicle (ibid., s.v.) extends through rather less than one third of the reproductive region of the body. Its course is straight. It is the widest portion of the genital tract, ·41 in diameter, the lumen distended with spermatozoa. It is often constricted near the middle of its course. The wall consists of an epithelial layer, which does not show cell-outlines. Where the tube is distended the epithelium is flat; where it is contracted the epithelium is thrown into ridges. The muscular layer consists of circular fibres.

The ductus ejaculatorius (Pl. 8, fig. 31) is the shorter,

terminal portion. Its lumen is narrow, but this is due to the thickness of the walls, as the diameter over all measures ·306 mm. The epithelium is the thickest layer; it is columnar, and shows a remarkable intra-cellular structure. The protoplasm is differentiated into two layers, an outer which stains with hæmatoxylin, and an inner which stains with eosin. The former projects into the body of the cell in a fingerlike process which surrounds the nucleus.

The muscular layer is well developed, and consists, as usual, of circular fibres. Muscular trabeculæ pass from the body-wall in the neighbourhood of the lateral lines, and converge toward the midventral line; they are attached to the outer surface of the ductus.

The ductus opens into the cloaca. This is formed by an invagination of epidermis, the wall consisting of cuticle, and a protoplasmic layer continuous on the one hand with the epithelium of the ductus and on the other with the epidermis (subcuticular).

The spiculæ are attached to the dorsal wall of the cloaca, and pierce this wall to project through the external aperture.

Ascaris capsularia (Rud.?).

Excretory Gland.

In almost every cod numerous nematode embryos are to be found encysted under the peritoneum. They occur in largest numbers on the surface of the liver and among the pyloric cæca, but are also common in the mesentery. They certainly belong to an Ascaris, but to what species I am unable to say. As usual with Nematode embryos, they occur coiled up like a watch spring and surrounded by a capsule of badly-formed fibrous tissue. They measure 22 to 28 mm. in length. The head is blunt, with three papillæ—one dorsal, two subventral—around the triangular mouth. The body tapers more gradually toward the head than toward the tail. There are no lateral membranes. The anus is subterminal. There is no diverticulum

at the junction of œsophagus and intestine. The nerve-ring lies shortly behind the mouth and is surrounded by a ganglionic collar. Generative organs indistinguishable as such. One of the most prominent internal organs is the gland which will be described below.

I believe that this form is identical with A. capsularia (Rud.) found in salmon and Gadidæ, but as there are very few points to distinguish Nematode larvæ from one another, this may not be the case, or several distinct embryos may be included under the one name. Dujardin (**2 a**) gives the length as 27 mm., von Linstow (**11 a**) 19 mm. I have therefore given the above description.

In addition, A. capsularia is, of course, merely an embryo; it should therefore not have a separate specific name, except for convenience, until the adult form is identified.

The gland which I propose to describe is interesting from the fact that it is homologous with the ventral gland of free Nematodes, and with, e.g., the poison glands of Strongylus filaria (Rud.).

It extends through the anterior six tenths of the body, lying ventral to the alimentary canal (Pl. 8, fig. 32, *vg.*), and its duct, after a short course, opens in the midventral line between the two subventral oral papillæ and immediately in front of the ganglionic collar which surrounds the nerve-ring.

The body of the gland (Pl. 8, fig. 32 *a*) is composed of a single gigantic cell, 15 mm. in length, ·255 mm. in greatest breadth, somewhat flattened between the body-wall and the alimentary tract, tapering to the posterior extremity, and rather blunt at the anterior.

The body of the cell is composed of finely granular acidophil protoplasm, the outer layer of which appears somewhat more condensed, and stains with hæmatoxylin.

The nucleus (*n.*) is a remarkable structure lying in the anterior half of the cell. It is 6 to 7 mm. in length. In transverse section its outline varies from linear to circular or biconcave. It has a wall identical in appearance with the outer wall of the cell; this encloses a vacuole containing

a finely granular substance which stains intensely—almost black—with hæmatoxylin.

A fine canal (*can.*), 0·0075 mm. in diameter, traverses the entire length of the cell, and becomes continuous with the duct. Its course is in places slightly tortuous. It receives numerous smaller canaliculi which traverse the protoplasm. Its walls are also composed of condensed protoplasm; its contents when fixed are very finely granular and acidophil.

The short duct (Pl. 8, fig. 33) runs from the anterior pole of the cell to the midventral line, and in the substance of this line to the external aperture. Its wall is composed of protoplasm continuous with the protoplasm of the cell; one or two small nuclei occur in it. There is a very fine cuticular lining continuous with the external cuticle.

Hamann describes a similar organ in the embryos found in Zeus faber. It again consists of a single cell, in front thread-like, at its greatest breadth extending from one lateral line to the other, is always in connection with the *dorsal* median line, contains a canal lined by a "glashelles membran," which opens *dorsally* behind the lips. [The italics are mine.]

Cells occur in the body cavity identical with the basophil cells of Oncholaimus vulgaris.

Lying between the ventral gland and the right lateral line is a solid cellular mass, possibly the rudiments of the gonads.

Comparative Morphology of the Excretory Glands in Nematodes.

This subject has been very fully worked up by Jägerskiöld in a masterly paper (**9**). I have, however, a few points to add. I shall commence with a brief summary of his results.

He finds that the excretory organs of Nematodes can be classified into four groups:

(1) The ventral gland of the most free living forms (Pl. 9, fig. 35).

(2) The unilateral asymmetrical excretory organ, in its anterior part flattened and band-shaped, and with a highly modified nucleus, as in A. decipiens (Pl. 9, fig. 37).

(3) A similar organ, but without the band-like enlargement, as in A. clavata (Pl. 9, fig. 38).

(4) The bilateral organ of, e. g., A. megalocephala (Pl. 9, fig. 40).

In all these types the organ consists of a single large cell, with an intra-cellular system of canals, and with a duct formed in many cases by an invagination of epidermis. All four are homologous, an intermediate type between (2) and (4) being found in A. rotundata, in which a small limb crosses from the main stem of the gland on the left side to the right (Pl. 9, fig. 39). The cause of the change in type from (1) through (2) and (3) to (4) is to be found in an increase of work thrown on the gland, probably by change of habits in the animal. The gland is compelled to enlarge, and adapts itself to the narrow body-form by elongating, and following the line of least resistance, applies itself first to one and then to both lateral lines.

Bastian (1) had previously (and he is quoted by Jägerskiöld) stated the same opinion, that the ventral gland of free Nematodes was homologous with the excretory organ of parasitic forms.

The results given above supply another link in the chain. The excretory gland in the embryo above described (Pl. 9, fig. 36) is a typical ventral gland, inasmuch as its opening, although situated close to the mouth, is also immediately in front of the nerve-ring (corresponding with the situation in Oncholaimus vulgaris), and as it lies free in the body space, comes into contact only with the left lateral line, never being in continuity with it.

On the other hand, in many points it resembles the excretory organ of A. decipiens. Its duct is formed by an ingrowth of cells from the ventral line; its anterior portion is broad, almost band-like, and contains the nucleus; its posterior portion is narrow and thread-like. It contains a central canal

with branches ramifying through the protoplasm. The nucleus is highly elongated, in places band-like, and its structure very strongly resembles that of A. decipiens and allied forms.

We should expect in the embryo of a parasitic form to find a transition between the type found in the free-living forms and that in the adult parasite. As I have shown, our expectations are fulfilled.

A step further can be taken in pointing out homologies; it appears probable that the excretory organ of Nematodes, in whatever form it occurs, is a nephridium homologous with the nephridia of, e. g., Platyhelmia or Chætopods.

A nephridium is defined by Ray Lankester (**11**) as follows: "Nephridia are distinguished by their independent origin, each from a single superficially placed cell, which often is seen to be derived from ectoderm, and probably must be traced to that layer even when it appears as part of the mesoblast. They are also distinguished by their structure, which is primarily that of a number of perforated or drain-pipe cells placed, as it were, end to end."

The excretory organ of Nematodes is a cell perforated by an intra-cellular canal. That it consists of a single cell and not of a number placed end to end does not militate against the homology suggested, since the chains of cells originate from a single cell.

Jammes (**7**) and Jägerskiöld (**9**) agree that it is ectodermal in origin. The condition as found in the ventral gland of free-living forms by many observers, and as described for Oncholaimus vulgaris by myself, certainly suggests an ectodermal origin.

Hamann (**5**) is the only modern authority who regards it as mesodermal.

We are accustomed to think of nephridia as paired organs, but a single organ arising from the midline is as much bilateral as two arising one on each side of the midline.

If the excretory organ when specialised resembles a nephridium, in its most simple form, it bears a strong

resemblance to an unmodified skin-gland. Jägerskiöld (**10**) has pointed this out also, stating that physiologically at least the ventral gland and the skin-glands are interchangeable. The results given above agree with this; in the female of Oncholaimus vulgaris the functions of the ventral gland are taken over presumably by the vulvar and lateral line glands, as the animal reaches maturity.

It is hardly necessary to insist on the structural resemblance between the ventral gland on the one hand and the tail-glands and glands of the lateral lines on the other.

The Cœlom.

It seems probable that the chief reason why a cœlom has not been recognised in Nematodes is to be found in the manner in which the reproductive organs have been described. The terms "testis" and "ovary" have been made to cover, not only these organs themselves but also the spaces which contain them, and although the spaces have of course been recognised, sufficient morphological importance has not been attached to them.

The condition may be summarised as follows: In all Nematodes hitherto described there is to be found either a single median space or two bilateral spaces, lined by a characteristic flat epithelium. These spaces with their walls I have described as testicular and ovarian regions. A specialised patch of this epithelium forms the testis or ovary. From the space, or spaces, a duct, or ducts, leads to the exterior: where there are two ducts they unite before reaching the exterior. In regard to origin, the cavity and, no doubt, the ducts as well are mesodermal. This is the view of Jammes (**7**), and in Hallez's (**6**) figures it can be seen that the gonad tubes arise from two groups of cells which in the early embryo lie in the blastocœle, one on either side of the gut.

The cavities are, then, typical gonocœls or protocœloms (**11**, pp. 35, 36), and the ducts (unless they should prove to be formed by ingrowth of epidermis, which there is no reason to expect) are typical gonoducts or cœlomoducts.

The fact that in parasitic Nematodes the cavities form highly convoluted tubes of great length should not be allowed to obscure this truth. In the forms which I have described we have a series. It commences with the simple gonocœl found in the immature female or mature male of Oncholaimus vulgaris; next, in the mature female the gonocœl is slightly more complex; there is a narrow conical outgrowth from the original cavity, which is represented by the ovarian cæcum; finally, in A. clavata the entire gonocœl has become enormously elongated and convoluted.

The cause for this latter development is obvious—viz. the call for an enormous increase in the reproductive products on changing from a free to a parasitic life.

In the description of Oncholaimus vulgaris and Ascaris clavata above I have refrained from the use of the terms "gonocœl" and "gonoduct," since I did not wish unnecessarily to bring theory into the strictly descriptive part of my article. I wish now, however, to repeat that I believe that the "testicular" and "ovarian regions" correspond accurately with the male and female gonocœls respectively, and that the remaining portions of the genital tracts (uterus and vagina and ductus ejaculatorius in Oncholaimus vulgaris; oviduct, receptaculum seminis, uterus and vagina, vas deferens, vesicle, and ductus in Ascaris clavata) correspond with the gonoducts. The wall of the former consists of an exceedingly fine squamous epithelium only; in the wall of the latter the epithelium is cubical or columnar (except when the duct is much distended), and there are other coats.

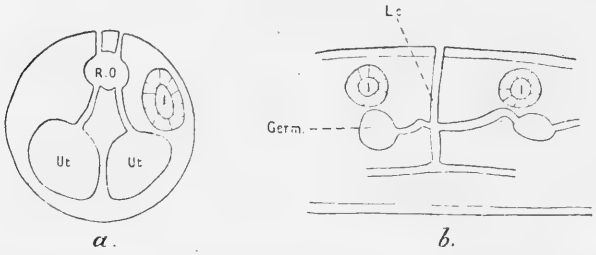
Comparative Morphology of the Gonenteric Canals.

The only organ in any way similar which has been previously described in Nematodes is the remarkable "Röhrenförmige organ" with its ducts, found by De Man (**12**) in Oncholaimus fuscus. This consists of a tube lined by epithelium, lying dorsally in the body-space, communicating

by two canals with the exterior and by two canals with the two uteri.

This condition and that found in O. vulgaris suggest at once a comparison with Trematodes. The former reminds us

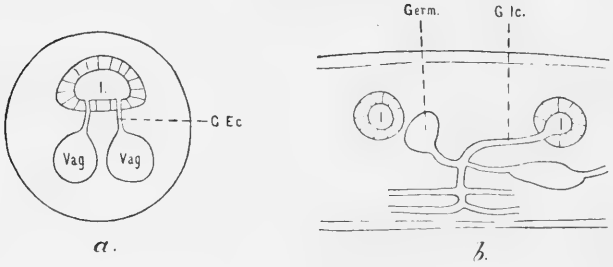
Text-fig. 8.



of Laurer's canal as it occurs in Malacocotyleans, the latter of the genito-intestinal canal of Heterocotyleans. The diagrams (text-figs. 8, 9) illustrate the point better than much writing. The diagrams of the Trematodes are copied from the article in Ray Lankester's 'Treatise on Zoology,' Pt. IV, p. 87.

Text-fig. 8 *a* represents diagrammatically a transverse sec-

Text-fig. 9.



tion through O. fuscus. The "Röhrenförmige Organ" (R. O.), with its ducts, is shown. Text-fig. 8 *b* represents a Malacocotylean. *Germ* is the germarium, *L. c.* Laurier's canal. Text-fig. 9 *a* represents O. vulgaris, and shows the gonenteric canals, to be compared with the genito-intestinal canal (*g. i. c.*) of the Heterocotylean (text-fig. 9 *b*).

In text-figs. 8 *a* and 9 *a* the two uteri and vaginæ are, for fairness of comparison, represented as if they lay side by side.

Literature referred to.

1. Bastian.—"Anatomy and Physiology of Nematoids, Parasitic and Free," 'Phil. Trans. Roy. Soc.,' 1866.
2. Bastian.—"Monograph of the Anguillulidæ," 'Trans. Linn. Soc.,' vol. xxv, 1866.

2a. Dujardin.—'Systema Helminthum.'

3. Golowin.—"Beobachtungen an Nematoden," II, 'Excretorishes Apparat.,' Kasan, 1902. (Summary, 'Zool. Centralblatt,' ix, p. 449.)
4. Hamann.—'Centralblatt f. Bakteriologie,' vol. xi, p. 501.
5. Hamann.—"Die Nemathelminthen," II, 'Die Nematoden,' Jena, 1895.
6. Hallez.—"Developpement de quelques Nematodes," 'Recherches sur l'Embryogenie,' Paris, 1885.
7. Jammes.—'Ann. des Sciences Natur.,' t. xiii, p. 321.
8. Jammes.—'Recherches sur l'Organisation et Developpement des Nematodes,' Thesis, Paris, 1894. (Summary, 'Zool. Centralbl.,' Jahrg. ii, p. 398.)
9. Jägerskiöld.—"Beiträge zur Kentniss d. Nematoden," 'Zool. Jahrb. Abtheil. Anat. u. Ontog.,' Bd. vii. p. 449.
10. Jägerskiöld.—'Weitere Beiträge z. Kentniss der Nematoden,' Stockholm, 1901.
11. Ray Lankester.—'Treatise on Zoology,' part ii.

11a. von Linstow.—'Fauna Arctica' (Schaudinn and Römer), Bd. i, p. 119.

12. de Man.—'Anat. Untersuchungen über Freilebende Nordsee-Nematoden,' Leipzig, 1886.
13. Nassonow.—'Zool. Anz.,' xx, p. 203.
14. Zur Strassen.—'Zeitschr. f. wiss. Zool.,' vol. liv.
15. Wandollek.—'Arch. f. Naturgeschichte Jahrg.,' lviii, Bd. i, 1892.

EXPLANATION OF PLATES 7—9,

Illustrating Dr. F. H. Stewart's paper, "The Anatomy of Oncholaimus vulgaris, Bast., with Notes on two Parasitic Nematodes."

Reference Letters.

a. c. Acidophil cell. *a. g.* Anal ganglion. *a. t.* Anterior testis. *a. t. r.* Anterior testicular region. *b. c.*[1] Basophil cell in ganglionic collar. *b. c.*[2] Basophil cell behind collar. *b. m.* Basement membrane. *Can.* Canal, excre-

tory. *Cl.* Cloaca. *Cl. ap.* Cloacal aperture. *cut.* Cuticle. *D. E.* Ductus ejaculatorius. *D. L. M.* Dorso-lateral muscle-field. *D. M.* Dorsal muscle-field. *ex. ap.* Excretory aperture. *g. c.* Ganglionic collar. *G. Ec.* Gonenteric canal. *g. s.* Germinal syncytium. *g. w.* Gonocœl wall. *I.* Intestine. *L. L.* Lateral line. *L. L. g.* Lateral line gland. *M. D. L.* Median dorsal line. *M. V. L.* Median ventral line. *m.* 'Mesoglœa.' *m. c.* muscle cell. *m. l.* muscular layer. *m. n.* 'Mesoglœa' nucleus. *n.* Nucleus. *n. g. w.* Nucleus of gonocœl wall. *n. r.* Circumœsophageal nerve-ring. *œs.* Œsophagus. *œs. d.* Œsophageal gland-duct. *og.* Oogonium. *ov. c.* Ovarian cæcum. *ov. r.* Ovarian region. *ph.* Pharynx. *P. S.* Protractor spiculorum muscle. *p. t.* Posterior testis. *p. t. r.* Posterior testicular region. *rh.* Rhachis. *Rec.* Rectum. *R. S.* Retractor muscle. *s.* Spermatozoon. *sc.* Subcuticle. *sg.* Spermatogonium. *shg.* Shell-gland. *sm. l.* Submedian line. *sp.* Spicule. *S. V.* Seminal vesicle. *T.*[1] Epidermal cell or nerve-cell, type 1. *T.*[2] ditto, type 2. *tg. ap.* Tail-gland aperture. *tg. d.* Duct of tail-gland. *T. R.* Testicular region. *ut.* Uterus. *vag.* Vagina. *v. d.* Vas deferens. *v. g.* Ventral gland. *v. g. c.* Vaginal or vulvar gland-cell. *v. g. d.* Duct of ventral gland. *V. L. M.* Ventro-lateral muscle-field. *V. M.* Ventral muscle field. *Vu.* Vulva. *Vu g. ap.* Aperture of vulvar gland.

PLATE 7.

Fig. 1.—Anterior extremity, O. vulgaris. Adult female. × 80. (*a*) A large cell of the body space.

Fig. 2.—Head, O. vulgaris. × 350. (*a*) Circumoral papilla; (*b*) circumoral ring of hair; (*c*) dorsal row of hair; (*d*) dorsal tooth.

Fig. 3.—Transverse section, O. vulgaris, male, shortly in front of nerve-ring. × 350. Stain: Safranin, picronigrosin.

Fig. 4.—Transverse section, O. vulgaris, male, through the nerve-ring. × 350. Stain: Safranin, picronigrosin.

Fig. 5.—Tail, O. vulgaris, male. × 350.

Fig. 6.—Transverse section, male, through cloaca. × 350. Stain: Thionin, eosin.

Fig. 7.—Gland-cell of lateral line. × 800. Stain: Safranin picronigrosin.

Fig. 8.—Part of a transverse section in posterior œsophageal region. × 800. Stain: Safranin, picronigrosin.

Fig. 9.—Type 1, epidermal cell. × 800.

Fig. 10.—Part of a transverse section in posterior œsophageal region. × 800. Stain: Safranin, picronigrosin.

Fig. 11.—Coarsely granular acidophil cell of the body space. × 800. Stain: Safranin, picronigrosin.

Fig. 12.—Transverse section through ventral gland, showing nucleus. × 350. Stain: Thionin, eosin.

Fig. 13.—Transverse section at the level of the termination of the œsophagus. Immature female. × 350. Stain: Safranin, picronigrosin.

Fig. 14.—Part of a transverse section in intestinal region. Mature female. × 800. Stain: Thionin, eosin.

Fig. 15.—Part of a transverse section, same as 14.

Fig. 16.—Transverse section through anterior testicular region, showing the germinal syncytium. × 350. Stain: Thionin, eosin.

Fig. 17.—Transverse section through posterior testicular region, ductus ejaculatorius, and intestine. × 350. Stain: Safranin, picronigrosin.

PLATE 8.

Fig. 18.—Transverse section through ovarian cæcum, containing ripe ovum with yolk granules. × 350. Stain: Thionin, eosin.

Fig. 19.—Transverse section through ovarian region (first part) and shell-gland. × 350. Stain: Thionin, eosin.

Fig. 20.—Transverse section through ovarian region (first part), and uterus. × 350. Stain: Hæmatin, picronigrosin.

Fig. 21.—Transverse section, showing gonenteric canal opening into intestine. × 350. Stain: Borax-carmine, picronigrosin.

Fig. 22.—Gonenteric canal (between the sections represented in figs. 21 and 23). × 350.

Fig. 23.—Gonenteric canal opening into vagina. × 350.

Fig. 24.—Transverse section through vulva. × 350. Stain: Borax-carmine, picronigrosin.

Fig. 25.—Immature female. Transverse section through gonocœl. × 350. Stain: Thionin, eosin.

Fig. 26.—Immature female. Transverse section through gonoduct not far from vulva. × 350. Stain: Safranin, picronigrosin.

Ascaris clavata.

Fig. 26 *a*.—Transverse section, ovarian region. × 350. Stain: Hæmatoxylin, eosin.

Fig. 27.—Transverse section, oviduct. × 350. Stain: Hæmatoxylin, eosin.

Fig. 28.—Transverse section, uterus. × 350. Stain: Hæmatoxylin, eosin.

Fig. 29.—Transverse section, testicular region. × 350. Stain: Hæmatoxylin, eosin.

Fig. 30.—Transverse section through vas deferens at its opening into seminal vesicle; portion of wall of latter shown. × 350. Stain: Hæmatoxylin, eosin.

Fig. 31.—Portion of transverse section, ductus ejaculatorius. × 350. Stain: Hæmatoxylin, eosin.

Ascaris capsularia.

Fig. 32.—Transverse section, showing ventral gland. × 80. Stain: Hæmatoxylin, eosin.

Fig. 32*a*.—Transverse section through ventral gland, anterior portion. × 350. Stain: Hæmatoxylin, eosin.

Fig. 33.—Oblique section through duct of ventral gland. × 350. Stain: Hæmatoxylin, eosin.

PLATE 9.

Diagrams illustrating the development of the excretory apparatus in Nematodes.

(Figs. 37–40 are based on the descriptions given by Jägerskiöld [9]).

Fig. 34.—Skin-gland of lateral line.

Fig. 35.—Ventral gland of eg., Oncholaimus vulgaris.

Fig. 36.—Ventral gland of Ascaris capsularia.

Fig. 37.—Excretory organ of A. decipiens.

Fig. 38.—Excretory organ of A. rotundata.

Fig. 39.—Excretory organ of A. clavata.

Fig. 40.—Excretory organ of A. megalocephala.

The Hæmoflagellates: a Review of Present Knowledge relating to the Trypanosomes and allied forms.[1]

By

H. M. Woodcock, D.Sc.(Lond.).

(With Text-figures.)

Contents.

[1] All the more important papers published up till February 1st, 1906 have, so far as the author is aware, been considered in drawing up this review.

Class.—**Mastigophora.**
Sub-class.—Flagellata.
Order.—Lissoflagellata.
Sub-order.—Monadina.
Family.—Trypanomorphidæ, n. fam.
Genus.—Trypanomorpha,[1] n.g.
Sub-order.—Heteromastigina.
Family.—Trypanosomatidæ, Dofl. emend.
Genera.—Trypanophis,[2] Trypanoplasma, Trypanosoma.

Section I. Characteristics.

The Hæmoflagellates, although possessing in common a uniform type of organisation, are probably not to be considered as all belonging to a single, well-defined group of monophyletic origin. They constitute, rather, an assemblage of forms springing from at least two different stocks, the resemblances which they exhibit being due to convergence, brought about by the acquirement of similar adaptations in response to their similar and highly-specialised mode of life. They are entirely parasitic, their characteristic habitat being the blood of a Vertebrate. It is unlikely, however, that, in the majority of cases, the whole life-cycle is undergone in that host. The transmission of the parasites from one Vertebrate individual to another is by means of a blood-sucking Invertebrate, which, in several instances, is now known to be a true alternate host, and not merely a carrier; indeed, it is becoming more and more probable that an alternation of hosts normally occurs in each life-history.

[1] The name for this genus has been kindly suggested by Prof. Léger. The writer desires, here at the outset, to warmly thank Prof. Léger for much advice and assistance, especially in connection with the section on the derivation and phylogeny of the Trypanosomes.

[2] Although Trypanophis is most probably not a hæmal parasite, it is included in this article since it is undoubtedly closely related to Trypanoplasma.

The Hæmoflagellates possess either one or two flagella, inserted into the body, with few exceptions, at or near its anterior end. When there are two flagella, one is free and directed forwards; the other is attached for the greater part of its length to the side of the body, by means of an undulating membrane, and terminates ultimately in a free portion directed backwards. When only one flagellum is present, it is invariably attached in this manner, but the flagellum is

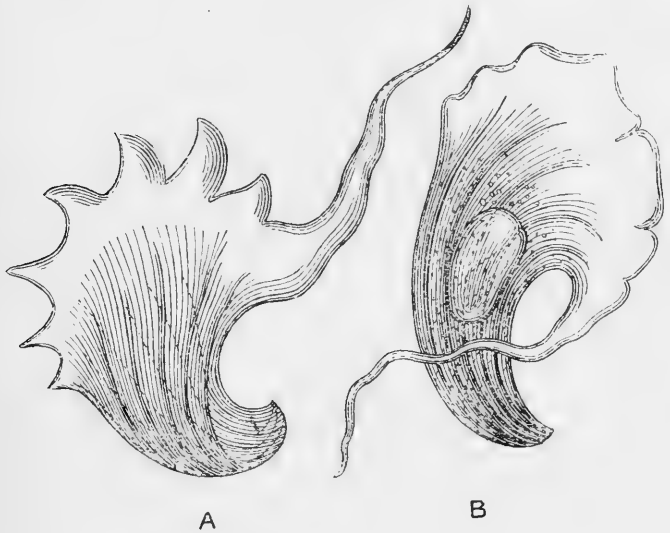


FIG. 1.—"Undulina ranarum," Lankester, 1871. In B, the nucleus is shown. (From Lankester.)

probably not to be considered homologous in all these cases. In certain forms, which are to be derived from a Monadine ancestor, it is, of course, the single anterior flagellum that is represented; in others, however, which are rather to be regarded as descended from a Heteromastigine ancestor, it is the trailing, posteriorly-directed flagellum that persists. There are two nuclear bodies, one, the trophonucleus, regulating the trophic life of the cell, the other, the kinetonucleus, directing its kinetic activities.

The most general method of reproduction is by binary,

longitudinal fission, but multiple division or segmentation is also met with. The complete life-history, where known, is very complicated. It includes true bi-sexual conjugation, which takes place in the Invertebrate; and it appears very likely that, in most instances at any rate, this host is to be considered as the definitive and primary one, and the Vertebrate as the intermediate or secondary one.

Section II. Introductory.

A Study of Recent and Rapid Growth.—Even more marked than in the case of the Sporozoa has been the recent great and rapid increase in our knowledge of the Trypanosomes. The bulk of the important research on these organisms has been accomplished, indeed, within the last four or five years, culminating, for the time being, in the remarkable and far-reaching discoveries announced by Schaudinn at the beginning of 1904. The realisation of the extreme economic importance of these parasites is mainly responsible for this advance. Until almost the commencement of the present century they had been very little studied from a purely zoological point of view. Apart from the work of Danilewsky in the eighties scarcely anything had been previously done towards elucidating their morphology and life-history. Reasons are not far to seek which explain, at any rate, to a certain extent, this lack of interest.

Occurrence.—The minute size of the parasites, together with their habitat in the blood, renders them, unlike the majority of Sporozoa, very inconspicuous; and they are consequently overlooked unless specially searched for. In the light of recent investigation, however, it cannot be maintained that Trypanosomes are at all limited in distribution. For, although they are restricted,[1] so far as is known, to blood-sucking Insects and leeches among Invertebrates, they

[1] The allied form Trypanophis is an exception, being parasitic in certain Siphonophora.

appear to be widely distributed among the principal classes of Vertebrates; and, at the present time, hardly a month passes without a new host being added to the list. It is more difficult to be certain with regard to the frequency with which individual species of the parasites occur, the data being, as yet, somewhat scanty. In one or two instances, however, they are known to be of fairly common occurrence, the Trypanosoma lewisi of rats, for example, being quite as abundant as many Gregarines. This form is met with in all parts of the world, having accompanied the Rodents in their ubiquitous migrations. The proportion of hosts infected varies usually from 10 per cent. to 30 per cent., according to the locality, but, in Berlin, Rabinowitsch and Kempner have found that it may be as high as 41 per cent.

Parasitism in General.—Another reason accounting for the comparative neglect of the Trypanosomes has been the fact that the forms prevailing throughout the greater part of Europe are non-pathogenic—that is, they do not, under ordinary circumstances, give rise to any obviously harmful effects in the animals which harbour them. Attention has not therefore been directed to them by anything comparable, for example, to the devastating epidemics of coccidiosis or myxosporidiosis which sometimes occur.

Animals liable by their natural distribution to the attacks of a given parasite may be divided into two classes according to their behaviour towards it. Either they are immune—this term being used to signify that the attacked animal is actively repellent[1] to the parasitic organism, which is thus unable to gain a footing—or they are susceptible. The reaction between any given parasite and its host, in the latter case, may be regarded as the resultant of several factors. The host, on its part, in many, perhaps in most instances has become accustomed or inured to the invader, and is, apparently, practically indifferent to its presence. Again, to consider

[1] The terms "repellent" and "tolerant" are suggested by Lankester ('Quarterly Review,' July, 1904) in his interesting discussion of the biological relations between a parasite and its host.

the matter from the point of view of the parasite, it may not be advantageous for this to cause, by its ravages, the functional disorganisation or premature death of its host. For one group of parasites especially would such a procedure be likely to have disastrous consequences, namely, the Hæmatozoa, which are dependent for the completion of their life-cycle upon being able to pass into an alternate Invertebrate host at the moment when it sucks the blood of the Vertebrate. In this case, therefore, we may say that such mutual toleration [1] exists between the parasite and its host, as, in ordinary circumstances, enables a proper balance to be maintained on both sides. This equilibrium is disturbed only when the situation is affected by adverse influences (e. g. an unusually strong infection, or weakness of the host owing to unfavourable seasonal or nutritive conditions, etc.).

Pathogenicity of Trypanosomes.—These considerations may afford some explanation of the non-occurrence of trypanosomosis,[2] or illness due to a Trypanosome, under normal conditions in nature. It is a very different matter when animals and parasites belonging to distinct regions are brought into contiguity to one another. A Hæmatozoan, and especially a Trypanosome, produces marked pathogenic effects upon gaining an entry into organisms which, previously, have never been, by their distribution, liable to its invasion. As Lankester (l. c.) points out, such a state of affairs is con-

[1] It is, perhaps, desirable to emphasise the fact that Hæmatozoa, whether we regard Hæmosporidia or Trypanosomes, do not, in natural conditions, cause any serious injury to their hosts. Consider, for instance, the Hæmosporea and the Trypanosomes of cold-blooded Vertebrates; and, again, the very great number of Avian hosts in which malarial parasites appear to be more or less generally present (cf. the observations of Galli-Valerio [**23**], Sergent [**102**], and others).

[2] This term is adopted in preference to various others in use for the following reasons: (*a*) it has the right of priority, having been proposed by Brumpt in 1901; (*b*) it agrees with the nomenclature of all other Protozoan diseases, e. g. coccidiosis, piroplasmosis, etc.; and (*c*) it is pure and not a latinized hybrid, like trypanosomiasis, for example (see Blanchard, 'Arch. Parasit.,' viii, p. 572).

stantly being brought about by the never-ceasing, restless activity of man. With the march of civilisation into the "hinterlands" of the various colonies, man, together with the numerous domesticated animals which accompany him, is brought into close proximity to big game and other wild animals, and, what is more important, into the zone of the par-

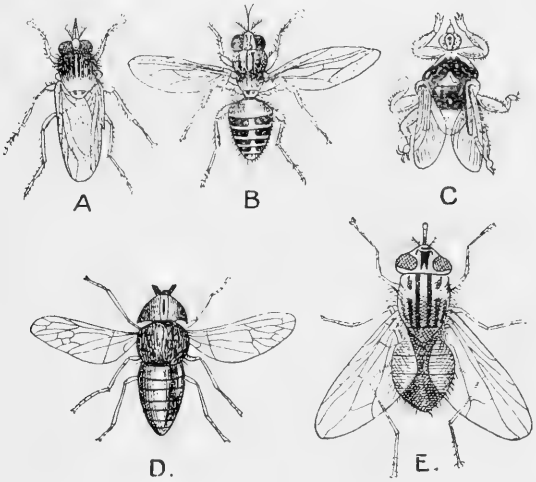


FIG. 2.—Various blood-sucking flies. A and B. Glossina morsitans (transmits the Trypanosome of Nagana, T. brucii) × 2; C, Hippobosca rufipes (thought to transmit the parasite of "Galziekté," T. theileri) × 1½; D, Tabanus lineola (probably conveys the Surra parasite, T. evansi) × 1½; E, Stomoxys calcitrans (suspected in connection with T. equinum, of Mal de Caderas [see, however, under Systematic]) × 2½. (A and B, from Laveran and Mesnil, after Bruce; C, after L. and M.; D and E, after Salmon and Stiles.)

ticular blood-sucking Insects which prey upon them. The new arrivals thus render themselves liable to infection by parasites to which they, unlike the indigenous animals of the neighbourhood, are quite unaccustomed. The new kind of host, being, of course, totally unadjusted to the special environment in which it finds itself and insufficiently supplied with reactive or defensive powers, is unable either to exert a repellent influence on the parasite or to maintain a proper balance between

itself and the latter. The parasite, on its part, enjoys, at first, a more lusty and vigorous development than usual in the new and fertile soil, rapidly gains an ascendancy and overcomes its host, which, sooner or later, almost invariably succumbs.

Thus it happens that the discovery of these minute and inconspicuous blood parasites has usually been the result of an endeavour to ascertain the cause of various perplexing maladies (malaria, trypanosomosis, piroplasmosis, etc.) to which "civilised" man and "imported" animals in these regions are subject. It follows, however, from what has been said above, that the animals for which these parasites are markedly pathogenic cannot be regarded as their true or natural hosts, which are rather to be sought among the native, tolerant animals of the locality concerned.

Abnormal and Involution Forms.—This method of discovery, moreover, tends, unfortunately, to militate against a thorough investigation of the morphology and life-cycle of the parasites themselves. The medical authorities by whom, in most of these cases, new disease-causing parasites have been first discovered were, as of course was only natural, chiefly intent upon an investigation of the malady and its prevention and cure. The parasite was studied, more or less incidentally and from a pathological point of view, attention being focussed principally upon the relation of its different forms to the different phases of the illness, etc. But when the reaction between the attacked organism and the invader is particularly severe (as in trypanosomosis, for example) great care is required in determining the exact relation in which any given form of the parasite stands to the typical life-history of the same. Especially is this so in the case of a Trypanosome, for here, at any rate, the parasite by no means has matters all its own way. A strenuous fight for life is made by the host's cells and tissues in which many of the organisms, notwithstanding their remarkable vitality, certainly suffer. As Laveran and Mesnil, two prominent researchers on this group, have pointed out, there can be no

doubt that many authors have not taken this factor sufficiently into account in constructing what they regarded as the normal life-history of the Trypanosome concerned, and that much of the variety in form and mode of division which has been described is due to abnormal and altered appearances of the parasite. These involution forms in reality represent the commencing degeneration of the Trypanosome and are to be carefully distinguished from its typical phases.

Schaudinn's Work on the Life-cycle of a Trypanosome.—The normal life-cycle of a Trypanosome can be studied much more easily and, it may be said, only thoroughly when it is undergone in the host or hosts to which it is by nature specially adapted, and which, on their part, have become accustomed to that particular parasite. It is highly significant that when an investigation under these conditions has been carefully undertaken, as recently by Schaudinn (**98**), in the case of two common, well-tolerated Trypanosomes of the Little Owl,[1] a more complete and comprehensible account of the whole life-history is made known as the result than had, up till then, been given for all the Mammalian Trypanosomes put together. Especially noteworthy is Schaudinn's revelation of the part played by the "carrier" of these parasites—a gnat. Bruce was the first to demonstrate, in a brilliant manner, the carrying function of the Tse-tse fly[2] in Nagana or the Tse-tse fly disease, and he showed that this Insect acts as the intermediary between wild game (tolerant of the Nagana Trypanosome, and serving as a reservoir) on the one hand, and domesticated animals on the other. Following his methods, much has since been ascertained by various workers concerning the bionomics of other species —their probable source, mode of infection, carrying agents, etc. Yet in no single instance had it been proved whether the Invertebrate is a true alternate host, one, that is, in which definite stages of the parasite's life-history are passed through, until the publication of Schaudinn's work.[3]

[1] Athene noctua. [2] Glossina morsitans.

[3] It is casting no reflection on this author's brilliant work to say that,

The main facts elucidated by this author's epoch-marking research may be here stated. His description relates to two distinct organisms, Trypanosoma (here called Trypanomorpha) noctuæ and Trypanosoma ziemanni. The latter Trypanosome is remarkable for the great resemblance it offers in certain phases to the Bacterial parasites, possessing the form of spiral threads, which constitute Ehrenberg's genus Spirochæta (allied to Spirillum).[1] In both these Hæmoflagellates, the general plan of the life-cycle shows a fundamental agreement. The gnat is a true host, and, indeed, the principal or definitive one, since in it the sexual process is undergone. During much of the time spent in the blood of the bird, the parasites are attached to or penetrate into a blood-corpuscle, acquire a resting, "gregariniform" condition, and become, in fact, what have hitherto been recognised as characteristic Hæmosporidia. The first-named Trypanosome passes into a species of Halteridium, the latter into a species of "Hæmamœba," or "Leucocytozoon." In other words these two Hæmosporidian parasites represent, respectively, only a transient phase in the life-cycle of a particular Hæmoflagellate.

Is a Similar "Alternation" Common to the Majority of Trypanosomes?—These facts will serve to indicate the great gap at present existing between our knowledge of these two parasites of the owl and that of most Trypanosomes. In many cases, indeed, almost the only facts with regard to the life-cycle as yet known with certainty are that the parasites possess the faculty of "agglomeration," and that they multiply by longitudinal division. There is, however, already a certain amount of evidence to hand—and such evidence is rapidly increasing—which tends

dealing as it does with revelations of such fundamental importance in the study of the Hæmatozoa, the corroboration recently afforded by Sergent's investigations (**103**) is highly welcome.

[1] So marked is this similarity, indeed, that Schaudinn was at first inclined to consider this Trypanosome as exhibiting the typical characters of a Spirochæta (see Appendix).

to prove that other forms agree with the examples mentioned above, at least so far as regards the broad features of their life-history. For instance, with respect to the question of their unity in possessing an alternation of true hosts, the Trypanosomes are at the moment[1] in a position quite similar to that in which, until lately, the Hæmosporidia were. It has been customary hitherto to sharply separate the Hæmosporidia of cold-blooded from those of warm-blooded Vertebrates, notwithstanding their close agreement in habitat and morphology, on the ground that the former had no alternation of hosts. Recently, however, Siegel (**105**) demonstrated such an occurrence in the case of Haemogregarina stepanovi, parasitic in a tortoise, the discovery being at the same time extended by Schaudinn for another member of the Hæmosporea, namely, Karyolysus lacertarum. The alternate hosts, in which in both cases the sexual process is undergone, are respectively a leech and a tick.[2] Hence it may be said with practical certainty that a definitive Invertebrate host is common to all the Hæmosporidia, and that being so the distinction between the two sub-orders vanishes. The arguments in favour of a similar fundamental agreement in the case of the different Trypanosomes may be discussed under two principal headings:—(1) the important and, in fact, essential part of transmitter of the parasites played by a blood-sucking Invertebrate, which is in some cases known, and strongly surmised in others, to be a true alternate host; and (2) the evidence which points unmistakably to a close connection between, at any rate, certain Hæmoflagellates and certain Hæmosporidia. The various observations, etc., coming under each heading are, however, considered in detail below (see especially section 9).

[1] Since this was written Prowazek (**88**) has described in detail the life-cycle of T. lewisi. Many of the phases, including sexual conjugation, are undergone in a louse (Hæmatopinus), which is a true alternate host for this parasite. This important discovery helps to bring Mammalian Trypanosomes into line with the rest.

[2] Placobdella catenigena and Ixodes ricinus.

Section III. Historical.

The first observation of a Trypanosome is probably to be ascribed to Valentin (**117**), who, in 1841, announced his discovery of Amœba-like parasites in the blood of a trout. In the two or three years immediately following, Remak, Berg, and others recorded the occurrence of Hæmatozoa which were undoubtedly Trypanosomes in various fishes. The observers usually remarked upon the transparent, membranous portion of the body, with a denticulate fringe or border,—the well-known appearance presented by the undulating membrane when in motion. The parasite of frogs appears to have been first seen by Gluge (1842), and in July, 1843, Mayer (**73 a**) described and figured certain corkscrew-like and amœboid organisms from the blood of the same animal, which he termed variously Amœba rotatoria, and Paramœcium costatum or loricatum. A few months later (November) Gruby (**25**) also published an account of this organism, to which he gave the new generic name of Trypanosoma. The same parasite was subsequently described and figured by Lankester (**30**) in 1871, who, unaware of Gruby's work, called it Undulina ranarum; this author was the first to indicate the presence of a nucleus (fig. 1 B) in the organism. The next discovery was that by Lewis, in 1878, of the form parasitic in Indian rats. This Trypanosome was named Herpetomonas lewisi[1] by Kent, and has since been shown to be of common occurrence in sewer-rats throughout the world. Trypanosomes were first met with in cases of disease by Griffith Evans, who in 1880 found them in the blood of horses suffering from Surra. The organisms were thought by him to be Spirilla. Steel (**110**) rediscovered the same form five years later in transport mules in British Burmah which were suffering from an "obscure and fatal disease." He took a similar view with regard to its affinities, and named it Spirochæta evansi. An early description, with figures, of this parasite was given by Crookshank (1886).

To Mitrophanow (1883 to 1884) and Danilewsky (1885 to 1889) we owe the first serious attempts to study the comparative anatomy of these Hæmatozoa. The work of the latter in particular does not appear to have received as much attention as it deserved. This author examined many birds and fishes, and endeavoured to fit the various phases of the parasites met with in each case into their proper place in the life-cycle. Considering the difficulties of technique with which Danilewsky had to contend, his researches merit great commendation. Some of his figures of a Trypanosome of birds are reproduced in fig. 3. Unfortunately the complete absence from his writings of any system of nomenclature, which leads to the same form being often

[1] This form is now placed in the genus Trypanosoma for reasons which will be given in the Systematic section.

referred to under distinct names, renders it sometimes quite difficult to follow him correctly. The thoughtful character of his work, however, is well illustrated by the following passage taken from the "Parasitologie comparée du sang des oiseaux" (**18**). The author draws attention to the analogy between the spirilliform flagella of "Polymitus" (i. e. the male gametes of a malarial parasite) and the Spirochæta obermeieri found in the blood in relapsing fevers, and goes on—"One may very likely suppose that S. obermeieri also is, by origin, not a free Bacterial form, but in all probability represents only a stage [in the life history] of a Hæmatozoan, more

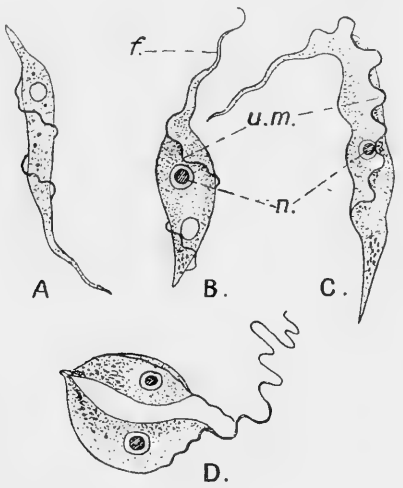


FIG. 3.—A—C. Different forms of "Trypanosoma sanguinis avium," Danilewsky; D, the same parasite dividing longitudinally. *n* = nucleus; *u. m.* = undulating membrane; *f.* = flagellum. (After Danilewsky.)

complex than is yet known, which at some period may even be intracellular (a Hæmocytozoon). Although this surmise has not been verified (so far) for that particular organism, it has been proved for one very Spirochæta-like parasite (T. ziemanni), and it seems by no means unlikely that it will be found to be true in the case of certain others also (cf. Appendix). It shows, at any rate, that Danilewsky was fully alive to the manifold possibilities in connection with these organisms.

The discovery by Bruce, in 1894, of the South African parasite (Trypanosoma brucii) in the blood of cattle and horses suffering from Nagana may be said to have inaugurated a rapid and considerable increase in the number of known forms, the knowledge of which has

in many cases thrown light upon the etiology of maladies hitherto obscure. Thus Rouget, in 1896, ascertained that a Trypanosome is the cause of the illness known as Dourine, which afflicts horses, mules, etc., in Northern Africa and the Mediterranean region. A very deadly malady of horses in South America, known as Mal de Caderas (hip-paraplegia) was shown to be due to one of these parasites by Elmassian, Sivori and Lecler, and Voges, working independently, in 1901. Similarly Theiler showed (1902) that another species, a very large one, causes a distinct disease of cattle in the Transvaal, known as Galziekté (bile disease). Since then, moreover, other varieties of trypanosomosis have been observed in different regions of Africa, but the exact specific nature of the parasites causing them remains, in many cases, problematical.

Finally, there is the discovery of the human parasite. The credit for first recognising a Trypanosome in human blood, and describing it as such, must undoubtedly be assigned to Dr. Nepveu (1898), although it is possible that Barron, who some years earlier reported having found Flagellate organisms in the blood of an anæmic woman at Liverpool, was in reality the first to notice these parasites in man. His description of them is, however, much too meagre to render this at all certain. Trypanosomes were next seen in Senegambia, in 1901, in the blood of a European suffering from intermittent fever. Forde first found the parasites, but was uncertain of their nature; he showed them to Dutton, who recognised them as Trypanosomes, and gave this form the name of Trypanosoma gambiense. A year later (1902) Castellani discovered a similar parasite in the cerebro-spinal fluid of patients suffering from sleeping sickness in Uganda, and it has since been conclusively proved by Bruce and Nabarro that this organism is the true cause of that ghastly disease. In all probability the species is the same as that investigated by Dutton (see below, under "Effects on host," p. 177, and also in the Systematic section).

More important, from the standpoint of zoology, than these interesting medical discoveries, have been the investigations by Laveran and Mesnil, Léger, Schaudinn and others during the last two or three years upon numerous "tolerated" species (many of them new) which supply, indeed, nearly all the material for the sections on morphology, life-history, and taxonomy. At the present time, scarcely a month passes without some new form being described by one or other of these indefatigable researchers, and it may be confidently expected that with such a rate of progress our knowledge of the complete life-cycle will not for long remain dependent on so few observations as is at present the case.

Section IV. Mode of Infection and Habitat; Effects on Host.[1]

A. Relation of the parasites to the Invertebrate host.

Schaudinn (l. c.) has minutely described the manner in which the infection of Athene noctua, on the one hand,

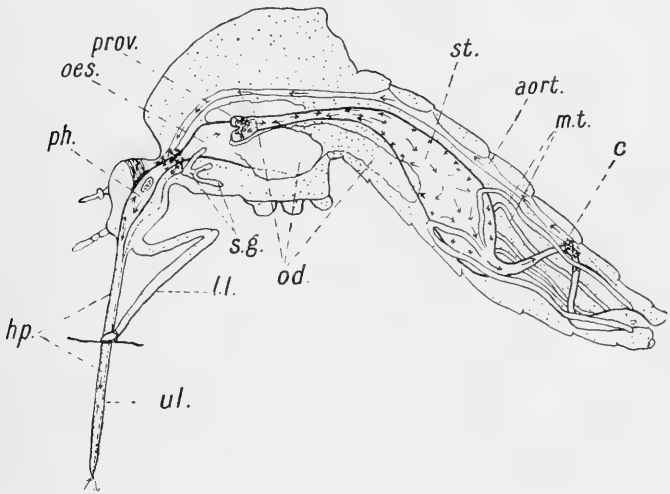


Fig. 4.—Diagrammatical longitudinal section through Culex pipiens to show the distribution of the parasites in the body. The arrows indicate the direction of their movement, the clusters of stars the places of agglomeration. *ul.* = upper lip; *l.l.* = lower lip; *hp.* = hypopharynx; *ph.* = pharynx; *s. g.* = salivary gland; *œs.* = œsophagus; *od.* = œsophageal diverticula (gas reservoirs); *prov.* = proventriculus; *st.* = stomach; *m.t.* = Malpighian tubes; *c.* = junction of ileum and colon; *aort.* = aorta. (After Schaudinn.)

and Culex pipiens (females[2]), on the other, is brought about. For a detailed account of the complicated part played by the different organs of the gnat in the act of biting, the

[1] The habitat and effects upon its host of "Piroplasma" donovani are discussed in Section X, it being thought preferable to consider all the facts relating to this parasite at the same time.

[2] Only the females of gnats and mosquitoes suck blood.

reader is referred to this work. It must suffice here to give some idea of the manner in which the passage of the Trypanosomes to and fro, and their wandering through the body of the Insect, is effected.

The first act after penetration of the proboscis or upper lip (*ul.*, fig. 4) is a sudden, particularly vigorous, respiratory contraction of the abdomen, which causes the blood in the body of the gnat to rush forwards. Pressure is thus exerted on certain sac-like diverticula of the œsophagus (*od.*) and on the salivary glands (*s. g.*), the gaseous and liquid contents of which are thereby expelled through the long tubular hypopharynx (*hp.*) into the wound, carrying with them, in the case of an infected Culex, loose masses of agglomerated Trypanosomes situated at the junction of the pharynx and œsophagus (shown by the cluster of stars in the figure). In this way is brought about the entrance of the parasite into the Vertebrate host. The quantity of saliva secreted is small, and serves principally to digest the blood. Schaudinn finds that the poisonous effects caused by the gnat's bite are due, not so much to the saliva, as to the irritant enzyme of a Fungus (related to the Entomophthoreæ), which is a very common commensal of the Insect, and lives in the œsophageal diverticula (the so-called "sucking-stomachs"). Here it gives rise, during the processes of metabolism, to carbonic-acid gas, and when the contraction of these "gas-reservoirs" takes place, the secreted gas, together with a small quantity of the Fungus in a pullulating condition, is injected into the wound. The author also thinks that rapid coagulation of the blood (before it can pass into the Insect's stomach) is prevented by the gas rather than by the salivary juice.

With the cessation of the respiratory contraction, a small area of negative pressure occurs at the junction of the gas-bubble in the wound and the capillary-like hypopharynx, and the liquid (chiefly blood) rushes up the narrow tube into the pharynx, carrying with it any Trypanosomes it may contain. From the pharynx the blood is pumped through a valve into the œsophagus and its reservoirs, which become filled before the next respiratory contraction takes place. When this happens the blood is driven from these œsophageal diverticula into the stomach (*st.*), where digestion goes on. The whole process may be repeated until the stomach becomes filled, often to overflowing.

The chances against a successful infection of the gnat appear to be, however, considerable. The author found from experiment that frequently infection did not occur at all, many of the gnats either not biting the birds, or at other times being unable to digest the blood, which was then evacuated practically unaltered together with the parasites.

Again, many individuals, as well as races of gnats, appear to have acquired immunity against the parasite, i. e., they are repellent to it. Moreover, even if the Trypanosomes, in the requisite phase of their life-history,[1] gain a footing in the Insect, their further development may be hindered by the existing presence of a different parasite. On the other hand, if the infection is too strong and the development of the organisms too lusty, the gnats are unable to withstand them, and, instead, succumb. A like consequence, it is interesting to note, may ensue if the commensal Fungus (normally, as has been seen, of much utility to the Insect) obtain too great an ascendancy.

The distribution of the Trypanosomes in the body of the gnat is intimately connected with the process of digestion.

As the imbibed blood passes through the posterior part of the œsophagus, the cuticular lining of the latter becomes altered into a gelatinous layer, which is cast off and envelopes the blood in a kind of sheath, the whole passing on into the mid-gut or stomach. Except during the actual time of feeding the hinder region of the œsophagus is invaginated into the anterior part of the stomach, the narrow neck (or proventriculus) of which is expanded to receive it (fig. 4, *prov.*). The epithelial regeneration which takes place at the conclusion of digestion begins in this region soon after the reinvagination of the œsophagus.

Towards the end of the digestion (which may take from two to six days) the Trypanosomes,[2] after a period of multiplication, enter upon a resting phase, and are found either attached to or between the epithelial cells. After a second meal, when the fresh quantity of blood has become digested and ready to be assimilated, a second period of multiplication of the parasites takes place, and the organisms gradually collect in the anterior part where, since the folds of the invaginated œsophagus are non-absorptive, the nutriment remains longest unabsorbed. Here the parasites commence to

[1] It must be remembered that, so far as is known in the case of the Hæmosporidia, only sexual forms are able to stand the transfer from the Vertebrate to the Invertebrate (see the account of the malarial parasites by Minchin [**75**]); and Schaudinn finds the same to be true in the case of the Trypanosomes he examined.

[2] This account refers to the first of the two parasites described by Schaudinn, namely Trypanomorpha (Trypanosoma) noctuæ; certain minor differences exhibited by the other one are mentioned below.

cluster. This is an especially favourable position for them to become attached, since the œsophageal epithelium has only lately shed its cuticle to form the gelatinous sheath around the second quantity of blood, and its cells are being actively regenerated prior to secreting a fresh one. The Trypanosomes, therefore, are able to penetrate the delicate surface of this layer, to which, indeed, as many as possible cling. With the increasing scantiness of nutriment elsewhere more parasites are drawn into the immediate neighbourhood, and these press in between those already attached until finally, at the close of the second digestive period, an enormous mass of parasites has accumulated at this spot, arranged in rows and layers, and all in a resting condition. By this time the new cuticle has become firm and chitinous, and when, at the next meal, the œsophageal invagination is withdrawn out of the neck of the stomach, it leaves behind it the cuticle, serving as a base of attachment for the mass of agglomerated Trypanosomes.

The next (the third) inflow of blood drives this mass before it, in the form of a rolled-up ball, until it reaches the junction of the ileum and colon (fig. 4, *c*), the narrowest point of the intestine. The wall is here very thin and easily ruptured on distension. In this way most of the Trypanosomes are enabled to pass through it, into the vascular lacunæ around, whence they are carried to the heart. From the heart, the Trypanosomes are borne through the aorta into the sinus surrounding the pumping-organ of the pharynx; and between the latter and the pharyngo-œsophageal valve[1] they become at length arrested. The parasites continue to slowly multiply and gradually collect again into agglomerated masses, which surround this region of the pharynx and press on its walls, owing to the narrow throat of the Insect. By the close of the third digestive period, these clumps of Trypanosomes have broken through, and partly block up the cavity of the pharynx. In the next biting act they are forcibly ejected thence into the blood of the owl, as above described.

Thus the parasites cannot leave the gnat until the fourth meal, including that which effected their entry, or not until the third meal after infection has taken place. Schaudinn found that the shortest time elapsing between entrance and exit was seven or eight days; this is the case when the Insects are maintained at the optimum temperature for digestion.

Not all the Trypanosomes, however, are able to leave the gnat. Those which become attached to the epithelium of the stomach, instead of to that of the œsophageal invagination, are not carried backwards to the colon, and so into the circulation, but remain behind in the mid-gut. These are chiefly females, and they can produce a general recurrence of the parasites

[1] A ring of muscular tissue, by means of which the cavity of the pharynx can be shut off from that of the œsophagus.

during the next digestive period. Hence if a gnat becomes successfully infected, it remains so throughout life.

The other parasite described by Schaudinn, Trypanosoma ziemanni, differs slightly in its behaviour in the gnat. Towards the end of the first digestive period the active parasites, instead of collecting in the anterior part of the stomach, press backwards and enter the Malphigian tubules, where, after undergoing multiplication, they come into relation with the excretory epithelium and assume a resting-phase. When the next supply of food has entered the stomach, the Trypanosomes again become active, and, after the process of epithelial regeneration, are carried along with masses of disintegrated cells into the colon, where the subsequent course of events agrees with that just related. The author adds that he has only twice found this form in the salivary glands, and does not think this is a normal habitat of the parasites.

Before leaving the consideration of the Trypanosomes in their relation to the Insect, a very interesting discovery of Schaudinn's must be mentioned, namely, the occurrence of true hereditary infection. Both the parasites described (and also, indeed, the commensal Fungus) may be inherited by the gnats. After breaking through the wall of the colon some of the Trypanosomes, instead of being carried forwards, may pass to the ovarian follicles, there penetrate into the eggs of the youngest, and so infect a succeeding generation. As a rule only few parasites, mostly females, thus penetrate into the ova; once there they assume a gregariniform resting-condition, in which they remain throughout the development of the embryo. When at length the imago first sucks blood these female forms undergo parthenogenesis, and the body of the gnat becomes overrun with Trypanosomes. Sometimes, however (in cases of ample nutrition), the parasites multiply in the yolk of the growing eggs, and may become so numerous that castration results.

Schaudinn has also ascertained that a similar hereditary infection of Anopheles with the tertian parasite occurs. Thus true hereditary infection in the Sporozoa is by no means limited to the case of Glugea bombycis, and it is highly probable that the infection of the progeny of ticks (Rhipicephalus = Boophilus) by Piroplasma also belongs in this category, although the parasites have not yet been demonstrated actually in the ova of the mother-individual.

In the case both of Culex and Anopheles the number of individuals inheriting the parasites appears to be limited. In contra-distinction to Rhipicephalus, where this is, apparently, the only mode in which infection is transmitted, Schaudinn thinks that the infection of gnats by this means is not common in nature. It probably occurs chiefly in the autumn, when the Trypanosomes penetrate into the young eggs, there to pass the winter in a quiescent condition.

Thanks to the elaborate and painstaking investigation of Schaudinn, we are thus enabled to form a very good idea of the manner in which the Trypanosomes are transmitted from the gnat to the owl, and vice versâ. There can be little or no doubt that the process is of an essentially similar nature in the relations of Mammalian Trypanosomes to other biting Insects. In fact, the elucidation of the many factors upon which infection and re-infection are dependent, and of the adaptive modifications of the parasite to the biology of the Insect, goes far towards explaining why many previous investigators, unsuccessful in ascertaining anything definite of the parasites in various "carrying" insects, have concluded that these are not true hosts.

These views are borne out by the recent work of Prowazek (l. c.), who finds that the behaviour of T. lewisi in Hæmatopinus and its passage through the body of the Insect agrees in the main with that above described for the Avian Trypanosomes in Culex. Such differences as there are stand in close relation, on the one hand, to the somewhat different mode of feeding and of absorption of nutriment in the louse, and on the other hand to the fact that this parasite appears to be more resistant to "external" influences.

B. Relation of Trypanosomes in general to their Vertebrate hosts.

Once an entrance into the blood is effected the parasites pass rapidly into the general circulation, and are thus carried to all parts of the body. In considering the distribution and numerical abundance or otherwise, of the Trypanosomes in any given individual, it is necessary to bear in mind whether they are in a tolerant host or in an unaccustomed one. Dealing with the former case first, the general trend of

observation so far is to show that the parasites are, as a rule, never abundant, but, on the contrary, usually rare,[1]—at least, in the Trypanosome form.[2]

One reason for this numerical scarcity is, undoubtedly, the fact that multiplicative stages are hardly ever met with. Multiplication of the Trypanosomes, as such (although in all cases, so far as is known, facultative), appears to be generally held in abeyance, except for a short period at the commencement of the infection. This has been well shown by Laveran and Mesnil (**40**) in the case of T. lewisi of the rat. After inoculation of an uninfected animal with these parasites multiplication goes on briskly for a time, but then slackens and finally ceases. In ordinary, naturally infected rats, multiplication forms are scarcely ever seen. Sivori and Lecler (**106**) alone have described them from very young sewer-rats, doubtless only just infected. Similarly in the case of the great majority of Trypanosomes of cold-blooded Vertebrates, dividing forms are extremely rare in naturally infected hosts. The two parasites of the owl appear, however (according to Schaudinn, l. c.), to behave differently in this respect. At stated intervals rapid, successive multiplication goes on for a short time, after which a period of rest and growth ensues. The reason for this is probably to be sought in connection with the fact that neither of these forms undergoes multiplication when in the Hæmosporidian phase (i. e. as a Halteridium or a "Leucocytozoon"), this absence of "schizogony" being a quite exceptional occurrence, up till now, among Hæmosporidia.

The Trypanosomes in the active motile form are, of course, always free in the blood-plasma (interglobular). But it can no longer be maintained that none are ever, at any stage of life, ecto-[3] or endo-globular. Of the two parasites in the owl, one (Trypanomorpha) comes into intimate relationship with the erythrocytes, and the other (Trypanosoma ziemanni) with the leucocytes and also

[1] It need hardly be again pointed out, that in unusual circumstances, such as an unusually strong infection, mal-nutrition of the host, or captivity, the Trypanosomes may overrun even a tolerant host, and give rise to (in more or less degree) the general symptoms of a pathogenic case. Such instances are noted by Léger (**64**), Plehn (**84**), and Hofer ('Allg. Fischerei Zeitung,' xix, p. 48, 1904).

[2] See, however, below (Section IX B) relative to the possibility of their occurrence in a Hæmosporidian guise.

[3] The term ectoglobular is here used to denote superficial attachment to the blood corpuscle.

with hæmatoblasts, which then fail to become red blood-corpuscles. In the former genus young, non-sexual or "indifferent" individuals attach themselves firmly to the erythrocytes by the flagellar end and come to lie parallel to the surface of the corpuscle. They then enter on a resting period and sink slightly or nestle into the latter, incurving its surface but nevertheless remaining ectoglobular.[1] The only part of the substance of the corpuscle used up by the parasite is that represented by the space which it has hollowed out, and upon which it pressed. After growing for a certain time the parasite leaves the erythrocyte without having decolourised or apparently injured it to any serious extent. Female forms, on the other hand, penetrate into the corpuscle and become endoglobular,[1] growing at the host-cell's expense, and eventually absorbing all its hæmoglobin, forming therefrom the well-known pigment, just like the human malarial parasites. A slightly different course is followed by Trypanosoma ziemanni with respect to the white corpuscles. In the first place the parasites become attached by the non-flagellate end, and secondly, the sexual forms of this Trypanosome are so large that it becomes, here, rather a question of the parasite taking up the leucocyte than the reverse.[2] Fig. 33 B shows a fully-grown female form just attached to a uninuclear leucocyte. According to Schaudinn, the parasite (which proceeds to enter upon a resting-phase as soon as attachment is effected) draws up, as it were, into itself the leucocyte, so that this

[1] Schaudinn finds an exactly parallel behaviour in the case of the indifferent ("schizont") and female forms of the tertian parasite; hence there is equal truth both in his and in Argutinsky's views with regard to this point. See also Minchin (l. c., p. 240).

[2] Both Danilewsky (l. c.) and Ziemann (**121**) agree with Schaudinn in attributing to this parasite a leucocytic habitat (whence its original name of "Leucocytozoon"), and the latter author, moreover, is inclined to admit the possibility, at any rate, of the leucocytes being enveloped by the parasites. Laveran (**37**) on the contrary, in his recent description of this form in the "Hæmamœba"-phase, regards the parasites as invading hæmatids (red blood-corpuscles) which become greatly altered and fusiform owing to their presence.

comes to lie between the ectoplasm and the endoplasm of the Trypanosome,[1] becoming greatly distended and elongated, and more or less spindle-shaped (fig. 33 c). The nucleus of the corpuscle also becomes drawn out and band-like. The ectoplasm of the parasite is apparently then transformed into a protective envelope, and finally cast off with the remains of the leucocyte at the close of this period.

There are, as well, one or two important observations showing that Mammalian Trypanosomes also may come into relation with the blood-corpuscles. Voges (**119**) often noticed individuals of T. equinum attached by the non-flagellate end, and also by the side (cf. Trypanomorpha noctuæ above), to red blood-corpuscles. In some cases, moreover, it appeared as if the parasite had actually penetrated the corpuscle and was destroying it. Similarly Buffard and Schneider (**14**), in the case of T. equiperdum, frequently observed the temporary fixation or attachment of the parasites by the non-flagellate extremity. On the other hand, Prowazek (l. c.) could find neither endoglobular nor ectoglobular phases of T. lewisi, and considers that the habitat of this parasite is restricted to the plasma.

With regard to the distribution of the Trypanosomes throughout the body, they are to be met with practically wherever the blood circulates. They are frequently more numerous in the spleen, bone-marrow, kidneys, and liver, than elsewhere[2]; and Schaudinn finds, in the case of his Avian forms, that it is when passing through the capillaries of these organs (especially of the hæmatopoëtic ones), where the circulation is more sluggish, that the parasites usually leave one host-cell, or seek a fresh one. Danilewsky, again,

[1] The author does not explain further how this feat is accomplished. Remarkable it certainly is; for the ectoplasm appears to be a well-defined layer without anything in the nature of a mouth-orifice, and normally, of course, the parasite absorbs food osmotically.

[2] One or two other points in connection with the distribution are more conveniently noticed below, when considering the pathogenic effects caused by the parasites.

says that the forms he examined in the Roller-bird (Coracias) were numerous in the bone-marrow, where multiplication went on actively, the physiological conditions there obtaining being very favourable for the infection of new host-cells by young parasites. Multiplication may also go on, of course, in the general peripheral circulation, and, in the case of newly-infected fishes or rats, dividing stages of Trypanosomes have been usually described from this situation.

Before passing on to consider the Trypanosomes as pathogenic agents, one very important point may be mentioned, namely, that hereditary infection of the Vertebrate does not, so far as is known, take place in normal circumstances.[1] In the case of Mammals, whether tolerant or unaccustomed hosts, the parasites appear to be unable to traverse the placenta unless this has been in some way injured. Several instances of the delivery of perfectly healthy young from infected mothers have been noted; and in no case have the organisms been found in the blood of a fœtus, where gestation was being accomplished without unfavourable incident.

A detailed description of the effects produced by the Trypanosomes upon unaccustomed Mammalian hosts, into whose blood they may pass, would be out of place in this article, and is, besides, unnecessary, since medical writers have paid great attention to this side of the subject. For full particulars, and also for lists of the various mammals for which a given Trypanosome is pathogenic and their degree of susceptibility, the works of these authorities should be consulted; here, it must suffice to give a general idea of the course of events.

The parasites may either remain infrequent or rare in the blood, sometimes, indeed, being unnoticed until shortly before death, or, on the other hand, they may soon become numerous (fig. 5), and go on increasing more or less constantly until the end. Speaking generally, it may be said that the former case usually occurs in those animals (Bovidæ, Equidæ, etc.)

[1] It must also be remembered that no instance of the inheritance of a Hæmosporidian infection by a Vertebrate host has been recorded.

which are especially liable to suffer naturally from the various maladies (Nagana, Surra, Dourine, etc.), while the latter condition is more often met with in small Mammals which have been artificially inoculated with one or other of those Trypanosomes. While, in this case, the disease is of an acute character and rapidly fatal, in the former it is more chronic and lasts much longer (often several months), with, however, nearly always the same termination. Even when microscopical examination of the blood is unsuccessful in finding the parasites, their presence in it is proved by the fact that, after the injection of a small quantity into another more susceptible host, the Trypanosomes soon appear in the blood of the latter.

There is, moreover, often considerable variability (particularly in chronic cases of Surra and Mal de Caderas, for example) with regard to the appear-

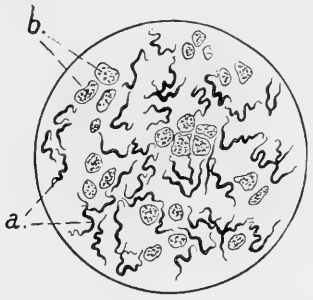


Fig. 5.—Trypanosoma equiperdum (of Dourine), in the blood of a rat eight days after inoculation. *a* = parasites; *b* = blood-corpuscles. (After Doflein.)

ance and number of the parasites in the blood at any moment. Occasionally and at irregular intervals, evidently following upon a period of multiplication, the Trypanosomes may be fairly numerous, their appearance often (though not invariably) coinciding with an access of fever. At other times they seem to vanish almost entirely from the peripheral circulation. Why, exactly, this should be so is not certain. Some authorities attribute it to the rise in temperature, as being unfavourable to the parasites; others think it is due to the more potent operation of chemical and physiological defensive agencies of the host at a higher temperature. It is supposed that certain of the organisms, more resistant than the majority, and situated, perhaps, in some more favourable region of the body, survive and give rise later to a fresh succession of parasites in the blood.

The main features of the illness show a general agreement, whichever variety of trypanosomosis is considered; one symptom may be more marked than another in any particular disease, but a fundamental similarity in type is usually noticeable—so much so that it was, for instance, a long time

uncertain whether Nagana and Surra were distinct diseases or only two varieties of the same. It may be here mentioned, in passing, that the morphological differences between the organisms themselves are sometimes so slight that it is impossible to say, from these alone, whether or no one is dealing with distinct species; the minute distinctions observed might be due to the parasites being in different hosts, for it is known that the same form often varies somewhat (e. g. in size), according to the host in which it is. Laveran and Mesnil, however, have performed a series of instructive experiments (see **52** and **53**) tending to prove that an animal which has been successively immunized against one Trypanosome and its disease is still liable to, at any rate, certain others. Hence there is great probability that a trypanosomosis of any particular region (when it is not, obviously, one which has been transmitted thither from another locality) is produced by a distinct species of parasite. This view is also supported by the specialised and limited facilities for distribution which Trypanosomes possess.

The pathogenic effects are nearly all referable to disorganisation either of the circulatory, or of the nervous system, or of both combined. Fever always occurs, at some time or other, during the course of the malady. Its manifestation is extremely irregular, both in character and in time of occurrence, and it is therefore usually readily distinguishable from malarial fever. It may be variable or continuous; in the former case it appears to be generally remittent rather than intermittent, the temperature, although varying considerably, remaining, for the most part, above the normal. There are, however, often periods of apyrexia, and the temperature may also fall below the normal, especially towards the close of the illness. There is, particularly in chronic cases, marked and progressive anæmia and emaciation, leading to pronounced enfeeblement, which is, in fact, the most characteristic symptom of naturally occurring trypanosomosis. The loss of red blood-corpuscles is frequently great (the number may diminish by as much as 50 per cent.), and hæmaturia is also met with, though never to the same extent as in piroplasmosis. Another common feature is the occurrence of œdematous swellings in various parts, chiefly in the neighbourhood of the genitals, of the abdomen, and around the eyes. The parasites are often more numerous in the bloody serosities bordering these places than in the general circulation. This fact is of great importance in connection with the transmission of Dourine. In this disease the parasites are extremely rare in the blood, but are generally numerous in the immediate neighbourhood of the œdematous excoriations on the penis, so that, in coitus, they come into contact with the vaginal mucous membrane of a healthy mare, through which they are able to pass. Among other externally visible symptoms which are met with in certain instances and to varying degrees may be mentioned the following:—"staring of the coat," or localised bristling of the hair; appearance of small naked areas of skin owing to the falling out of the hairs; occurrence of sanguineous subcutaneous clots, which usually

furnish a rich source whence to procure the parasites, and are doubtless the result of embolism of the capillaries or small vessels by the same.

Nervous symptoms may be only slightly noticeable (e. g. a dull and lethargic tendency towards the close of the illness), or they may be strongly in evidence, especially in Dourine, Mal de Caderas, and sleeping-sickness. In the two former more or less general paralysis of the posterior part of the body frequently sets in; Mal de Caderas of horses in South America is, indeed, often called "hip-paraplegia." In neither of these two diseases, however, have the parasites been observed actually in the nervous system itself, although the brain and spinal cord show considerable histological alteration. But in sleeping-sickness the Trypanosomes penetrate through the membranes surrounding the brain and spinal cord, and can usually be found upon centrifugalising a sufficient quantity of the cerebro-spinal fluid; they have also been seen, in post-mortem examination, in the lateral ventricles of the brain. It is this invasion of the nervous system by the parasites that marks the transition of the case from one of "Trypanosoma-fever" (while the parasites are confined to the blood) to one of sleeping-sickness. The results of the change are soon apparent in the onset of apathy, lassitude, tremor, and the other associated nervous symptoms which characterise this dreadful malady.

Death from trypanosomosis is generally due either to weakness and emaciation (in chronic cases), or to blocking of the cerebral capillaries by the parasites (where these are abundant and the disease consequently acute and rapid), or to the disorganisation of the nervous system (paraplegic and sleeping-sickness forms). Laveran and Mesnil have expressed the opinion that some factor in addition to the presence of the parasites themselves—especially when these are rare—is requisite to explain the severe effects produced, and suggest that the Trypanosomes secrete a toxine. Neither they nor other investigators have, so far, been able to discover traces of any such substance. In post-mortem examination the most obvious pathological feature is hypertrophy of the spleen, which is generally met with, and sometimes to a very considerable degree. Hypertrophy of the liver, and of the lymphatic glands, also occurs; the glands in the neck, inguinal region, etc., are occasionally greatly swollen, and contain numerous parasites.

The spleen and lymphatic glands are, undoubtedly, the

organs which react most strongly to the parasites, and their enlarged condition is probably to a great extent due to enhanced activity in elaborating blood-corpuscles, leucocytes, etc., to cope with the enemy. Laveran and Mesnil (**40**) frequently noticed, in the peritoneal exudations of rats artificially infected with T. lewisi, instances of phagocytic action by leucocytes upon the parasites (Fig. 6). Bradford

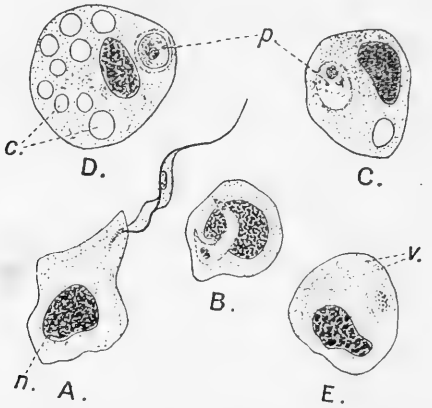


Fig. 6.—Phagocytosis of T. lewisi. In A the leucocyte is commencing to engulf the Trypanosome; in B the latter is completely intracellular; C—E show the gradual dissolution of the parasite, the two nuclear elements remaining longest recognisable. p = parasite; n = nucleus of leucocyte; c = ingested blood-corpuscles; v = vacuoles remaining after their dissolution. (After Laveran and Mesnil.)

and Plimmer (**6**) describe the same taking place in the spleen of rats and mice infected with T. brucii, and also in the blood of "spleenless" animals (i. e. those from which this organ has been extirpated). These authors conclude that, at any rate in the earlier stages of the disease, a good deal of phagocytic action takes place in the spleen. Castellani (**15**), again, has observed phagocytosis of T. gambiense.

But it is probable, also, that the hæmatopoëtic organs or lymphatic glands secrete some chemical or physiological substance which exerts a harmful action upon the Trypanosomes and causes them to undergo involution, assuming

"amœboid" and "plasmodial" forms.[1] These evidences of commencing degeneration or slow death of the parasites are often numerous in the spleen, lymphatic glands, and the bone marrow (especially of spleenless hosts), and, of course, are also met with in the general circulation. Bradford and Plimmer say that they have observed phagocytosis only of such forms, and not of typical adults. This is in favour of the view that these forms are abnormal, already weakened by some agency of the host, and, therefore, less capable of resisting ingestion by the leucocytes. It is these altered forms which are especially liable to block the cerebral capillaries. Their morphology will, however, be more conveniently discussed after considering that of typical Trypanosomes.

Section V. General account of Trypanomorpha (Trypanosoma) noctuæ (Celli and San Felice).

(A scheme indicating the principal phases of the life-history, and serving as a general summary, is given on p. 180.)

In the life of Trypanomorpha noctuæ,[2] parasite of Athene noctua and Culex pipiens, the Trypanosome phase is so frequently lost sight of—the parasite passing into the Hæmosporidian phase, when it takes the form of a Halteridium—that certain stages in development are most easily

[1] McNeal (**74**) would assign the destruction of the Trypanosomes to the cytolytic agents in the peritoneal fluid, which bring about their immobilization and gradual solution, rather than to phagocytosis.

[2] This parasite has been selected as an example of the complete life-cycle of a Trypanosome, not as intending to imply that it is in every way typical of the majority, but because, when the plan of this article was arranged, choice was limited to one of the two forms described by Schaudinn. Trypanomorpha (and still more, Trypanosoma ziemanni) has advanced further in the direction of the Hæmosporidia than, for instance, Mammalian Trypanosomes probably have. Nevertheless, there can be little doubt that, as regards the chief features of its biology, morphology, and life-history, this parasite may be considered as a representative Hæmoflagellate (see below, under "Comparative account of the life-cycle").

Scheme of the Life-cycle of Trypanomorpha noctuæ (Celli and San Felice).

A. In the gnat (Culex pipiens):

Microgametocyte (introduced with the blood of the owl) $\times$[1] 8 microgametes
Megagametocyte „ „ ⟶ megagamete
} + Copula or Zygote ⟶ { Ookinete of indifferent character [1]. „ male „ [2]. „ female „ [3].

[1] ⟶ indifferent Trypanosome × (by binary fission) ..... [x daughter-Trypanosomes ⟶ resting ("gregariniform") ⟶ active × .....] .....xy daughter-Ts., which { pass into the owl [B. 1]. ⟶ male forms (homologous with [2]). ⟶ female „ „ „ [3]).

[2] ⟶ male form (microgametocyte) × 8 male Trypanosomes (microgametes) —||.

[3] ⟶ female Trypanosome (megagametocyte) ⟶ resting-phase (gregariniform) ⟶ (by parthenogenesis) { indifferent form [1]. male form [2]. female form again [3], which { passes, if young, into the owl [B. 3]. ⟶ resting-phase.....

B. In the Little Owl (Athene noctua):

[1] Young indifferent Trypanosome (from the gnat [A. 1]) ⟶ [attached to blood-corpuscle (gregariniform) ⟶ free in plasma..... (active) (alternates 6 times) ⟶ adult T. × .....x daughter-Trypanosomes ⟶] ⟶⟶ { microgametocyte [2]. megagametocyte [3].

[2] Microgametocyte × 8 microgametes (but only in the instant of passing into the gnat [A]).

[3] Megagametocyte (young female Trypanosome) ⟶ endoglobular ⟶ free in plasma.....(slowly alternates until) ⟶ full-grown endoglobular megagametocyte, which { passes into the gnat [A]. ⟶ (by parthenogenesis) { [1]. [2]. [3].

---

[1] Explanation of symbols:—

⟶ signifies becomes, passes into; × signifies gives rise to, by division; } + indicates conjugation; { signifies alternatives; ..... indicates repetition; [ ] signifies all thus enclosed may be repeated; and — || signifies dies off.

and fittingly described by means of the conventional Hæmosporidian terminology.[1]

It is most convenient to commence the account of the complicated life-cycle with the zygote or copula, which results from the union of a microgamete with a megagamete in the stomach of the gnat. Even before nuclear fusion is complete, the copula has acquired the vermiform, motile condition, in which it is known as an ookinete (fig. 7). Its hyaline and more refractive anterior end is capable of considerable and rapid changes of shape, now being extended and pointed, now bluntly rounded off. Behind this follows, usually, a region containing one or more larger or smaller clear vacuoles, then a denser cytoplasmic part with the nuclear spindle, and, lastly, the rounded posterior end containing pigment and other grains. The movements of the ookinete are identical with those of the corresponding phase in other malarial parasites. The ookinetes next proceed to get rid of unnecessary material, including the pigment-grains and the reduction-nuclei left over in the cytoplasm after fertilisation. These are expelled from the hinder end, enclosed in a portion of the cytoplasm, which is cut off at the same time, and forms a gelatinous investment to the mass (figs. 8 B and E, 9 B, and 10 B).

While this is going on, the complete fusion of the male and female pronuclei into the definitive nucleus ("synkaryon") is being slowly accomplished. This, however, by no means includes all the nuclear apparatus present, which is of an exceedingly complex character. In T. noctuæ the nuclear material is highly differentiated and organised. Not only is there a definite and constant number of chromosomes, but the individuality or "separateness" of the principal nuclear constituents, according to their particular function, is very marked. This is revealed in two distinct ways: (*a*) in the sharp resolution of the nuclear material into trophic and

[1] In order to avoid recapitulatory explanation of terms, readers are reminded that a full account of malarial parasites is to be found under the heading "Hæmosporidia," in Minchin's article (l. c.) on the Sporozoa.

kinetic constituents, which are practically separate and independent, at any rate, during the trypanosome phase; and (*b*) in the looseness of the union between the male and female elements. The fertilisation spindle or definitive nucleus is to be regarded as representing the trophic portion, and it will be convenient, therefore, to distinguish it as the trophonucleus. When reconstituted and in the resting condition this body possesses, as the normal number, eight distinct

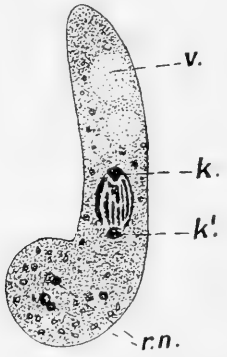


FIG. 7.—Ookinete of Trypanomorpha (or Halteridium) noctuæ (Celli and San Felice). The fertilisation spindle has not yet become rounded off (see text). *k*, *k'* = kinetonuclear elements of the male and female gametes, not yet united; *v* = vacuole; *r.n.* = residual nuclei and pigment grains. (After Schaudinn.)

chromatic aggregations or chromosomes (fig. 8 A, *t.chr.*).[1] Close to either end of the spindle is another chromatic body (fig. 7, *k*, *k'*). These two masses also come from the micro- and megagamete respectively. They proceed to fuse, and the resulting body, which may be termed the kinetonucleus, passes into the now rounded trophonucleus, where it takes up a central position. The kinetonucleus also possesses eight peripherally situated chromosomes (fig. 8 A, *k.chr.*), embedded in a plastinoid matrix; near its centre lies a centrosomic granule (*c*), surrounded by a clear zone. As the name

[1] Strictly speaking, these ought perhaps to be regarded as half-chromosomes.

implies, the kinetonucleus represents that part of the nuclear material which regulates the locomotor activities of the cell.

Different ookinetes, though, at first, essentially similar in nuclear constitution, exhibit considerable variation in the minute structure of the cytoplasm, the quantity of reserve material this contains, and the size of the body as compared with that of the nucleus. In fact, the ookinetes are differentiated into three distinct types, leading to the formation of indifferent, male, or female Trypanosomes, with widely different subsequent histories; and these cytoplasmic variations afford the earliest indications of the direction of further development in any given case. Schaudinn considers that this variability in character stands in intimate relation with the previous history of the sex-cells (gametes) which may have been, as will be seen later, extremely diversified.

(A) Ookinete of Indifferent Character and its subsequent history in the Gnat.

The cytoplasm of an indifferent ookinete (fig. 8) is fairly clear and faintly staining; it usually possesses one or two large vacuoles in the anterior part, but little, if any, reserve material is noticeable. The pigment grains in the hinder region may not all be eliminated at the first attempt, a second expulsion sometimes taking place (fig. 8 E), which leaves the cytoplasm free. Meanwhile, important nuclear changes are occurring. The kinetonucleus becomes amœboid and gives up its material to the trophonucleus (B). The result is that the eight chromatic elements of the former become united, by the aid of the plastin basis, with those of the latter, leaving the above-mentioned granule in the middle. This granule divides in a dumb-bell-like manner, producing a small axial spindle (fig. 8 C, *a.s.*), around which the eight compound chromosomes arrange themselves. These next split, and the halves pass to either end, forming a diaster which is markedly heteropolar (C). The right

(or dorsal) half, the kinetic[1] portion, is perceptibly smaller,

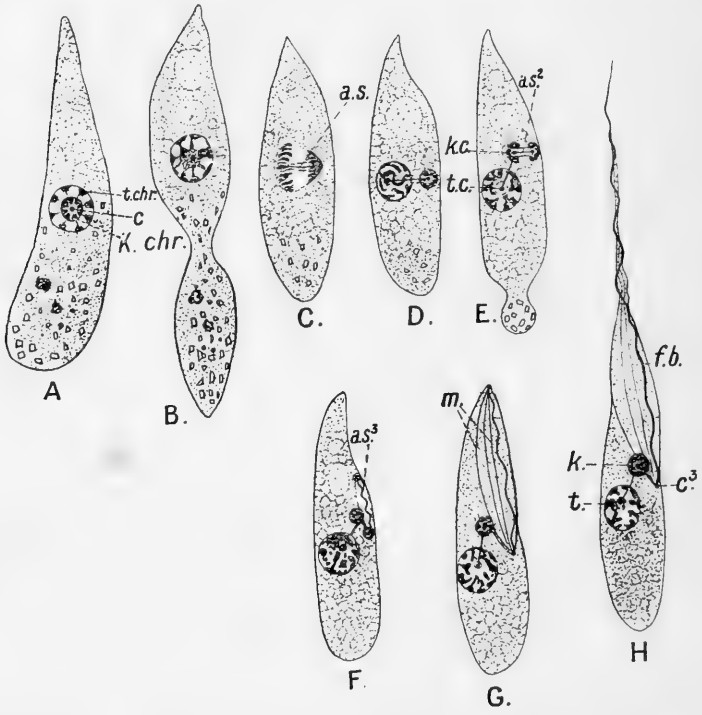


FIG. 8.—Development of an indifferent Trypanosome from an ookinete of indifferent character. *t.chr.* = trophonuclear chromosome; *k.chr.* = kinetonuclear do.; *c* = centrosomic granule; *a.s.* = first axial spindle; $a.s^2$, $a.s^3$ = second and third do.; *t* = trophonucleus; *k* = kinetonucleus; *k.c.* = kinetonuclear centrosome; *t.c.* = trophonuclear do.; *m* = myonemes; *f.b.* = flagellar border of undulating membrane (third axial spindle); c^3 = its proximal centrosome. (After Schaudinn.)

[1] It is here inferred (though not explicitly stated by the author) that the splitting merely separates again the trophic and kinetic constituents which had united, male and female kinetic elements, on the one hand, and trophic ones, on the other, remaining together. There is no evidence that, in the indifferent ookinete, each of the two nuclei thus formed contains both trophic and kinetic parts (i. e., that each is entirely unisexual, one having male, the other female chromosomes only). This is the more unlikely, since, in that case, the nuclei of the indifferent Trypanosomes would not be comparable with those of the others (cf. below, pp. 188 and 191).

but denser, richer in plastin, and more deeply staining than the other.

In this manner, therefore, two distinct nuclear bodies are formed, of different size and structure. The larger one, lying nearer the middle of the body, which rapidly reconstitutes itself (fig. 8 D), is the trophonucleus; its chromosomes have become long, winding threads. The eight chromosomes of the other, the kinetonucleus, are only to be made out distinctly in maceration preparations. Situated near the middle of each nucleus is a small centrosomic grain. These two intra-nuclear centrosomes are connected by a fine achromatic thread, which represents the remains of the axial spindle.

While the trophonucleus now enters upon a resting phase, the kinetonucleus proceeds to form the characteristic locomotor apparatus of the Trypanosome. It passes forward slightly, and takes up a position at the periphery of the endoplasm, lying, indeed, against the limiting ectoplasm.[1] Its centrosome divides again in a dumb-bell-like manner,[2] and forms another axial spindle (E, *a.s.*[2]) at right angles, as before, to the length of the organism. The chromatin becomes aggregated round either end, again in a slightly heteropolar fashion, the dorsal half being somewhat the smaller. There are now two daughter-kinetonuclei, the one on the right being quite in the ectoplasm; they also remain connected together by the drawn-out central spindle, which, as before, joins the two centrosomes. The peripheral daughter-nucleus forms yet another spindle (fig. 8 F, *a.s.*[3]), whose axis is now, however, longitudinal. This assumes large proportions, and spreads forward to the anterior end of the body

[1] Schaudinn thus mentions the existence of a definite ectoplasmic layer but does not describe it, nor do his figures indicate it (apart from the undulating-membrane) at all clearly; see, however, below, p. 209, *et seq.*

[2] The behaviour of this organella strongly recalls that of the "nucleolo-centrosome" of Euglena, during division, and also that of the karyosome of many Coccidia in schizogony; quite possibly, it should be considered in the double light of a "karyo-centrosome."

(G), the whole lying in the ectoplasm, which becomes greatly developed to form the undulating membrane.

In T. noctuæ the undulating membrane arises by the anterior part of the body becoming much flattened laterally, and, to a certain extent, drawn out dorsally by the spindle, the two ectoplasmic surfaces thus coming close together. The well-developed, sinuous, axial spindle has now become excentric in position, and strengthens, or rather itself constitutes the free (dorsal) edge of the membrane, forming a flagellar border to the same (H, *f.b.*) A supporting framework is formed by eight myonemes, representing the eight elongated chromosomes, four of them being arranged on each lateral surface. The flagellar spindle does not stop on reaching the anterior limit of the body, but continues to elongate, drawing out with it the undulating membrane, which narrows and finally thins out. The myonemes then unite with the spindle to form the free flagellum, which gradually tapers away at its distal extremity. The centrosome at this end disappears, as such, but that at the basal or posterior end of the spindle persists (c^3). The other daughter-kinetonucleus has now become rounded off as the functional kinetonucleus (*k*); it remains connected with the complicated locomotor apparatus by means of the delicate thread which represents the second axial spindle. In older stages the kinetonucleus may pass backwards behind the trophonucleus (*t*), pulling with it the associated structures, which thus become even more extended (cf. fig. 13 G).

The characteristic Trypanosome-form is now attained, and the "indifferent" parasite next enters upon a period of multiplication by binary fission. The details of the process are, Schaudinn says, too complicated for explanation without the aid of numerous figures, so that only the main features can be outlined here. The division of the nuclear apparatus is the first to occur; either the tropho- or the kineto-nucleus may lead the way. This is followed by the duplication of the locomotor apparatus, which begins at its basal end, starting either from the still undivided kinetonucleus or

from the new daughter-one. The new flagellum, myonemes, etc., are laid down independently alongside and parallel with the old organellæ, arising, exactly as these did, by the great extension of a kinetonuclear spindle. Lastly, the general cytoplasm divides, and two practically equal daughter-Trypanosomes result.

After active movement and multiplication (the latter taking place without any loss of motility) have continued for some time a resting condition succeeds. The parasites now become gregariniform, and strongly recall the similar phase described by Léger (**63** and **68**) in certain Herpetomonads.[1] The Trypanosome bores into an epithelial cell of the stomach by means of its flagellum, which becomes reduced to a short, rod-like organ, serving to anchor the parasite firmly. Binary fission may go on during this gregariniform condition, and this often leads to the formation of a dense layer of parasites all attached to the epithelium. Besides this superficial attachment they may also penetrate far in between the cells, when they assume a rounded form, and lose all traces of the flagellum. Upon the Trypanosomes again becoming active, or trypaniform, the flagellar apparatus is re-constituted by the kinetonucleus.

This alternation of resting and active periods, accompanied by division, has a limit, dependent upon internal causes (within the parasites themselves) and external ones (due to the reactions of the host). The course of succeeding events may be very varied. The indifferent forms may pass into the blood of the owl, or they may, in certain circumstances which Schaudinn was unable to ascertain, lose their indifferent character and become sexual, either male or female, having the same subsequent development as the male or female Trypanosomes resulting from ookinetes of corresponding character. Finally, if hunger ensue and the gnat is unable to make another meal, the indifferent Trypanosomes gradually die off.

[1] See below, in Section II.

(B) Ookinete of Male Character and its subsequent development in the Gnat.

Ookinetes which will produce male forms are easily distinguishable from those of indifferent character. The cytoplasm (fig. 9) is almost hyaline, and much clearer than in the indifferent ookinetes, which occupy, in this respect, a position intermediate between the other two forms. Reserve materials are completely lacking. The body is smaller than that of either of the other kinds of ookinete; the nuclear apparatus is, however, much larger relatively to the cytoplasm, and very rich in chromatin. The earliest nuclear changes which take place are, apparently, similar to those above described, and lead to the union of the chromatin of the two nuclear constituents. A heteropolar spindle is next formed, and the chromatic elements divide, half being drawn to either end.

It is clear from the subsequent development, however, that this division is in a different sense from the corresponding one in the indifferent ookinetes. Instead of the trophic constituents being separated from the kinetic ones, we must consider the male elements of both kinds as being grouped together and separated from the female ones. The smaller, more condensed half (9 B) is entirely male in character, while the larger, looser half is of female sex. This latter nuclear body, which remains centrally situated (C and D, *f.n.*), is not to be regarded, therefore, merely as a trophonucleus, but as containing the female elements belonging to both tropho- and kineto-nucleus. It rounds itself off, but takes no further part in development, gradually disappearing in situ and being finally left behind with the unused cytoplasm (fig. 9 F). The male nucleus, on the other hand, divides successively to form eight nuclei (C and D, *m.n.*), which become uniformly distributed throughout the body of the microgametocyte, as the male ookinete may now be termed. Each of these nuclei is, moreover, double, the

kinetic and trophic portions of each having separated to form a kineto- and a tropho-nucleus respectively, the former being, in this case, almost as large as the latter (D, *m.k.* and *m.t.*).

The microgametocyte itself never becomes trypaniform. Its cytoplasm assumes a rounded shape, and the eight double nuclei pass to the periphery and there take up a radial

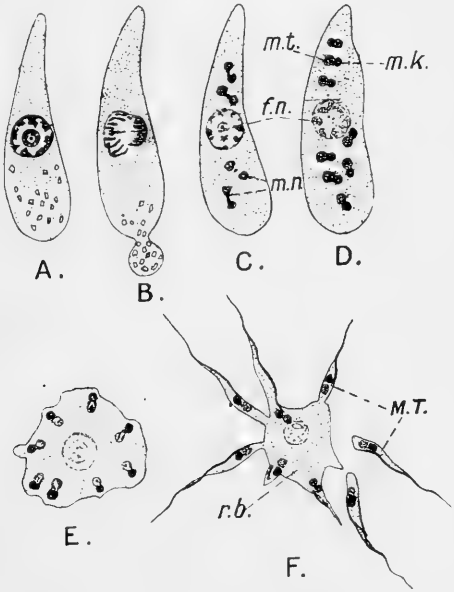


FIG. 9.—Development of microgametocyte and male Trypanosomes from an ookinete of male character. *m.n.* = male nuclei; *f.n.* = degenerating female nucleus; *m.t.* = male trophonucleus; *m.k.* = male kinetonucleus; *M.T.* = Male Trypanosome; *r.b.* = residual body. (After Schaudinn.)

position (E), the kinetonuclei being nearest the surface. The superficial cytoplasm opposite each forms a little prominence or hillock; these eight elevations grow out (accompanied by the eight nuclei, one to each), become narrower as they lengthen, and gradually assume the typical Trypanosome shape.[1] The kinetonucleus of each is producing, meanwhile,

[1] This process, it is interesting to note, takes place in a manner quite comparable to that by which the schizogony of Coccidia is effected.

the locomotor apparatus exactly as in the case of an indifferent Trypanosome, and finally the eight little male Trypanosomes (F, *M.T.*) break away from the central residual mass. They are easily distinguishable from the other forms by their minute size and by their flagellar apparatus, which is, relatively, much more strongly developed, and gives them an unusual degree of activity.

Schaudinn finds that these male Trypanosomes (homologous with microgametes) in the gnat are quite incapable of further development. They cannot divide and soon die off, even though they pass into the blood of the owl. It should be pointed out that the trophonuclei of the male Trypanosomes have already undergone reduction during the second nuclear division in the parent gametocyte,[1] and now possess only four chromosomes each; an important difference in this respect is shown by the kinetonuclei, which have not been reduced (cf. below, p. 197). Schaudinn considers that the reason for the inability of a male Trypanosome to live independently is to be found in this early reduction of its trophonucleus and the consequent derangement of metabolism.

(c) Ookinete of Female Character and its subsequent history in the Gnat.

The cytoplasm of a female ookinete (fig. 10 A) is fairly dense, with dark staining bodies of reserve material. The nucleus is somewhat smaller, relatively to the size of the body, than in an indifferent ookinete. Nuclear changes bring about the formation of a male and female nucleus, exactly as described for the male ookinete, but in the present instance it is the male nucleus which ultimately degenerates. As before, this gives rise, by successive divisions, to eight daughter-nuclei, which pass into the hinder region of the body (B and C, *m.n.*). Each of them becomes distinctly separated into trophic and kinetic portions, but after this the eight double-nuclei gradu-

[1] The author leaves a minute description of the process for his detailed memoir.

ally fade away and are eventually dissolved up in the cytoplasm. Concurrently, the large female nucleus has behaved in a manner recalling the division of the original compound nucleus of an indifferent ookinete, giving rise, by a heteropolar division, to a tropho- and a kineto-nucleus (C). The latter proceeds to form a complete but somewhat feebly-developed locomotor apparatus (D).

A female Trypanosome differs from an indifferent one by its plumper shape and its denser, more deeply-staining cyto-

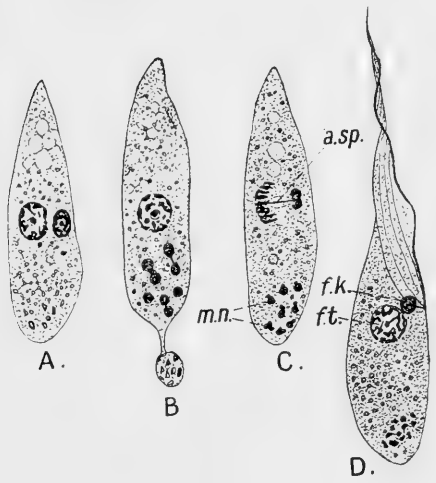


FIG. 10.—Development of a female Trypanosome from an ookinete of female character. *m.n.* = degenerating male nuclei; *a.sp.* = first axial spindle of female nucleus; *f.t.* = female tropho-nucleus; *f.k.* = female kinetonucleus. (After Schaudinn.)

plasm, containing granular reserve nutriment. The kineto-nucleus is smaller and the flagellum shorter; hence the movements of the parasites are feebler and slower, and they soon pass, for a time, into the attached, resting-phase, characterised, as before, by the retrogression of the locomotor apparatus. These forms appear to have quite lost the capacity of longitudinal division, either in the trypaniform or gregariniform phase. Growth is accompanied by a considerable

accumulation of reserve material, and the parasite's dimensions may attain thrice those of an indifferent Trypanosome. The older forms are no longer able to pass from the passive into the active condition, and can only perform slow movements of contraction, flexion, and the like.

In consequence of their reserve stores the adult females, in the gregariniform phase, are exceptionally resistant to external influences, and the most able to withstand unfavourable circumstances. During long hunger-periods of the gnat all the other stages of the parasite die off. Only the females, deeply seated between or beneath the epithelial cells, remain alive, slowly using up their supply of nutriment. With the advent of fresh blood into the stomach they undergo parthenogenesis, at the end of which the parasites are able to become either indifferent, male, or female forms again, the result being that once more fresh generations of Trypanosomes overrun the alimentary canal. It is, moreover, the gregariniform females which bring about the infection of succeeding generations of the gnat, remaining dormant in the ovaries throughout the winter until the eggs are laid and the larvæ develop in the following spring.

Parthenogenesis.—The cytoplasm of a parasite about to undergo parthenogenesis is poorer in reserve material, and now more or less vacuolated. No trace of the locomotor apparatus is visible, and the kinetonucleus lies in contact with the trophonucleus (fig. 11 A). The centrosome of the latter is now surrounded by a chromatic body which somewhat resembles the kinetonucleus when occupying this position in the compound nucleus of an ordinary ookinete[1] (cf. figs. 8 A, and 9 A); and the whole nucleus next undergoes a process apparently similar to that which occurs in an indifferent ookinete at that time. The formation of a heteropolar spindle leads to the separation of a smaller (kinetic ?) half from a larger half, the latter rapidly reacquiring a

[1] Perhaps this body represents an increase, during the resting-period, of kinetic nuclear constituents, in which a female form is apparently more deficient than either a male or an indifferent form.

resting condition. There are now two almost equal-sized nuclei lying contiguous to, and on opposite sides of, the larger, central, probably trophic one, namely, the old kinetonucleus and the newly-formed body. Each of them next divides twice (the old kinetonucleus may have commenced before, as in [B]), cutting off successively two reduction-nuclei (C and D, *r.n.*), which are gradually absorbed by the cytoplasm. The reduced kinetonuclear elements penetrate into the resting trophonucleus from opposite sides (D, *r.k.e.*), and fuse to form

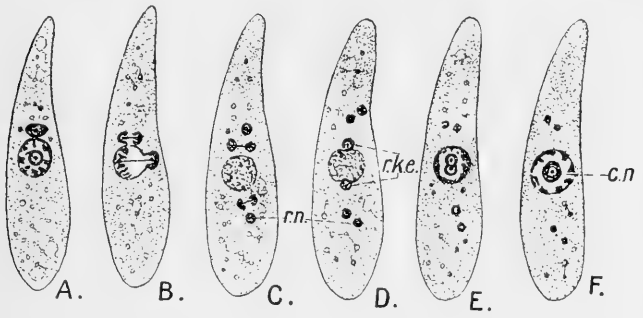


FIG. 11.—Parthenogenesis of a gregariniform female. *r.n.* = residual nuclei; *r.k.e.* = reduced kinetonuclear elements; *c.n.* = compound nucleus, equivalent to that of an ookinete. (After Schaudinn.)

the central kinetonucleus (E and F), exactly as do the reduced kinetonuclei of the male and female gametes, after fertilisation. After this double process of parthenogenesis and "self-fertilisation," the gregariniform parasite can develop along any of the three lines above indicated. The actual course taken is, doubtless, largely determined by the existing condition of the cytoplasm as regards nutritive material, and by its size relative to that of the nucleus.

The three types of parasite above described include all the varieties of form met with in the gnat. From the standpoint of reproduction the indifferent Trypanosomes are by far the most important, the capacity for longitudinal fission being,

indeed, limited to them. Hence these forms largely predominate in number, and many of them, especially small ones resulting towards the end of a multiplication-period, go to swell the ranks of male and female parasites. Correspondingly, while all the types can pass into the blood of the owl, the great majority of those which do so are indifferent forms. Before considering the Trypanosomes in the blood of the bird, however, there is one very important characteristic of the parasites which must be mentioned.

Agglomeration.—In common with many other Trypanosomes, Trypanomorpha noctuæ possesses the capacity for agglomeration. Agglomeration takes place upon the advent of unfavourable conditions (e. g. a period of hunger)

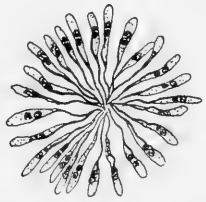


Fig. 12.—Cluster of agglomerated male Trypanosomes in the intestine of the gnat. (After Schaudinn.)

in the environment of the parasites, and all three types possess this faculty; it occurs to the greatest extent, however, among the male and indifferent forms. Agglomeration consists in the grouping or union together of the Trypanosomes around a common centre; this leads to the formation of rosette-like clusters (fig. 12), or even large masses. The parasites are invariably attached by the same end, which is, in the case of Trypanomorpha, the anterior, flagellate end; so that all the flagella are directed inwards, towards the centre of the rosette. If the unfavourable conditions continue the parasites remain in this agglomerated state until they die and disintegrate. If, however, a favourable change suddenly sets in, the Trypanosomes are able to undergo the inverse process, namely, disagglomeration.

The parasites free themselves from one another, and the rosette is dissolved. The entire set of phenomena also occurs in the blood of the owl. Schaudinn ventures no conjectures with regard to its biological significance.

(D) The Behaviour and Subsequent Development of the Trypanosomes in the Vertebrate Host.

All the Trypanosomes met with in the blood of the owl are easily recognised as belonging to one or other of the three categories found in the gnat. Even though the parasites in any given phase may not exactly agree in the two hosts so far as minute detail is concerned, a study of their previous and subsequent history in both cases renders it, nevertheless, manifest that the two forms are the homologues of each other; and, in fact, in all essential characters the Trypanosomes, as seen in the owl, agree with the corresponding type in the gnat. One distinction which may be noted is the presence, in the former case, of pigment in the cytoplasm, produced as the result of the alteration of the hæmoglobin of the red blood-corpuscles.

(1) Indifferent Trypanosomes.—The larger forms entering the blood continue to divide until the size of their descendants is sufficiently reduced. The small ones attach themselves directly to the erythrocytes, and enter upon a period of rest and growth. The mode of attachment has been described above (p. 171), and is shown in fig. 13 A. The locomotor apparatus disappears, and the kinetonucleus takes up a position in close contact with the trophonucleus (B and C). The form of the body is now quite that of a young Halteridium, and, after twenty-four hours, the first pigment grains appear in the cytoplasm. By this time the parasite has increased to about double its original size. It now becomes vermiform and active, reconstitutes its flagellum, etc., and leaves the host-cell (D), usually in the

night time,[1] to become once more a typical Trypanomorpha (E). After a (short?) period of movement free in the plasma, the parasite again becomes attached, resumes the Halteridium form, and grows until the next night, when the above changes recur. This cycle is repeated for six days, until the full size of the organism is attained (F and G). The adult Trypanosome then undergoes, in the active, trypaniform condition, a number of successive longitudinal divisions, until the resulting daughter-Trypanosomes have

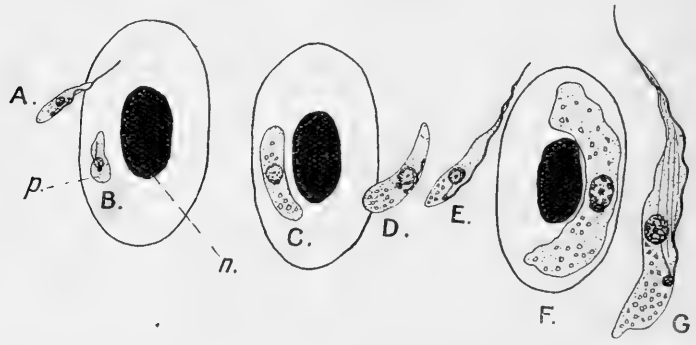


FIG. 13.—Stages in the growth of an indifferent Trypanosome in the blood of the owl. n = nucleus of red blood-corpuscles; p = young intra-cellular parasite. (After Schaudinn.)

at length reached a minimum size, whereupon the process of attachment and growth is begun anew. It is important to note that Schaudinn never observed any division of the parasites when in the gregariniform (Halteridium) condition by multiple fission or schizogony, such as occurs in other Halteridia and Hæmosporidia generally.[2]

(2) Microgametocytes and Microgametes (male forms and male Trypanosomes).—If any little male Trypanosomes from the gnat do succeed in gaining an entry

[1] The reason being, Schaudinn thinks, the fall in temperature of the bird, which occurs at this time.

[2] As the author remarks, this is probably of later phylogenetic development (see below, in Section II).

nto the blood they soon die off. The microgametocytes, here, arise from very young indifferent Trypanosomes, which, as they grow, reveal, by their pale, clear cytoplasm, coarse pigment, etc., their assumption of male characters. A careful examination of the adult microgametocyte shows that the apparently single nucleus, which previous authors have united in describing as, relatively, very large, is, in reality, a highly complex organisation, and consists of eight groups of double-nuclei (i. e. tropho- and kineto-nuclei in close association, compare above p. 189) aggregated together. As in the case of the corresponding forms in the gnat, the trophonuclear elements have undergone reduction, and now possess only four chromosomes each; the kinetonuclei still have the normal number. The formation of the eight microgametes and their separation from the parent-individual takes place in the manner already described.

Each microgamete is a very specialised organism, as is seen from fig. 14 A, and the accompanying diagram. The body is extremely slim and tapering, especially at the posterior end, in marked contrast to that of a male Trypanosome in the gnat; the anterior end, on the other hand, is not drawn out, but acutely conical. The trophonucleus is greatly elongated and has the form of a very long thread, extending nearly the whole length of the body (fig. 14 A, *t.*), to which it serves as an axis. The four chromosomes are strung upon it like beads, at regular intervals. The kinetonucleus (*k.*) is also somewhat elongated and shows distinctly eight chromosomes and an intranuclear centrosome (*k.c.*). It should be noted that the "tail" end is not the flagellar end, but a posterior, whip-like extension of the cytoplasm and trophic nucleus, and it has no relation whatever to the locomotor nucleus and apparatus. As a matter of fact there is here no free flagellum; in other words, the kinetonuclear spindle, which has formed in the usual manner the strongly developed border of the undulating membrane (*a.sp.*[3]) and the eight strengthening myonemes (*m.*), does not extend anteriorly beyond the limits of the cytoplasm. It ends in a

distinct centrosome (*a.c.*), which, as above-mentioned, disappears during the formation of the flagellum in the other trypaniform types. Posteriorly, the locomotor apparatus also terminates in a centrosome (*p.c.*) just in front of the last trophonuclear chromosome. This centrosome is connected

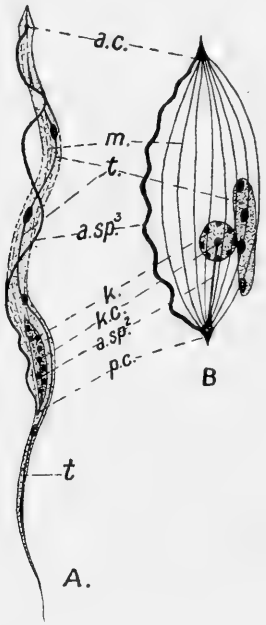


FIG. 14.—A. Structure of a microgamete. B. Schematic groundplan of the same. t = trophonucleus (already reduced); k = kinetonucleus; *k.c.* = kinetonuclear centrosome; $a.sp^2$ = second axial spindle; $a.sp^3$ = third do. (flagellar border of undulating membrane); *a.c.* = anterior centrosome of same; *p.c.* = posterior do.; m = myoneme; t (below) = tail-like prolongation of the body posteriorly. (After Schaudinn.)

with that of the kinetonucleus by a thread ($a.sp.^2$) representing the second axial spindle (see above, p. 185). (The mutual relations of the different nuclear constituents are perhaps, more easily apprehended from the schematic groundplan).

(3) Megagametocytes (female Trypanosomes).—

Large female forms laden with reserve materials are unable to pass through the proboscis of the gnat. Young females, on arriving in the blood, at once penetrate into the erythrocytes. Growth is much slower than in the case of the indifferent forms. Moreover, the parasites change host-cells less frequently, and the older ones appear to be no longer able to assume the Trypanomorpha-form. Such individuals leave one red corpuscle, wander about in the plasma, and then pass into another corpuscle while in the gregariniform condition. A ripe adult megagametocyte is incapable of movement, and remains enclosed by the now pallid and disorganised host-cell, the nucleus of which has been pushed to one side. Its general structure is already well-known through the older researches on Halteridium. Schaudinn points out that, in contact with the relatively small nucleus (trophonucleus), there can be seen a correspondingly small kinetonucleus.

As in the gnat the female Trypanosomes are the only forms able to survive unfavourable circumstances, so here, in the blood of the bird, the megagametocytes alone remain when the indifferent Trypanosomes and microgametocytes have all died off. Similarly, they are able to cause, at intervals, a recurrence of the infection by undergoing the process of parthenogenetic development above described, in the same way that the recurrence of malaria is brought about in the case of Plasmodium (see Schaudinn [**97**]).

(4) Maturation of the Megagametocyte, and Fertilisation of the Megagamete by the Microgamete.—Maturation and fertilisation do not take place until the sexual forms are transferred, with the blood, to the alimentary canal of the gnat. The main outlines of the process have been well described by MacCallum (**72**) for another species of Halteridium, so that our present author directs attention more especially to the cytological details.

As soon as the megagametocyte leaves the warm-blooded host it becomes rounded off, ruptures the delicate envelope of the host-cell still surrounding it, and is thus set free. The

chromatin of the trophonucleus becomes arranged in a long, spirally-wound thread. Its centrosome has disappeared. The chromatic thread is next segmented, both by longitudinal and transverse divisions, so that four separate tetrads result. Meanwhile the kinetonucleus has passed inside the trophonucleus, and appears to serve as the spindle for the reduction-divisions of the latter. After the first mitosis each resulting half has, of course, four dyads instead of four tetrads. In the second division of the germinal nucleus these four dyads are split into monads, or single chromosomes, in

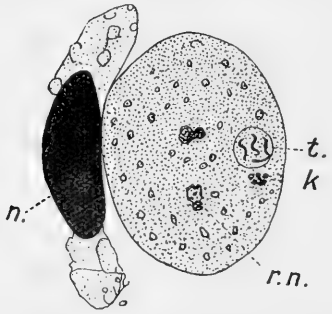


Fig 15.—A ripe megagamete liberated from its host-cell (lying to the left). n = nucleus of disintegrated corpuscle; t = reduced trophonucleus; k = reduced kinetonucleus; $r.n.$ = one of the degenerating reduction-nuclei. (After Schaudinn.)

the usual way. After this the kinetonuclear part resumes its old position outside, but contiguous to, the reduced trophonucleus. How, exactly, the kinetonucleus becomes reduced Schaudinn was not able to determine. The end-result of the process is seen in fig. 15. To the right lies the spherical female trophonucleus (*t.*), with its four thread-like chromosomes, and below it the kinetonucleus (*k.*), consisting of five deeply-staining masses, namely, the four chromosomes and the centrosome.[1] In the middle lie the two reduction-nuclei (*r.n.*).

[1] It is important to note that, in both gametes, the two kinds of nuclear element are present. Hence, on the one hand, neither is the trophonucleus solely female in character nor the kinetonucleus solely male; and, on the other hand, neither is the former merely somatic, nor the latter purely sexual.

The female element is now a ripe megagamete or ovum ready for fertilisation. The microgamete penetrates it at a receptive cone, which arises from the cytoplasm on the side where the female nuclei are situated. Its flagellar apparatus disintegrates and disappears, and, in fact, the only parts remaining distinct are the reduced male trophonucleus and the still unreduced male kinetonucleus.[1] The latter next undergoes two reduction-divisions (not clearly made out), and then the two trophonuclei unite to form the well-known elongated "fusion-spindle," the kinetonuclei taking up a position at either end of it, as in fig. 7. With this act the zygote or copula arrives at the stage with which this description began, and the complete cycle is now accomplished.

Section VI. Comparative Morphology of Trypanosomes.

The body varies greatly with regard to size. Even in one and the same species this is frequently noticeable, particularly under different conditions of life; and since, moreover, different authors often give different estimates of the size of a particular parasite, it is evident that any dimensions given for purposes of comparison can only be considered as approximate. The common Trypanosoma rotatorium of frogs (fig. 17 A and B) is, taking it all in all, one of the largest forms so far described. Its length[2] varies from 40—60 μ,[3] while its greatest width dorso-ventrally[4] is from 8—30 μ; in the very wide examples breadth is gained more or less at the expense of length. Conversely, T. gambiense, the human parasite (fig. 16 C), is one of the smallest forms known.

[1] See footnote on previous page.

[2] The length is always inclusive of the flagellum, unless otherwise stated.

[3] The forms known as T. mega and T. karyozeukton, which are closely allied, but probably distinct, species are somewhat longer.

[4] Adopting Léger's convention by which the convex side, bearing the undulating membrane, is distinguished as dorsal; the measurements of width always include the undulating membrane.

Its average length is about 21—23 μ, and its width 1½—2 μ. The majority of Mammalian Trypanosomes are fairly uniform

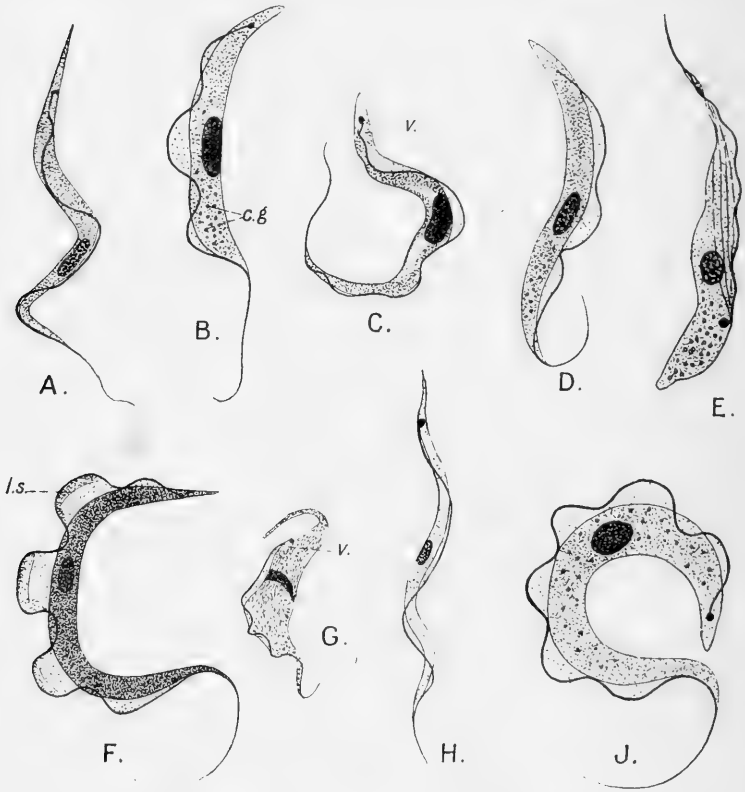


Fig. 16.—Representative Mammalian, Avian, and Reptilian Trypanosomes, to illustrate the chief morphological characters. The figures (excepting E) are all drawn to the original magnification, given where stated. A, Trypanosoma lewisi, after Bradford and Plimmer; B, T. brucii, after Lav. and Mesn., × 2000; C, T. gambiense (blood, *T.*-fever), after Bruce and Nabarro; D, T. equinum, after L. and M., × 2000; E, Trypanomorpha (Trypanosoma) noctuæ, after Schaudinn; F, T. avium, after L. and M.; G, Hanna's *T.* sp. from Indian pigeons; H, T. ziemanni, after Schaudinn; J, T. damoniæ, after L. and M., × 2000. *c.g.* = chromatoid grains; *v.* = vacuole; *l.s.* = fold or striation.

in size (fig. 16 A, B, D), the only noteworthy exception being T. theileri (fig. 49), which is much larger than the rest,

varying, indeed, from 30—65 μ in length. The Piscine Trypanosomes, on the other hand, though possessing an equally great range, exhibit a much more regular gradation. Starting with relatively small forms like T. remaki, var. parva, with a medium length of 30 μ, parasites of all sizes are to be met with up to T. granulosum (fig. 17 K) and T. rajæ, which are among the longest Trypanosomes known, attaining a length of 80 μ.

There is equally great variation in respect of form. Typically, the body is elongated, more or less curved and spindle-shaped, and tends to be slightly compressed laterally. It may be, however, anything from extremely slender or vermiform, to thick-set and stumpy; while, in some cases, the parasites show little or no trace of the spindle-form, but are squat and elliptical. Some authors are inclined to group the parasites according to type of form; the writer does not think, however, that anything is to be gained by so doing. It is very difficult to draw any hard and fast distinctions, because of the individual variation. Apart from the fact that a fully-grown adult, ready to divide, is, in many cases, very much plumper than a young adult (cf. T. lewisi, fig. 27 A and B), there can be no doubt that considerable polymorphism[1] also sometimes occurs; illustrations of this are given below. Some of the chief variations in form, as found in the different groups of Vertebrates, may now be discussed a little more fully; it will be seen that no particular type can be said to be peculiar to a given class of hosts.

On the whole the greatest uniformity is seen in the Mammalian and Piscine Trypanosomes. Among the former the typical fusiform shape prevails. The parasites may be very slender (as in T. lewisi, fig. 16 A, and some forms of T. gambiense, fig. 48 B), fairly so (as in the majority of cases, fig. 16 B—D), or relatively thick-set (T. transvaaliense, fig. 50 C). The animals are usually either crescentic (fig. 16 B) or sickle-like (fig. 16 D). Piscine Trypanosomes are nearly always very elongated and often, relatively, quite as thin as,

[1] This is, of course, quite apart from degeneration and involution forms.

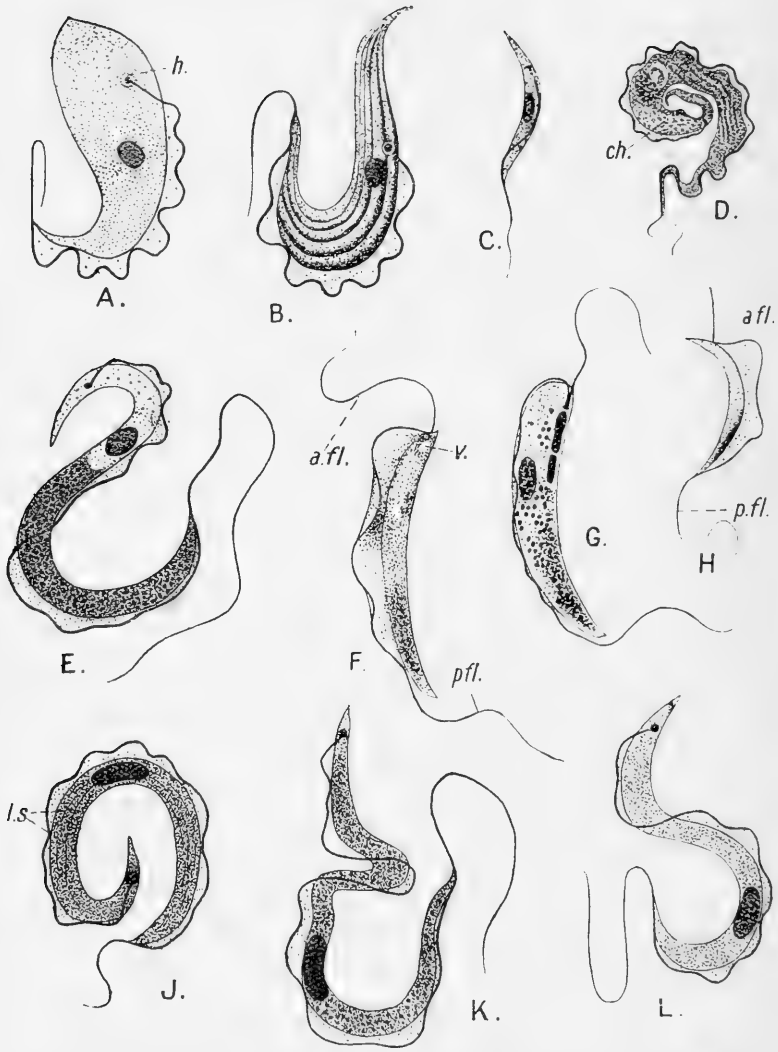


FIG. 17.—Representative Amphibian and Piscine Trypanosomes. Magnification as in Fig. 16 (except D). A and B, Trypanosoma rotatorium, after Lav. and Mesn., × 1400; C, T. inopinatum, after Sergent, × 1000; D, T. karyozeukton, after Dutt. and Todd, × 1000; E, T. nelspruitense, after L. and M, × 2000; F and G, Trypanoplasma borreli (living and stained), after Léger; H, T. cyprini, after Plehn; J, Trypanosoma soleæ, after L. and M., × 2000; K, T. granulosum, after L. and M.; L, T. remaki, var. magna, after L. and M., × 2000. *h.* = clear zone or halo around kinetonucleus; *ch.* = chain of chromatic rodlets running from trophonucleus to kinetonucleus; *a.fl.* = anterior flagellum; *p.fl.* = posterior do.; *l.s.* = longitudinal striations or myonemes; *v.* = cytoplasmic vacuole.

or even thinner than T. lewisi; T. granulosum (fig. 17 K), for instance, is extremely worm-like. In Piscine forms owing, probably, to their great length, the body is frequently coiled or rolled up on itself, as in T. soleæ (fig. 17 J), and T. rajæ (fig. 62 B). Trypanoplasma, as exemplified by T. borreli (fig. 17 F, G), differs from the majority of Piscine Trypanosomes in being short and relatively broad; the length (of the body alone) is 20—22 μ, and the width $3\frac{1}{2}$—$4\frac{1}{2}$ μ.

Coming to the Amphibian parasites, T. inopinatum (fig. 17 C) somewhat resembles a Mammalian Trypanosome, and T. nelspruitense (17 E) a Piscine form; finally, there is the well-known T. rotatorium, which, when most Trypanosome-like, has the form of a thick spindle (17 B). Frequently, however, this parasite is greatly flattened out, and, consequently, very broad and stumpy (figs. 17 A and 56). A similar polymorphism of form is described by Léger (**64**) in Trypanoplasma borreli. Certain individuals are more massive than others, and often, indeed, very short and squat (fig. 18 B).

There can be no doubt that, in some cases at any rate, this variation indicates a difference in sexuality. We have already seen that this is so in Trypanomorpha; and Léger interprets, in the same manner, the broad distinctions between different individuals of Trypanoplasma just mentioned (see also below, in Section IX B).

It is particularly necessary to bear this factor in mind in considering the Avian Trypanosomes. For it is here, perhaps, that we find the extreme types of form; and we are, unfortunately, at present largely in the dark as to how far these represent different parasites and how far polymorphism. That one and the same species may appear entirely different in different phases of the life-history is manifest on comparing the chief "forms" of Trypanosoma ziemanni described by Schaudinn. The asexual or indifferent type is extremely thread-like (fig. 16 H), and, indeed, greatly resembles a Spirochæta. On the other hand both male and female individuals (the latter especially) have the form of a

wide spindle (fig. 33 A and D). In the case of other Avian Trypanosomes, whose life-cycle has not yet been worked out, the parasites have generally been given distinct names according to their appearance when observed. Thus, the attenuated or "spirochætiform" type is exemplified by T. johnstoni (fig. 51) from a small bird (Estrelda) in Senegambia; while the broad fusiform type is represented by Hanna's Trypanosome from Indian birds, and particularly by Dutton and Todd's parasite (also from Estrelda), which is almost rhomboidal in shape (fig. 52). According to Laveran's figure, T. avium (fig. 16 F) occupies an intermediate position. Novy and McNeal find, however, considerable polymorphism in this species, and include herein some of the above forms.[1]

No one doubts that the anterior extremity of the body in the biflagellate or Heteromastigine forms (Trypanoplasma and Trypanophis) is that from which spring the two flagella. In these the anterior end may be acutely or obtusely conical (fig. 17 F) or bluntly rounded (figs. 17 G, 18 A and C). At the tip is often to be noted a little active ("metabolic"), sensitive beak or rostrum; although this points to one side in Trypanophis, it is probably morphologically terminal. Equally certain is it that in the uniflagellate form Trypanomorpha (Trypanosoma) noctuæ, whose life-cycle has been described above, the end of the body bearing the free portion of the flagellum is the anterior one. But with regard to the correct orientation in the rest of the uniflagellate Trypanosomes (collectively included in the genus Trypanosoma), the greatest confusion exists. At present,[2] for the sake of clearness, the terms flagellate and non-flagellate end will be used.

In Trypanosoma the non-flagellate extremity presents considerable variation, often in different individuals of the

[1] It is uncertain how far the conclusions of these authors are warranted, since their "determinations" appear to have been based largely on the different forms of the parasites observed in cultures. See, however, below, in Systematic Section.

[2] The whole question is fully discussed in Section 11.

same species; as Laveran and Mesnil point out, it is particularly plastic. Hence, in the following examples, it must not be inferred that the parasites always conform to that particular description, but merely that such and such a mode of termination is usually to be noted in their case. The non-flagellate end may be much drawn out and pointed, as in T. lewisi, T. ziemanni, T. avium, and T. inopinatum; or shorter, and sharply acute, as in T. gambiense, T. granulosum, T. equinum (fig. 16 D). In other instances it may be obtuse or even rounded off at the tip, as in individuals of T. brucii (fig. 42 B), T. equiperdum (fig. 42 C), and, sometimes to a marked degree, in T. dimorphon (fig. 49 I); although these are all relatively slender forms. Nor does it always follow that the non-flagellate end is blunt in the thick, fusiform parasites; it tapers very finely in T. paddæ (fig. 54), and in Hanna's Trypanosome it is extremely long and attenuated (fig. 16 G). Lastly, figs. 17 A and B, 56 show the different modifications in this respect in T. rotatorium. In one or two instances, the extreme tip evinces a certain degree of contractility,[1] as seen in fig. 42 D of T. equiperdum, where it is retracted, so that the extremity appears forked or bifid. Dutton and Todd seem to have observed a similar appearance in the case of T. rotatorium.

The flagellate end of the body is more uniform and nearly always tapering; it sometimes thins away so gradually (e. g. fig. 16 F) that it is difficult to be quite certain of the exact point where it passes into the flagellum. In one or two cases, however, it ends rather abruptly (e. g. T. soleæ, fig. 17 J).

There are two flagella only in Trypanoplasma and Trypanophis. They are inserted into the body very close to the anterior end, just to one side of the rostrum. The two flagella are quite separate from each other, and, while one (that most anteriorly situated) is entirely free and directed forwards, the other at once bends backwards and is attached to the convex (dorsal) side of the body, throughout the greater part or all the length of the latter. Posteriorly this

[1] Compare the active, sensitive, beak of Trypanoplasma borreli.

flagellum becomes free, and is directed backwards like a tail. A comparison of the degree of development of the two flagella in different cases is very instructive in a phylogenetic connection.

In Trypanosoma there is only one flagellum, which is invariably attached to the body in the manner characteristic of the posterior flagellum of the biflagellate forms. The point of insertion of the flagellum into the body is generally near the non-flagellate end, but may vary considerably; it is in close relation with the position of the kinetonucleus, which is discussed below (p. 216). Although there is generally a free continuation of the flagellum, this may be short or absent. It is very short in T. paddæ and indifferent individuals of T. ziemanni, while in T. johnstoni (fig. 51) and female forms of T. ziemanni (fig. 33 A) the flagellum ceases with the limit of the body. Laveran and Mesnil (**54**) maintain that the same occurs in the case of T. dimorphon (fig. 49 I), but Dutton and Todd, who first described this parasite (**21**), figure a distinct, free flagellum, sometimes short, sometimes long (fig. 49 II). Further investigation is necessary to decide this point.

Along the dorsal side runs a characteristic fin-like expansion of the body, the undulating membrane. This always begins proximally at the place where the attached flagellum emerges from the body; hence, its distance from the non-flagellate end is dependent upon the point of insertion of the flagellum. The free edge of the membrane is more or less sinuous in outline, which gives the structure, even when at rest, a wavy appearance. The edge itself is really formed by the attached flagellum. Distally the membrane thins away concurrently with the body, and when it ceases the flagellum becomes, with one or two exceptions, free. Probably, in fact, in forms with a very tapering flagellar end, the last portion of the body is constituted mainly or entirely by the undulating membrane, which has been drawn out in some such manner as in the case of the anterior end of Trypanomorpha. The membrane may be only slightly developed,

narrow, and chiefly discernible by its thickened flagellar border, as in T. inopinatum (fig. 17 C), sometimes in T. lewisi, and particularly in certain individuals of T. ziemanni (fig. 32) and T. johnstoni (fig. 51); in these cases it is comparatively straight, and of about equal narrowness throughout. In most Mammalian Trypanosomes it is moderately developed, and usually more or less curved and wavy. Lastly, in T. rotatorium (fig. 17 A and B), T. avium (fig. 16 F),[1] T. damoniæ (fig. 16 J), Trypanoplasma and most Piscine Trypanosomes it is very well developed, and often thrown into broad folds or pleats of varying number.

Minute Structure.

The body appears to be in all cases naked, without distinct limiting membrane or cuticle. This is probably an adaptation to the peculiar habitat, and would undoubtedly facilitate nutrition, which, of course, takes place here solely by osmosis. The occurrence of any differentiation of the peripheral cytoplasm in the form of an ectoplasmic layer has only seldom been noted. Most writers simply ignore the point; Laveran and Mesnil (**56**) say that they have not succeeded in differentiating any ectoplasmic layer, either in T. lewisi or in the other Trypanosomes they have investigated. Wasielewsky and Senn (**120**),[2] however, observed such a layer in the case of T. lewisi, distinguished from the rest of the

[1] According to Laveran and Mesnil there is a slight peculiarity in the undulating membrane of T. avium, a well-marked line running in the middle, parallel with its contour (fig. 16 F, *l.s.*); it does not appear to be continuous, being interrupted at the narrow parts of the membrane. These authors consider that it represents a fold. It rather recalls the strengthening filaments or ribs in the membrane of Trichomonas, and perhaps serves a similar function.

[2] These authors use the botanical term "periplast" to denote this layer. Not to enter here into a discussion of the various forms which Senn's "periplast" assumes, it may be merely stated that, at any rate so far as the Trypanosomes are concerned, the zoological designation of ectoplasm undoubtedly best indicates its nature and character.

cytoplasm by taking a slightly different tint; and Prowazek, in his recent work on this parasite, remarks to the same effect. In Trypanophis, Keysselitz (**28**) finds a prominent, highly-refractive, and finely-alveolar ectoplasmic layer, especially well developed near the anterior end, where it forms a little cap (fig. 41, *e.c.*). Unfortunately, even now very few direct references to the subject are available.

It appears most likely that the undulating membrane is largely, if not entirely, an ectoplasmic development. This is usually clearer, more hyaline, and less granular than the rest of the cytoplasm, and in these characters it agrees with a typical ectoplasm. The fact, too, that it is so closely associated with locomotion supports this view.[1] Similarly, with regard to an ectoplasmic differentiation surrounding the body generally, the occurrence of distinct, superficial, longitudinal striæ, probably comparable with myonemes (see below, p. 219), points to the existence of such a layer, since myoneme fibrillæ, when they occur, are always situate in the ectoplasm. Dutton and Todd (l. c.) found that, in injured individuals of T. mega, a delicate membranous (?) envelope, continuous with the undulating membrane, could be easily separated from the rest of the body. In it were fine, pink-staining lines, having a looped arrangement. There can be little doubt this structure was an ectoplasmic sheath with myoneme striations. In short, it seems probable that, in the majority of Trypanosomes, there is such a layer around the body, although, apparently, often poorly developed and ill-differentiated when compared with the undulating membrane.

The general cytoplasm may be of a clear, finely granular, or alveolar nature, presenting a fairly homogeneous appearance, as in T. lewisi, T. equiperdum, and T. gambiense (some individuals), although even in these cases it rarely stains up quite uniformly. It may be coarser and relatively dense, as in T. avium, T. rajæ, T. scyllii (fig. 62), and others. The cytoplasm of male forms is, in general, much clearer and less granular than that of female forms. Accord-

[1] Cf. also its formation in Trypanomorpha (p. 186).

ing to Dutton and Todd the cytoplasm in T. mega and (though to a less extent) in T. karyozeukton shows marked differences in different regions of the body. In the third of the body in front of the nucleus (fig. 58) it is very spongy, and appears loose and alveolar in character; behind the nucleus it is arranged in alternating light and dense, deep-staining bands ("hyaloplasm" and "spongioplasm"), running more or less longitudinally. In Trypanophis there are one or two rows of highly-refractive, yellowish inclusions running the length of the body (figs. 40 and 41). The larger ones lie in the row nearer the convex side, close to the undulating membrane, and these may be oblong in shape, arranged at right angles to the length of the body. These grains are probably not comparable with those next described. Keysselitz thinks they represent collections of fatty and oily substances.

In many forms deep-staining grains or granules, of a chromatoid nature, and of varying size are to be observed in the cytoplasm.[1] These are few and minute in T. danilewskyi (fig. 60 A) and T. tincæ (60 B), somewhat more numerous in T. equinum (16 D), and T. theileri (fig. 50), and relatively large and numerous in T. brucii, and certain individuals of Trypanoplasma borreli (fig. 17 G). In most instances the granules are, if not confined to, chiefly distributed in the flagellate half of the body (in the case of Trypanoplasma, the posterior half). In T. nelspruitense (17 E) and T. granulosum (17 K) the grains are large and particularly numerous, and, in the latter parasite, spread forwards almost to the non-flagellate end.

In certain Trypanosomes a vacuole is often, though by no means constantly, to be observed, situated at a varying distance from the non-flagellate end. This vacuole is well defined, usually of oval shape, and sometimes very prominent, especially in certain Mammalian forms, e.g. T. brucii (fig. 44), T. gambiense (figs. 16 C, 48), and T. evansi (fig. 45). Prowazek (l.c.) also describes, for the first time, the occurrence of one in T. lewisi. Laveran and Mesnil are

[1] For their probable origin and nature see below, p. 229.

not inclined to regard this structure as a normal constituent of the cell,—comparable, for instance, with an excretory vacuole. They only describe its occurrence very rarely (fig. 42 c); their preparations showing it are of Trypanosomes which were not in the blood, but in another medium, e.g. serous, or cerebro-spinal fluid, and they consider that it is an artifact caused by the imperfect fixation of such fluid, in which, of course, the parasite is bathed. There are, however, several considerations to be set against this view. All the above figures are from blood preparations, and in the Reports of the Sleeping Sickness Commission there are some realistic figures of T. gambiense from the blood, in most of which this vacuole is well marked.[1] Moreover, Dutton and Todd's figures (l. c.) of dividing stages of T. dimorphon show clearly that an oval vacuole is present and also divides (fig. 49 II). Again, it is important to note that a similar structure has been described in the case of parasites in their natural (tolerant) hosts, and in what there can be no question were absolutely normal conditions. Thus Léger (l. c.) describes a clear, oval space near the anterior end of Trypanoplasma, which, he says, probably represents a true vacuole, and is not to be confused with certain rounded vacuoles that sometimes appear in unhealthy individuals[2]; Hanna figures a small vacuole in his Trypanosome from Indian pigeons (fig. 16 G); and lastly, Schaudinn describes one or two large ones in the indifferent forms of Trypanomorpha noctuæ, near the apparently opposite, flagellate end be it noted. Hence it appears almost certain that a vacuole, probably excretory in function, may occur normally in many Trypanosomes.[3]

[1] The writer would add that he has seen some of these, and other preparations of Mammalian Trypanosomes, in which the parasites appeared perfectly normal and exhibited this vacuole.

[2] Progressive vacuolisation on a large scale is often met with in atypical or abnormal conditions of the parasites (see below, p. 229).

[3] It may possibly be that the technique used by Laveran and Mesnil is not suited for demonstrating this particular point (see below, p, 217).

There can be little doubt that much has yet to be ascertained concerning the details of nuclear structure in most forms. We have above described the complexity of the nuclear apparatus in Trypanomorpha noctuæ, its division into two distinct parts, trophic and kinetic, and the intimate connection of the latter with the locomotor apparatus. It must be remembered, moreover, that Trypanomorpha is not the only Trypanosome in which this highly complex condition exists. Schaudinn finds a complete parallel in Trypanosoma ziemanni (the "Spirochæta"-form); in this parasite the nuclear apparatus is even more complicated, owing to the fact that the number of "chromosomes" is sixteen, as compared with eight in the first-named form. Again, according to Prowazek's recent investigations, T. lewisi and T. brucii also possess the same fundamental type of nuclear structure. Indeed, the system of axial spindles produced by successive divisions of the karyocentrosome is even more elaborate in the former parasite than in Trypanomorpha. In both forms, the number of trophonuclear chromosomes was clearly seen to be eight; those of the kinetonucleus were more difficult to make out, owing to the compacter form of the latter body. The only other case in which a definite number of chromosomes has been made out is the trophonucleus of Trypanoplasma borreli. Here Léger describes eight dumbbell-shaped chromosomes[1] radially arranged around a central grain (karyocentrosome). These instances would seem to suggest that, with finer and more detailed investigation, the nuclear apparatus of Trypanosomes will be found to show a greater uniformity of complex organisation than is so far known. Our present knowledge of the nuclear elements in the majority of Trypanosomes relates chiefly, however, to their position and general appearance, and is soon summarised.

The trophonucleus (nucleus) occupies a position usually about the middle of the body (figs. 16 B—D, F, 17 J and K); it may, however, be either in the flagellate half, as in T.

[1] They may be divided, forming 16 small chromatic masses.

lewisi (16 A), T. remaki (17 L), and T. rajæ (62 B), or in the non-flagellate half, as in T. damoniæ (16 J) and in T. nelspruitense (17 E); in Trypanoplasma borreli it is often comparatively far forwards (fig. 18). In some cases, at all events, the position is by no means constant (cf. T. equinum [figs. 16 D, 47] and T. evansi [figs. 42 A, 45]). The trophonucleus presents no striking variations in size, which, indeed, appears to be often independent of the size and shape of the parasite. Thus in T. rotatorium it is scarcely larger than that of many slender Mammalian or Piscine forms, in which it occupies almost the entire breadth of the body. The trophonucleus attains, perhaps, its greatest size in the large T. theileri and T. rajæ. In form it is generally ovoid, the longer axis being directed longitudinally, but in the Trypanosome described by Dutton and Todd from Senegambian birds, and, similarly, in Hanna's Trypanosome, the long axis is transverse to that of the body (figs. 52, 53). The shape of the trophonucleus in the latter instance is also unusual, resembling an isosceles triangle. The minute structure is generally described as consisting of a more or less compact aggregation of chromatin grains embedded in a plastin-like base or matrix; these may be uniformly distributed throughout the nucleus, or more closely packed in the peripheral part, leaving a clearer central area (figs. 19, 44, 56). No mention is usually made of a nuclear membrane or distinct reticulum.[1] In T. remaki, possibly also in T. soleæ, and in Trypanophis, there is a large, deeply-staining granule in the centre of the nucleus, surrounded by a clear area, which probably represents the trophonuclear centrosome (karyocentrosome); in Trypanophis this granule appears to divide by simple mitosis (fig. 41).

Of the highest importance is Schaudinn's revelation of the true nature of that hitherto enigmatical and much-discussed chromatic body, which is situated near the root of the

[1] Prowazek describes a nuclear reticulum in both T. lewisi and T. brucii.

flagellum.[1] In view of its essential nuclear character and the fact that it serves as the directive centre for the locomotor activities of the cell, and since, moreover, none of the other

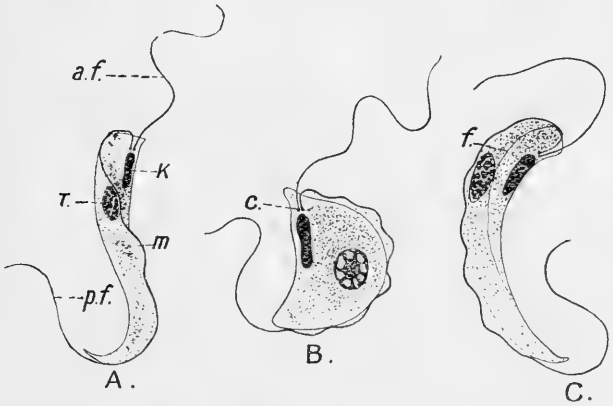


FIG. 18.—Trypanoplasma borreli, Laveran and Mesnil. *a.f.* = anterior flagellum; *p.f.* = posterior do.; *m.* = undulating membrane; *T.* = trophonucleus; *K.* = kinetonucleus; *f.* = fibril (myoneme); *c* = centrosomic granule at base of flagellum. (After Léger.)

names given correctly indicates its real nature, the term kinetonucleus has been adopted in this article to designate

[1] According to Wasielewsky and Senn the "Geisselwurzel" (to call the body for the moment by a non-committal name) is a blepharoplast, i. e. a purely superficial thickening or ectoplasmic differentiation (in botanical language, a periplastic or kinoplasmic development), and bears in its origin no relation to the nucleus. This view is now completely out of court. Rabinowitsch and Kempner (**89**) considered it as a nucleolus, notwithstanding the fact that it is nearly always extranuclear. The other two well-known theories, namely, the centrosomic view of Laveran and Mesnil and the micronuclear one of Bradford and Plimmer, have each a certain modicum of truth. But, as Schaudinn points out, the kinetonucleus is much more than a centrosome—possesses, in fact, a centrosome of its own—and, on the other hand, it has not much in common with the "micronucleus" of Infusoria, beyond the fact that it is of nuclear origin. A micronucleus is essentially a sexual nucleus, its rôle being played when that of the mega- or somatic nucleus is finished; whereas here the kinetonucleus has primarily a kinetic function, the other, the trophonucleus, being just as important sexually.

this structure. For it can scarcely be doubted that this characteristic organella of Trypanosomes is homologous in all cases, and agrees, in origin and significance, with the kinetonucleus of Trypanomorpha above described. Although, unfortunately, very little is known in most cases about the minute structure of this body, owing to its propensity for staining up deeply, the evidence afforded by the work of Schaudinn and Prowazek is, we think, sufficient to justify this view.

One or two additional confirmatory points may be mentioned. In the first place there is the well-developed nature of the kinetonucleus in Trypanoplasma borreli, where it may be almost as large as the trophonucleus, and the same remark applies to the other species of this genus.[1] Again, in the ookinetes of T. barbatulæ (see below, Section IX), Léger has observed heteropolic division of the single large nucleus, doubtless leading to the formation of tropho- and kinetonucleus. Lastly, Bradford and Plimmer (**6**) have themselves observed the latter body given off from the larger, trophic nucleus (the "macronucleus" of these authors) in Trypanosoma brucii.

The kinetonucleus in an adult Trypanosome is always situated normally in the non-flagellated half of the body. Its distance from the end varies considerably, even in different individuals of the same species. The more slender and tapering the extremity, the farther away from it, usually, is the kinetonucleus. In certain forms, e. g. T. mega (fig. 58), T. transvaaliense (fig. 50 c), and some individuals of T. rotatorium (17 b, 56 a) and of T. inopinatum (17 c), it lies more centrally, and is often contiguous to the trophonucleus. The kinetonucleus is usually rounded in shape, but may be sometimes elongated or rod-like, as in T. inopinatum, T. johnstoni (fig. 51), T. transvaaliense, and Trypanoplasma borreli (fig. 18), with its axis either transverse or longitudinal. It attains its largest size in certain Piscine forms, e. g. T. soleæ; in these cases it frequently occupies

[1] To judge, that is, from Plehn's rather unsatisfactory figures (**84**).

the entire width of the body at that point (fig. 17, J and K). There is sometimes a clear zone or halo surrounding this organella, as in figs. 17 A, B, 23 C. Possibly this clear region represents the cytoplasmic vacuole of other forms, which usually lies close to the kinetonucleus (cf. figs. 52, 53). In T. equinum the kinetonucleus is extremely small and difficult to make out; according to Lignières (**71**) and Laveran and Mesnil it is a dot-like thickening at the root-termination of the flagellum (fig. 16 D)[1]; in this form it has apparently become secondarily reduced.

In intimate relation with the kinetonucleus is the flagellum. That portion lying in the general cytoplasm of the body is distinguished as the rhizoplast. When the kinetonucleus is situated near the non-flagellate end and, consequently, nowhere far from the surface, the rhizoplast is very short, and the flagellum leaves the cytoplasm almost at once, becoming, of course, the flagellar border of the undulating membrane. When the kinetonucleus is deeply situated, the rhizoplast is somewhat longer, and (in Trypanosoma) usually runs in an oblique direction to the point where it emerges (figs. 17 A, 58). In Trypanoplasma, the rhizoplastic portion of each flagellum is well-developed; the two are quite distinct, and lie parallel to each other (fig. 18).

Apart from Schaudinn's observations, only in very few cases up till now has the occurrence been recorded of a distinct centrosomic granule, from which the flagellum originates. Léger describes a well-marked granule ("diplosome") at the base of each rhizoplast in Trypanoplasma (fig. 18 B, *c*); and Keysselitz suspects the presence of similar ones in Trypano-

[1] It is most easily demonstrated by using Laveran's special stain. In this connection, and à propos of the genuineness of the vacuole mentioned above, it is interesting to note that Elmassina and Migone (**22**) describe (in preparations made according to their own method) a clear, refringent sphere, situated very near the point where the flagellum emerges from the body. This structure is not rendered visible, these authors find, by Laveran's stain, although this alone reveals the kinetonucleus. From Elmassina and Migone's description and figures it seems most likely that this refringent sphere is a cytoplasmic vacuole.

phis, although owing to the intensity with which the kinetonucleus stains he is unable to be certain. Prowazek frequently figures such a centrosomic granule, situated between the kinetonucleus and the base of the flagellum, in both T. lewisi and T. brucii. Sometimes this granule is closely attached to the kinetonucleus, and appears separated by a short gap from the flagellum; at other times it is attached to the flagellum and separated from the kinetonucleus. As the author points out, it is probably homologous with that at the base of the flagellum in other cases, and, indeed, as already stated, Prowazek finds the minute structure and development of this region entirely comparable to that described by Schaudinn in Trypanomorpha and Trypanosoma ziemanni.[1]

Bearing this in mind, it seems most likely that there is, as a rule, actual organic union between the flagellum and the kinetonucleus, even where there appears to be a gap between the two. Probably the delicate connecting-thread (or axial spindle of an earlier division, see above, p. 185) is not easily demonstrated.[2] In the majority of Trypanosomes the flagellum is described as joined to, and originating from, the kinetonucleus (figs. 16, 17). Even in those cases where there is a clear zone around the latter, and the flagellum seems to begin at the outer border of the halo, Laveran and Mesnil consider that there is, nevertheless, an unbroken union between the two organellæ; for, in involution forms of the particular Trypanosome concerned, when most of the organism has perished, kinetonucleus and flagellum still persist, intimately united.

[1] An interesting point bearing on the view that the flagellum represents the greatly elongated axial spindle of a nuclear division may be noted. In Trypanosoma johnstoni, where there is no free portion of the flagellum, this terminates (at the limit of the cytoplasm) in a small deep-staining granule, which is perhaps comparable to the distal centrosome of the axial spindle (compare also pp. 197-8).

[2] In one or two maceration preparations of T. brucii, Prowazek was able to see this connecting fibril.

The occurrence of prominent myonemes in the undulating membrane of Trypanomorpha, and their nuclear origin (as "mantle-fibrils") has been above described. Schaudinn finds a like development in Trypanosoma ziemanni, the fibrillæ being particularly noticeable in the male and female forms (fig. 33). In this parasite, however, they are not restricted to the undulating membrane, but are arranged laterally, half running down each side of the body, in the ectoplasm. Their total number is sixteen, corresponding to the number of chromosomes. According to Prowazek an exactly similar state of affairs exists in both T. lewisi and T. brucii, although the myonemes (of which there are here eight) are very delicate and difficult to make out. In two or three other forms longitudinal striations, comparable to muscle-fibrillæ, are well-marked; nothing is known, however, with regard to their origin. Thus in Trypanoplasma borreli there are two, which start in front, sweep round, one on each side of the body, and run backwards more than half-way, finally joining ventrally (fig. 18 C, *f.*). The myonemes are very prominent in the ribbed[1] forms of T. rotatorium (fig. 17 B), in which the surface of the body is thrown into longitudinal folds or ridges, often having a somewhat spiral course (56 A); the striations appear to lie in the furrows between the ridges. In T. soleæ, again, the longitudinal striæ are distinctly discernible (fig. 17 J); and Novy and McNeal (**81**) record the occurrence of six or eight in T. avium.

Section VII. Biological Considerations; Movement; Agglomeration; Abnormal and Involution Forms.

(A) Movement.

In general Trypanosomes are extremely active, as would naturally be expected from their powerful locomotor organs.

[1] Other individuals, distinguished as smooth forms (figs. 17 A, 56 B), do not show them.

According to the manner in which they are produced, two kinds of movement, broadly speaking, can be distinguished: (1) displacement of the body, usually rapid, and (2) movements of flexion, extension, and contraction, often comparable to "euglenoid" movements. The latter are brought about, in all probability, by the superficial myonemes above described (cf. the muscle-fibrillæ of Gregarines).

In Trypanoplasma the anterior end always moves first in displacement. According to Léger (l. c.) the principal organ concerned is the undulating membrane, which, by its rapid vibrations from side to side, is thrown into a series of curves or folds; the effect produced is that of rapidly succeeding waves, starting in front and running backwards.[1] The oscillations may be continued into the posterior flagellum, which is then a subsidiary organ of locomotion, of the type known as a "pulsellum,"—i. e. it acts in a driving sense, like the tail of a spermatozoon. Léger thinks, however, that this flagellum functions principally as a rudder. The anterior flagellum is not greatly, if at all, concerned in locomotion. During rapid displacement it is directed backwards, probably passively carried along by the movement of the animal, only, of itself, making slight wavy or circumrotatory movements. At other times it seems to function rather as a sensitive organ, being repeatedly thrust out, as it were, tentatively.

[1] It may be noted that Keysselitz (l. c.) takes a somewhat different view of the manner in which displacement occurs in Trypanophis. Keysselitz thinks that change of place is brought about by rapid, vibratile, or serpentine movements of the whole body substance, the membrane acting, on the contrary, as a curb or drag, which, of itself, would drive the animal in the opposite direction. The author bases his opinion on the observations of the parasites in the attached phase, when the typical swimming motions are often continued, and contends that were it not for this antagonistic working of the membrane the parasites would be driven against their base and swollen out of all shape. It may very well be that the membrane works in an opposite sense at such a time (cf. next page); but it is rather unlikely that the delicate body-substance can produce the vigorous movements shown by the actively swimming parasites, when its only muscular elements are perhaps myoneme fibrils (cf. the slow progressive movement of Gregarines).

The thick, stumpy parasites only accomplish jerky movements of flexion, which scarcely serve to displace the animal.

The manner of locomotion in Trypanosoma differs in one or two points from that in Trypanoplasma. In Trypanosoma the flagellar extremity generally leads the way in movements of displacement, though the parasites can, and sometimes do, move with the non-flagellate end directed forward. The movements may be very rapid and relatively considerable, as in T. lewisi, for example, which quickly darts across the field of the microscope and is lost to sight. T. evansi, again, also easily traverses the field, although it is somewhat slower; on the other hand, T. brucii scarcely ever leaves the field of view, its powers of active displacement being either insignificant or else very little used. There is some difference of opinion as to whether the undulating membrane or the flagellum plays the principal part in these movements. Probably the former does, though the flagellum doubtless acts to a certain extent as a "tractellum," especially in cases of very rapid movement. All Trypanosomes undergo, more or less continually, a vibratile or undulatory motion, caused by the membrane. This may be in either direction, i. e. commencing anteriorly or posteriorly. Movements of contortion are much in evidence in Piscine forms, which, as above mentioned, are frequently coiled up on themselves. In many Trypanosomes, especially the more slender or spirochætiform ones, the undulating membrane often appears spirally wound round the body, this being really due to a more or less pronounced torsion of the latter, which gives the animals a corkscrew-like movement. Euglenoid or semi-amœboid movements are common in the more sluggish parasites, constituting in many individuals of T. rotatorium, for instance, practically the only kind that there is to be observed.

Before leaving the question of movement it is essential to note that slow displacement of the body, occurring in quite a different way, has been observed in certain cases. Thus Léger (**66**) describes a creeping or crawling movement in T.

barbatulæ, which would seem to be quite comparable to a "gregarinoid" movement. Again, Gray and Tulloch (**24a**), in their description of T. gambiense in the fly (Glossina palpalis), say that the parasites also progress in a zig-zag manner, advancing by a series of contractions, which bend first one side of the body and then the other (cf. the flexion movements of sporozoites). In all such cases described, not the flagellate end, but the non-flagellate end, goes first.[1]

(B) Agglomeration.

Before considering the process itself, a few words are necessary with regard to its occurrence and causation. This characteristic phenomenon of Trypanosomes appears to take place chiefly or only upon the advent of unfavourable biological conditions in the surrounding medium. As said above, increasing scarcity of nutriment brings about its occurrence in the case of Trypanomorpha, when in the Insectan host. In the normal, unaltered blood, or other humour, of Vertebrate hosts, agglomeration has only seldom been observed. A tendency to it has been noticed in T. lewisi, and Prowazek has found it to occur in T. brucii, in the inner organs of guinea-pigs and rats. In these cases the phenomenon does not appear to be very persistent. Schaudinn also describes its occurrence in T. ziemanni in the owl. This variety is often termed self- or auto-agglomeration.

On the other hand, when blood containing some of the parasites is drawn off, defibrinated, and kept for some time at a low temperature[2] (in the refrigerator), agglomeration usually sets in partially, and continues more or less persistently, until the death of the parasites supervenes. It is readily and, one may perhaps say, typically produced, when the serum of an animal which has been two or three times inoculated with its specific Trypanosome (e. g. T. lewisi, in the case of the rat), and which is thus becoming repellent to, or acquiring immunity[3] against, that par-

[1] For the importance of this fact see below, in Section XI.

[2] It may be here mentioned that Trypanosomes appear to be much more resistant to a lowering than to an increase of temperature. Their vitality, in cold solutions, has a significant bearing upon the question of a cold-blooded, alternate host.

[3] Without entering here into the question of how immunity is acquired, it may be mentioned that this does not seem to stand in any relation with the property of agglomeration. For one thing, the action of heat upon any serum differs considerably as regards the destruction of its agglomerative

ticular form, is added to blood containing it; in such a case agglomeration is very rapid and frequently total—i. e. embracing the entire number of individuals present. Laveran and Mesnil consider that this peculiar occurrence is intimately connected with the development, in the blood of the host which is undergoing the immunisation, of a substance which has this specific property towards that Trypanosome; in other words, of a specific agglomerine, perfectly analogous to the agglutinines that cause the agglutination of Bacteria. Probably this specific agglomerine is present, or can be produced, in a rat infected for the first time, but only to a very slight degree; with successive reinfections its power is greatly increased. Moreover, sera other than that of the particular kind of host, for the time

FIG. 19. FIG. 20.

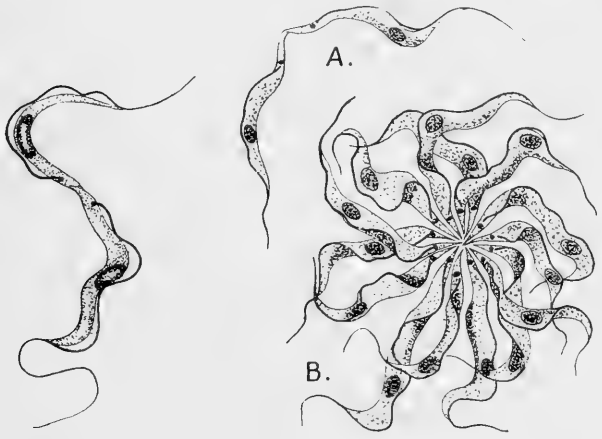


FIG. 19.—Binary union or agglomeration of T. brucii. (After Bradford and Plimmer.)

FIG. 20.—A, ditto of T. lewisi; B, agglomeration cluster or primary rosette of same parasite. (After L. and M.)

being, of a given parasite, may also possess, in a varying degree, this agglomerative property towards that Trypanosome; in such cases, of course, the agglomerine concerned is not specific. Lastly, agglomeration has also been produced by the addition of chemical solutions, and in artificial cultures of the parasites. For fuller details and examples of the compara-

---

and immunising powers. Again, certain sera which exhibit the former property towards a given Trypanosome may not be able to prevent infection by that form. Laveran and Mesnil consider that the "preventivity" of a serum is principally dependent upon its enhanced phagocytic activity.

tive behaviour of different sera, the reader is referred to the works of Laveran and Mesnil (**40, 48** and **56**), Lignières (**71**), Brumpt and Wurtz (**12**), Thiroux (**114, 115**), and others.

Agglomeration commences by two Trypanosomes coming together and joining (figs. 19, 20 A). In all cases in which the phenomenon has yet been witnessed in a natural (as opposed to an artificial[1]) medium, a particular form of Trypanosome always unites by the same end. In Trypanomorpha, as already described, the parasites join by the anterior, flagellate end. Agglomeration has not, up till now, been observed in Trypanoplasma, but there can be little doubt that, if it occurs, it will be found to take place there also by the anterior end. On the other hand, in all the species of Trypanosoma (including T. ziemanni) for which the occurrence has been so far described, the parasites unite by the non-flagellate end. The union may sometimes remain only binary. In other cases agglomeration is rapid and progressive, the union of two parasites being quickly followed by that of many others around the same centre, the whole forming a "multiple union" or rosette (fig. 20 B). Such a rosette is termed a primary agglomeration, and may be composed of as many as one hundred individuals. In many cases, especially where the agglomerine is specific and very powerful, the rosettes themselves become grouped together to form large tangled masses known as secondary agglomerations.

In "cultures," rosettes or clusters are frequently observed in which the arrangement of the parasites is different, and may vary even in the same species; that is to say, in some cases the Trypanosomes have their flagella at the periphery, while in others they are all attached by the flagella, which are directed towards the centre (fig. 29). This has been considered as indicating that the end by which agglomeration takes place cannot be

[1] Much attention has lately been paid—particularly by Novy and McNeal (**79–81**), Smedley (**107**), and Thiroux (l.c.)—to the cultivation of different Trypanosomes in artificial media, in the same way in which cultures of Bacteria are obtained. The writer does not propose to give the details of the composition of the various media tried, nor to discuss the technique and the great (but quite natural!) difficulty experienced in persuading the parasites to live and thrive [?]. For it cannot be too strongly insisted that this is not a zoological method of research, and that the results obtained do not add to our knowledge of the parasite's real life-history and biology, but must be accepted as normal phases only with the greatest caution. It is not merely a question of obtaining a "pure culture," as Novy and McNeal consider: the Trypanosomes are not Bacteria. As will be seen later, some of the opinions to which the authors are led, as a result of practically limiting themselves to this method of investigation, are—to say the least—not generally accepted.

regarded as of importance in determining the orientation of the body. It appears, however, that two entirely different processes are concerned. In most cases, if not in all, the clusters which have the flagella pointing centrally are not instances of agglomeration, but of rapid division (see below, Multiplication), where the parasites remain in contact and form large colonies (exs.: T. lewisi, L. and M., Smedley, McN., and others, T. avium and other forms, N. and McN., T. duttoni, Thiroux). On the other hand, even in cultures, true agglomeration clusters, formed by the union of independent parasites, are attached by the non-flagellate end, as in the blood (exs.: T. brucii and T. lewisi, McN., Smedley and others, T avium, N. and McN., T. paddæ, Thiroux).

These agglomerations differ strikingly from an agglutination of Bacteria, in that the Trypanosomes do not, in the slightest degree, lose their mobility. Each individual continues active movements, its flagellum lashing away at the periphery, and appears to be making strenuous endeavours to escape.

Another distinctive feature of the phenomenon is that of disagglomeration. The individuals constituting agglutinated Bacterial clusters are never known to detach themselves, with the resultant dissolution of the mass; and it is for this reason that Laveran and Mesnil use the distinct terms here adopted. Disagglomeration is in consequence of the retention of the power of movement by the parasites during the progress of agglomeration; thus the Trypanosomes are able to disengage themselves from the cluster, and so to cause the complete break-up of the rosette. Sometimes all the individuals, apparently quite unaltered morphologically, become thus dispersed. At other times the break-up is only partial, a certain number of the more feeble and less mobile parasites remaining together and slowly dying off. Even the larger secondary masses may be thus dissolved. The ability of the Trypanosomes to disagglomerate themselves stands in inverse relation to the strength of the agglomerating serum; where the agglomerine is powerful the parasites appear unable to liberate themselves.

Not only normal and actively-living Trypanosomes undergo this process, but also parasites which have been stupefied, paralysed, or even killed by chemical reagents or strong doses of a serum become united together. In such cases the Trypanosomes are quite irregularly and indiscriminately arranged, forming more or less compact masses of varying shape. Hence Laveran and Mesnil argue that the rosette-formation in typical agglomeration is determined solely by the fact that the parasites possess unimpaired mobility, and are actively striving to free themselves, the resulting figure being that of equilibrium.

Agglomeration does not of itself seem to have any ill effects upon the parasites. Unless disagglomeration occurs the rosettes and masses persist unaltered for some time, the agglomerated individuals retaining their vitality just as well as free individuals in the same surroundings. The only exception is seen in the case of a central rosette, which has served, as

it were, as the nucleus of a large secondary agglomeration; here, if dispersion does not soon take place, the individuals comprising it rapidly degenerate and die, owing to their confined and unfavourable situation.

The significance of the process has yet to be ascertained. By some it is considered as a purely involuntary proceeding on the part of the parasites, and brought about mechanically, by the operation of external influences.[1] The clusters of paralysed and dead Trypanosomes which may be formed are adduced in support of this view. Prowazek's explanation (l. c.) is that nuclear substances resulting from the partial break-up of the kinetonucleus are passed out, causing the surface of the body near that end to become sticky and viscous; and this brings about agglomeration. McNeal (l. c.) also considers that the agglomerating end of the parasites is sticky and adhesive. Neither he nor other workers, however, have described such a fragmentation of the kinetonucleus in Trypanosomes constituting typical rosettes (cf. fig. 20 B), this organella usually appearing quite normal.[2] In view, also, of the fact that the parasites may disagglomerate, it does not seem probable that the disorganisation of the kinetonucleus is the cause of agglomeration.

On the other hand some authors (e. g. Bradford and Plimmer [**6**], and Stassano [**108, 109**]) have seen in the binary unions of T. brucii an occurrence more or less comparable with the conjugation of Infusoria. There can be no doubt that this is too extreme a view to take, there being certainly not sufficient ground for supposing that conjugation in the strict sense, i. e. with nuclear fusion, etc., is here taking place. What evidence there is, is entirely against such a conclusion. In the first place agglomeration can scarcely be considered, as an integral part of the life-cycle. The process is the more entire and lasting, the more unfavourable the conditions which induce it. Indeed, complete fusion is not known to occur (even in binary unions) except in those cases where the parasites are powerless to liberate themselves, when they gradually coalesce and degenerate (see below). Again, it is almost certain that a true conjugation, in the form of the union of differentiated gametes, occurs at quite a different stage of the life-cycle.

[1] Laveran and Mesnil say that there is sometimes to be noticed, at the centre of a rosette, a leucocyte or hæmatoblast which may, perhaps, have served as a nucleus of attraction (cytotactic or chemiotactic) for the individuals of that cluster.

[2] It seems to the writer uncertain how far these parasites with the kinetonucleus broken up into fragments (as was frequently the case, Prowazek says, in agglomerated clusters) ought to be regarded as normal forms; for in addition, in one or two of Prowazek's figures, vacuolisation is much in evidence. Hence it is not unlikely the parasites were somewhat altered and commencing to degenerate (see also below, under "Chromatolysis").

Lastly, it must be remembered that, even if, in some of the binary unions, the kinetonuclei themselves join, as Bradford and Plimmer have supposed, these are not to be regarded as sexual nuclei, comparable to micronuclei (see above, p. 200, footnote).

Bearing in mind, however, the recent work of Calkins and others upon the essential meaning of fertilisation, a remark of Lignières (**71**) is not without interest in this connection. This author investigated the phenomenon in T. equinum, where binary unions are very frequent but fugitive, separation (disagglomeration) readily occurring. He considers it quite probable that, as a result of the close intimacy, a molecular interchange goes on between the associates. The process may be stimulating or recuperative, induced by the effect of the changes in the environment. It is, moreover, somewhat suggestive that the agglomeration is, generally, at first binary, and sometimes (though not often) tends to remains so. Can the process perhaps be considered as affording hints of a plastogamic union? It is evident that much has still to be learnt respecting the biological meaning of agglomeration.

(c) Abnormal and Involution Forms.

Involution and degenerative stages of Trypanosomes have received attention, and acquired an importance altogether undeserved, owing, chiefly, to the fact that many of the parasites have been studied, so far, only in strange and unaccustomed hosts, hosts to which they are unadapted, and for which they, on their part, prove markedly pathogenic. That these forms are the outcome of the unusual environment seems clearly proved by the fact that they are rarely or never described in the case of the many tolerated parasites now known. For being the first to suggest the real significance of the weird shapes often met with, and thus throwing light upon much that had greatly puzzled previous investigators, students of the group are indebted to Laveran and Mesnil, and this is, from the point of view of zoologists, not the least important of the many contributions of these authors to our knowledge of the Trypanosomes. Even as it is, the line of demarcation between forms which are to be regarded as typical and representing a phase in the life-history, and those which are abnormal and commencing to degenerate, is often sufficiently difficult to draw.

Trypanosomes appear to be, in most cases, able to support, for a longer or shorter period, unfavourable conditions of environment, whether due to the reaction of the host itself, or to the transference of the parasites to a strange medium. Moreover, although the organisms, sooner or later, feel the effects of such altered circumstances and show signs of involution, it by no means follows that they rapidly die off. On the contrary, a great number of these abnormal forms, on entering the blood of a fresh host, are able to infect it,

although they may have been, in certain cases, kept for a long while in artificial surroundings. Indeed, their vitality and ability to recuperate themselves and give rise to a fresh succession of parasites,[1] if involution has not proceeded too far, is sometimes nothing less than remarkable. For instances and experiments showing this, reference should be made to the works of Laveran and Mesnil, Lignières, Sivori and Lecler, and others.

The course which involution takes varies somewhat in different cases, and the process may be considered as following one or another of three lines,

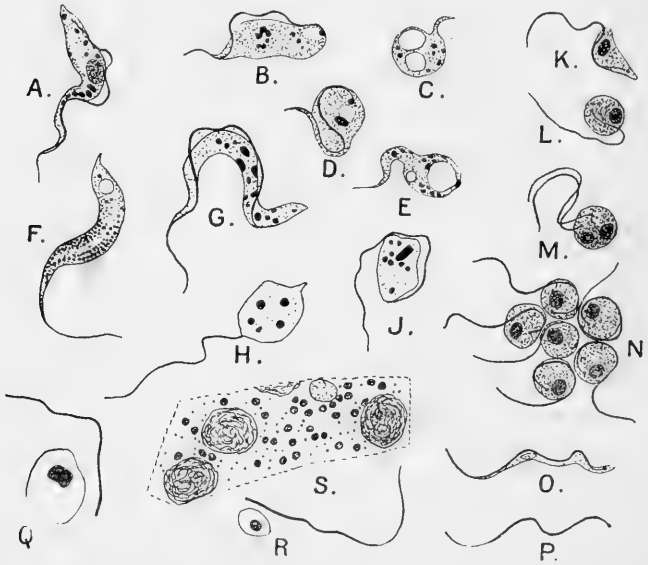


Fig. 21.—Involution and degeneration forms of different Trypanosomes. For description see text. A—E, T. gambiense (A, C, and E after Bruce and Nabarro; B and D after Castellani). F, K—P, T. brucii (F after Bradf. and Plim.; K—P after L. and M.). G—J, Q and R, T. equinum (after Lignières). S, T. brucii, plasmodial mass, from spleen-pulp (after Bradf. and Plim.).

which, though here dealt with separately for convenience, are, of course, occasionally to be met with in combination in any given abnormal form. These three directions are—(a) Chromatolysis, (b) vacuolisation, and (c) change of form.

(A). In chromatolysis, either there is a more or less complete loss by the nucleus (i. e. the trophonucleus) of its chromatic constituents, which in

[1] Hence their virulence and power of infection.

some way pass out into the cytoplasm, leaving, finally, only the faintly or non-staining plastinoid basis (fig. 21 A); or else direct fragmentation of the nucleus occurs (F—J), this being probably a modification of the former method. At other times it is apparently the kinetonucleus which undergoes fragmentation. This is so in T. lewisi, according to Prowazek, and from some of his figures of the parasites the process would certainly seem to bear the interpretation of abnormality. In any case, the result is much the same. Chromatic lumps and grains, varying greatly as regards size and number, become more or less generally disseminated throughout the cytoplasm. Lignières (l. c.) considers that this process is simply an abnormal development of what is a common occurrence in many Trypanosomes. It has been pointed out that in several forms chromatoid grains are frequently to be noticed in the cytoplasm. This author has followed the formation of such in T. equinum, and has seen them given off at intervals from the nucleus.[1] Moreover, it has been observed that these chromatoid grains increase considerably beyond their normal number in individuals placed in unfavourable surroundings, and in some cases the involution process, at any rate in this direction, appears to stop here.

(B). Vacuolisation may also be regarded as a normal function carried to excess. The frequent presence of a vacuole in many Trypanosomes has been mentioned above, and reasons adduced for considering that this structure represents a normal, though not necessarily constant, cell-organella. The first indication of abnormality in this direction is perhaps afforded when the vacuole increases very greatly in size, as in figs. 21 E, 22 E. This may be followed by the appearance of others in the cytoplasm (figs. 21 C and 22 G) when the involution becomes pronounced in character.

(C). Change of form. This is the most obvious, and at the same time the most far-reaching in the effects produced, of the chief lines of involution. Alteration in shape is presaged and accompanied by an increasing loss of mobility until the parasites can no longer move. The manifold varieties which abnormal Trypanosomes may exhibit in respect of shape can be most easily understood, when arranged according to the normal condition or phase which they represent, and of which they are the degenerative results. (i). Single forms. Examples of these are seen in fig. 21. The body becomes fat and stumpy (B—E), and may entirely lose its trypaniform shape, becoming ovoid or spherical—in fact, like a ball (C, L). The flagellum is limp and inactive, and is often partially coiled around the body (J). The undulating membrane can no longer be made out. (ii). Division forms. Certain individuals may have commenced longitudinal division when the change of shape just described sets in, and the process is not completed. Thus result more or less rounded

[1] Probably some process of nuclear excretion or readjustment is concerned.

forms with duplicated kinetonucleus (and sometimes also trophonucleus) and flagellum (M). At other times a quite irregular multiplication of the locomotor apparatus takes place, leading to the formation of distorted bodies, possessing three or four flagella at the corners, with or without associated nuclei (fig. 22 A, E, G). Sometimes again, in more massive forms, the cytoplasm becomes lobed and partly divided up, tending towards

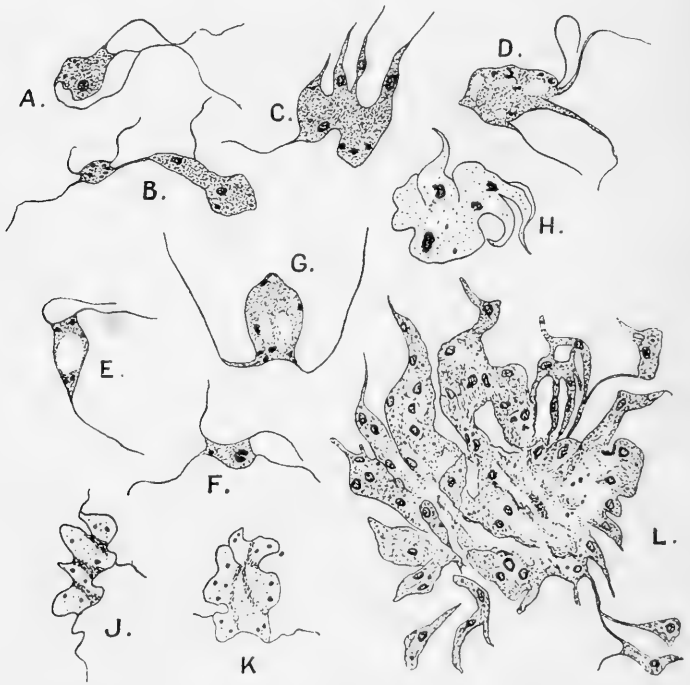


FIG. 22.—Involution and degeneration forms (continued). A—C, T. brucii, after Bradf. and Plim.; D—G, T. gambiense, after Castellani; H, T. brucii, after Martini (interpreted as a small degenerating agglomeration form); J—K, T. equinum, after Voges; L, T. brucii, agglomeration-cluster, commencing to form a plasmodium, after B. and P.

transverse division (J and K) or multiple segmentation (C and D). Perhaps the appearance seen at B is to be thus interpreted, the two halves only remaining joined by a thin cytoplasmic connecting bridge. (iii). Fusion forms. These result either from the grouping together (partial agglomeration) of individuals which had begun to show form involution (fig. 21 N),

or from the degeneration of more or less typical agglomeration rosettes. In the latter case the individuals fuse up into a common mass. The process begins in the centre and gradually extends to the periphery, the Trypanosomes losing their independence and distinctness (fig. 22 L). Thus are formed large plasmodial masses (so-called "plasmodia") consisting of great numbers of nuclei embedded in a now more or less hyaline cytoplasmic matrix (fig. 21 S).

If the organisms remain subjected to the unfavourable influences, or if involution has reached too advanced a stage, death and disintegration result. The cytoplasm is the first to disappear, becoming hyaline and colourless, and refusing to stain up (Q, R). The nucleus rapidly follows suit. The most resistant elements are the kinetonucleus and flagellum, which may persist long after other traces of the organism have vanished (P), the former as a little thickening at one extremity of the latter; sometimes the flagellum alone is left.

(*To be continued.*)

THE MARINE BIOLOGICAL ASSOCIATION

OF THE

UNITED KINGDOM.

——:o:——

Patron—HIS MAJESTY THE KING.

President—Prof. E. RAY LANKESTER, LL.D., F.R.S.

——:o:——

THE ASSOCIATION WAS FOUNDED "TO ESTABLISH AND MAINTAIN LABORATORIES ON THE COAST OF THE UNITED KINGDOM, WHERE ACCURATE RESEARCHES MAY BE CARRIED ON, LEADING TO THE IMPROVEMENT OF ZOOLOGICAL AND BOTANICAL SCIENCE, AND TO AN INCREASE OF OUR KNOWLEDGE AS REGARDS THE FOOD, LIFE CONDITIONS, AND HABITS OF BRITISH FOOD-FISHES AND MOLLUSCS."

The Laboratory at Plymouth

was opened in 1888. Since that time investigations, practical and scientific, have been constantly pursued by naturalists appointed by the Association, as well as by those from England and abroad who have carried on independent researches.

Naturalists desiring to work at the Laboratory

should communicate with the Director, who will supply all information as to terms, etc.

Works published by the Association

include the following:—'A Treatise on the Common Sole,' J. T. Cunningham, M.A., 4to, 25/-. 'The Natural History of the Marketable Marine Fishes of the British Islands,' J. T. Cunningham, M.A., 7/6 net (published for the Association by Messrs. Macmillan & Co.).

The Journal of the Marine Biological Association

is issued half-yearly, price 3/6 each number.

In addition to these publications, the results of work done in the Laboratory are recorded in the 'Quarterly Journal of Microscopical Science,' and in other scientific journals, British and foreign.

Specimens of Marine Animals and Plants,

both living and preserved, according to the best methods, are supplied to the principal British Laboratories and Museums. Detailed price lists will be forwarded on application.

TERMS OF MEMBERSHIP.

ANNUAL MEMBERS	£1	1	0	per annum.
LIFE MEMBERS	15	15	0	Composition Fee.
FOUNDERS	100	0	0	„ „
GOVERNORS (Life Members of Council)	500	0	0	

Members have the following rights and privileges:—They elect annually the Officers and Council; they receive the *Journal* free by post; they are admitted to view the Laboratory at any time, and may introduce friends with them; they have the first claim to rent a table in the Laboratory for research, with use of tanks, boats, etc.; and have access to the Library at Plymouth. Special privileges are granted to Governors, Founders, and Life Members.

Persons desirous of becoming members, or of obtaining any information with regard to the Association, should communicate with—

The DIRECTOR,
The Laboratory,
Plymouth.

New Series, No. 198 (Vol. 50, Part 2). Price 10s. net.

JUNE, 1906.

THE QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE.

EDITED BY

E. RAY LANKESTER, M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND OF THE ACADEMY OF THE LINCEI OF ROME, AND OF THE AMERICAN ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE ROYAL ACADEMY OF BELGIUM; HONORARY MEMBER OF THE NEW YORK ACADEMY OF SCIENCES, AND OF THE CAMBRIDGE PHILOSOPHICAL SOCIETY, AND OF THE ROYAL PHYSICAL SOCIETY OF EDINBURGH, AND OF THE BIOLOGICAL SOCIETY OF PARIS, AND OF THE CALIFORNIA ACADEMY OF SCIENCES OF SAN FRANCISCO; FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE AMERICAN PHILOSOPHICAL SOCIETY; DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE FULLERIAN PROFESSOR OF PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN; LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

FELLOW AND TUTOR OF TRINITY COLLEGE, CAMBRIDGE;

AND

SYDNEY J. HICKSON, M.A., F.R.S.,

BEYER PROFESSOR OF ZOOLOGY IN THE OWENS COLLEGE, MANCHESTER.

WITH LITHOGRAPHIC PLATES AND TEXT-FIGURES.

LONDON:
J. & A. CHURCHILL, 7 GREAT MARLBOROUGH STREET.
1906.

Adlard and Son, Impr.,] [London and Dorking.

CONTENTS OF No. 198.—New Series.

MEMOIRS:

In Memory of

WALTER FRANK RAPHAEL WELDON,

M.A., D.Sc., F.R.S.,

Linacre Professor of Comparative Anatomy in the University of Oxford, and Fellow of Merton College, Oxford,

WHO DIED APRIL 13th, 1906.

The Hæmoflagellates: a Review of Present Knowledge relating to the Trypanosomes and allied forms.

(*Continued from page* 231.)

By

H. M. Woodcock, D.Sc.(Lond.).

With Text-figures.

CONTENTS.

Section VIII. Multiplication.

Binary longitudinal division is, probably, of universal occurrence, and appears to be the usual method of multiplication,[1] at any rate, in the Trypanosome phase. T. lewisi,

[1] Certain authors (e. g. Rabinowitsch and Kempner, and Voges) have described stages in T. lewisi and T. equinum, which they consider as indicative of transverse division, but it is very unlikely that these represent normal dividing forms; none of the more recent investigators of the same (or other) parasites have observed such a method, and, in short, these stages are to be placed in the category of involution forms showing irregular segmentation, to which reference has just been made.

at any rate,[1] possesses another method in addition, namely, rosette-like segmentation, which is easily derivable from the former. Longitudinal fission in general follows, in its main outlines, the process above described in the case of Trypanomorpha noctuæ; the chief differences to be observed are slight variations in mode and order of procedure. Full-grown Trypanosomes about to divide are, as a rule, rather broader than the ordinary adults; in T. lewisi, this increase in size may be very marked, the parasites being not

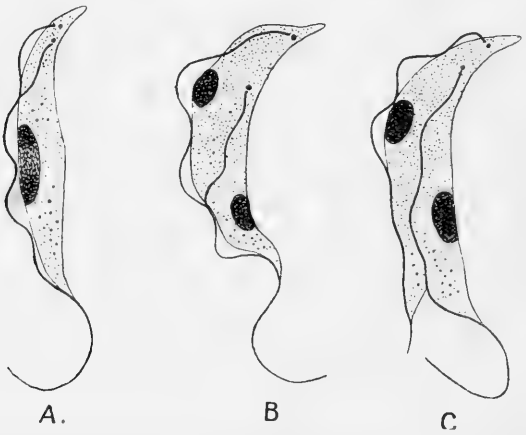


Fig. 23.—Stages in binary longitudinal fission of T. brucii. (After Lav. and Mesn.)

only much wider, but also longer (fig. 27 B). The kineto-nucleus is frequently the first to divide (fig. 23 A), but sometimes either the kineto- or the tropho-nucleus may do so indifferently; whichever leads the way, the other very soon follows suit (B).

The duplication of the flagellum always begins at its proximal end, which is in relation with the kinetonucleus. Until recently the process has always been considered as an actual longitudinal splitting of the flagellum, following upon the separation of the two daughter-kinetonuclei. The splitting

[1] See below, p. 240, for other possible instances.

has been described, either as extending to the distal end of the undulating membrane (i. e. as far as the flagellum acts as a border to the same [fig. 23 c]), after which the two halves separate; or, as being practically limited to the root-portion, which becomes thickened and then divided, one half breaking away as a new short flagellum, the further growth of which is basal and centrifugal (fig. 27 D). As above stated, however, Schaudinn finds that, in Trypanomorpha noctuæ, the whole of the flagellum, etc., is developed independently from the daughter-kinetonucleus and laid down alongside the old locomotor apparatus; moreover, Prowazek (l. c.) maintains that this is also the case both in Trypanosoma lewisi and T. brucii.[1] It appears uncertain, therefore, whether splitting of the flagellum really occurs.[2] However this may be, one of the resulting flagella (the new one) is often at first shorter than the other, either possessing only a small free portion (fig. 23 c) or none at all; more particularly is the latter the case when rapid successive multiplication has been going on.

Cytological details with regard to the behaviour of the nuclear apparatus, such as are given by Schaudinn (l. c.), are only to hand in one or two cases. For the most part, nuclear division has been, so far, described as consisting simply of aggregation of the chromatin at either end, followed by constriction in the middle and subsequent separation of the two halves as daughter-nuclei. Wasielewsky and Senn (**120**), however, mention and figure a kind of simple mitosis in a case of multiple division in T. lewisi. Prowazek (l. c.) has described the process in T. brucii more fully. The kinetonucleus becomes thickened and more or less spindle-like (fig. 24 A) Subsequently it assumes a dumb-bell-like appearance, and the two halves become further separated, remaining connected only by a long thread (B); this sometimes shows a delicate thickening (apparently divided in the fig.)

[1] McNeal (**74**) is of the same opinion with regard to the multiplication of T. brucii in cultures.

[2] The same applies equally, of course, to the formation of the undulating membrane. If the flagellar border splits, the membrane doubtless divides also; but where the flagellum is a new development, the undulating membrane is so too. Certainly, to judge from many of the published figures (cf. also some of Prowazek's), one would conclude that actual splitting of the flagellum is taking place.

in the middle. The trophonucleus increases in size, and its chromatin becomes arranged in eight rather elongated chromosomes, which next begin to divide in a similar dumb-bell-like manner (fig. 24 c). The trophonuclear karyosome (karyocentrosome) has frequently divided by this time (c); but in one case Prowazek observed it much drawn out and functioning as an intranuclear division centre (D), the chromatin having become aggregated around its two ends. In fig. 24 c the chromatoid grains in the cytoplasm are also seen dividing.

The division of the general cytoplasm takes place last of all. In the great majority of forms this is equal or sub-equal and the two resulting daughter-Trypanosomes are of approximately equal size (figs. 23, 25 c). Although the cytoplasmic fission usually begins at the flagellar end, it is important to

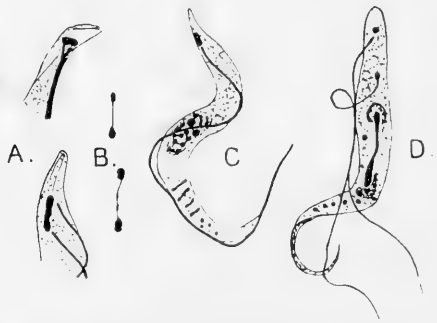


FIG. 24.—Nuclear details in the division of T. brucii. A—B, division of the kinetonucleus; C and D, of the trophonucleus. (After Prowazek.)

note that it may commence instead at the non-flagellate extremity. This is the case (according to both Lignières and Elmassian and Migone) in T. equinum, where the division starts indifferently at either end (cf. fig. 25 C and D). It would be very interesting to know at which extremity it begins in Trypanoplasma, but, unfortunately, only an early stage has, up till now, been described for this form (fig. 26), and many more details are needed.

In some instances (e. g. T. brucii, T. equinum [fig. 25 E], T. equiperdum [fig. 25 G]) the longitudinal fission is apparently multiple, the original individual giving rise, simultane-

ously, to three or even four descendants. This is most likely due to the successive division, before the common cytoplasm

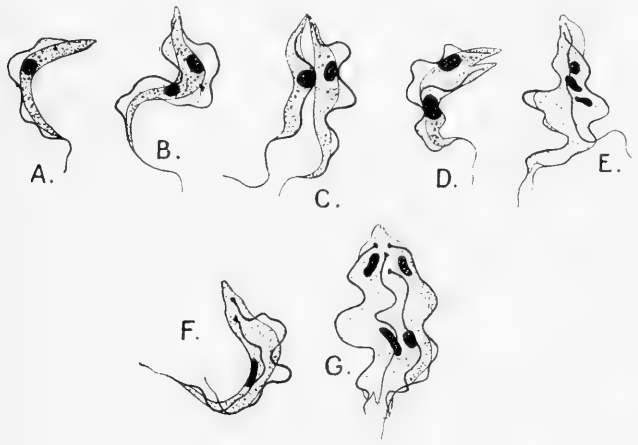


Fig. 25.—A—D, stages in binary longitudinal fission of T. equinum; E, multiple longitudinal division in same parasite; F and G, binary and multiple fission in T. equiperdum. (After Lignières.)

has divided, of the organellæ of one or both of the two halves resulting from the first multiplication.

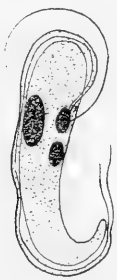


Fig. 26.—Early stage in binary fission of Trypanoplasma borreli. (After L. and M.)

T. lewisi differs from other Trypanosomes in that the cytoplasm divides in a most unequal manner (fig. 27). Indeed,

the process is more comparable to budding, since the larger, or parent-individual may produce, successively, more than one daughter-individual [1]; moreover, the progeny may them-

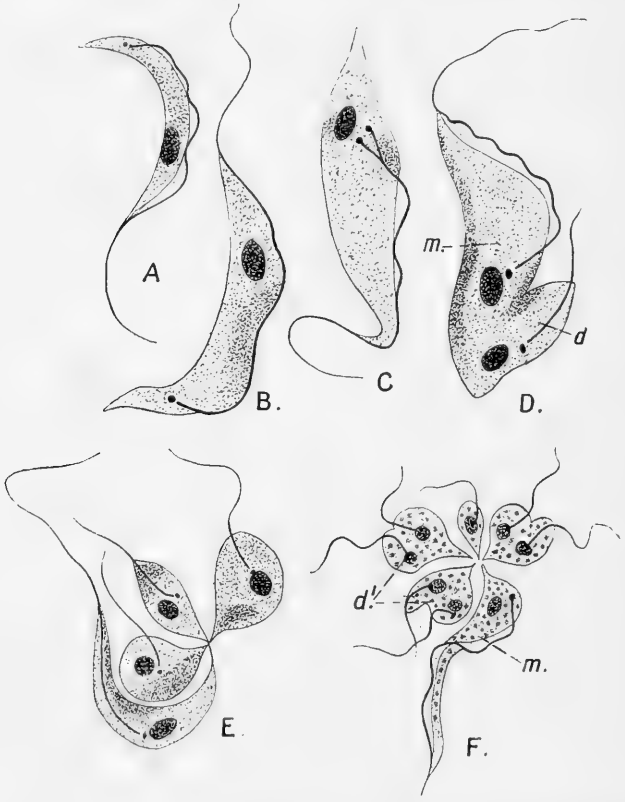


Fig. 27.—Unequal division in T. lewisi. *m.* = parent-individual; *d.* = daughter-individual; *d'.* = daughter-individual dividing. × 2000. (A—E, after L. and M.; F, after Wasielewsky and Senn.)

selves subdivide before separating, the whole family remaining connected together by the non-flagellate end, which is often much attenuated in each individual as a consequence

[1] Léger (66) instances a somewhat similar unequal division or budding in T. barbatulæ.

of the numerous divisions (in fig. 27, E represents a small family, and F a large one where the parent-individual is very distinct). It is important to note that the kinetonucleus changes its position during the commencing stages of division and comes to lie alongside the trophonucleus, or even passes to the other side of it, i. e. nearer the flagellar end.

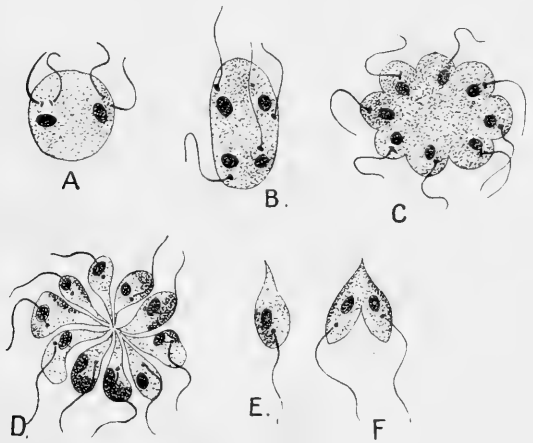


FIG. 28.—A—D, rosette segmentation in T. lewisi; E, daughter-individual; F, one dividing. × 1750. (After L. and M.)

This variety of division forms a direct transition between binary fission and the other characteristic method of T. lewisi, viz. segmentation; indeed, such a family as that just described often greatly resembles a rosette, but is generally distinguishable therefrom by the presence of a parent-individual. In rosette-formation, on the other hand, segmentation is multiple and equal.[1] The body assumes an ovoid to spherical

[1] McNeal (l. c.) doubts the occurrence of true equal segmentation. He thinks that the "budding" process is rather concerned in all cases, i. e. that a parent-individual can always be recognised. The fact, however, that equal multiple longitudinal fission occurs, in which there is no sign of a parent-individual (cf. fig. 25 G), at least makes it possible that the process of equal segmentation occurs. The condition seen in fig. 28 B is easily derivable from that in the former figure. See also next page.

form, and then repeated division of the nuclei (both kinds) and flagellum goes on, each daughter-kinetonucleus remaining in contiguity to the corresponding trophonucleus, and all taking up a position of uniform distribution near the margin of the cytoplasm (fig. 28 A—C). The cytoplasm next becomes lobulated peripherally, and gradually segregates around the nuclei, forming as many little, radially-arranged daughter-Trypanosomes as there are nuclear groups (D).[1] This method of multiple division offers, it will be seen, considerable analogy to the schizogony of Hæmosporidia, and the latter is probably to be regarded as a modification of it, adapted to an intracellular and gregariniform stage.

These small Trypanosomes so formed (E) differ from the typical adults by their stumpy, pyriform shape, the position of the kinetonucleus near the flagellar end of the body, and the absence, during the first part of their youth, of an undulating membrane. The parasites have at this period, it is to be noticed, a very Herpetomonas-like facies, the importance of which is discussed below (p. 276). These young individuals of T. lewisi can themselves multiply by equal binary fission (fig. 28 F), and give rise to little fusiform Trypanosomes. With growth the latter gradually assume the adult appearance, by the progression of the kinetonucleus past the trophonucleus, almost to the other end of the body, and the concurrent development of an undulating membrane as the extended flagellum takes up its regular superficially-attached position.

Wasielewsky and Senn (l. c.) also describe and figure multiple segmentation in this same parasite; again Rabinowitsch and Kempner, in a recent paper (**90**), mention that they have occasionally observed appearances and groupings of Nagana and Dourine parasites (T. brucii and T. equiperdum) which strongly suggest that these also possess, in addition to the usual method of equal binary fission, a similar modification. Apart from these instances, however,

[1] A multiplication-rosette is readily distinguished from a typical agglomeration-cluster by the different shape of the individuals, and the different position of the kinetonucleus (cf. fig. 20 B).

the writer has not come across any other observations of multiplication-rosettes being formed by the parasites while in the blood.

On the other hand, as above indicated, the occurrence of such rosettes has frequently been observed in artificial cultures of different Trypanosomes. Fig. 29 shows two such clusters in the case of T. lewisi. The larger ones are formed apparently by the successive divisions of the elements of the smaller ones; concurrently the individuals gradually lose their pyriform shape and become more elongate and fusiform. In this way colonies of hundreds of individuals are formed.

The origin of these rosettes appears to be by the multiple division of a single form. The radial arrangement and the general shape of the parasites in a small cluster suggests this (cf. fig. 29 A with fig. 28 D, showing

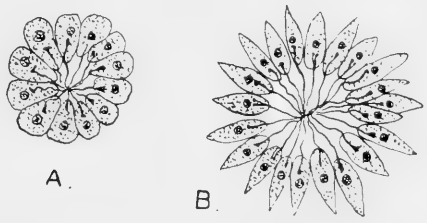


FIG. 29.—Multiplication-rosettes of T. lewisi from a culture. (After L. and M.)

segmentation in the blood). Novy and McNeal, moreover, describe and figure (**81**) early stages in such multiple division in T. avium and other Avian parasites, which they consider will lead on to the formation of a rosette. Probably, however, "segmentation" is soon replaced by rapid binary division. A noticeable distinction from the multiplication-rosettes of T. lewisi in the blood is that, in most cases described, the clusters of Trypanosomes so formed in cultures have their flagella and kinetonuclei centrally disposed.[1] It is by no means impossible that this results from the strange medium in which the parasites are; in so far as this is the case, the arrangement must be considered abnormal.

[1] This is not so in T. brucii, which, according to both Smedley and McNeal, preserves the "blood-type,"—i. e. the flagella are outwardly directed.

Section IX. Comparative Account of the Life-cycle.

(A) Life-history of Trypanosoma ziemanni compared with that of Trypanomorpha.

Besides demonstrating a complex life-cycle in the case of Trypanomorpha, Schaudinn has shown that another parasite of Athene noctua, Trypanosoma ziemanni, also has a similar history, and undergoes much of its development

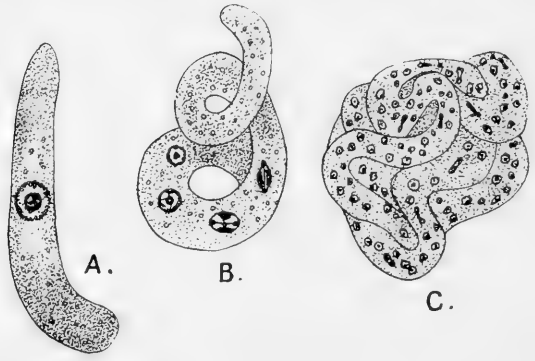


Fig. 30.—Growth and metamorphosis of an indifferent ookinete of Trypanosoma ziemanni. (After Schaudinn.)

in the same Invertebrate host, Culex pipiens. While agreeing in its main features with that of the first-named parasite, the life-cycle of this form presents certain important differences, which may now be considered.[1]

The most remarkable modification, and one which bears forcibly upon the relationship of the Hæmosporidia to this group, occurs almost at the commencement of the life-history. Instead of passing at once into the Trypanosome stage, all three types of ookinete first enter upon a period of

[1] For the general account of the life-history the reader is referred to the description of Trypanomorpha, and to the tabulated summary of the principal stages given on p. 180.

growth and nuclear multiplication. Following the process in an indifferent form (fig. 30), it is seen that growth results principally in an increase in length, and the ookinete becomes serpentine-like and then rolled and coiled up upon itself (B and C), finally assuming the appearance of a tangled ball or skein. Meanwhile nuclear division is proceeding. The nucleus here remains for some time in the compound condition. Nuclear division takes place in the same manner as in the megagametocyte of Trypanomorpha (Halteridium) noctuæ, and the kinetonucleus functions as a central spindle. By successive divisions a great number of nuclei[1] are at length produced, uniformly distributed throughout the coil (fig. 30 C). To each daughter-nucleus a small zone of cytoplasm is apportioned, and each of the "cell-territories" thus segregated becomes a little Trypanosome in the way above described. By a similar process male and female ookinetes

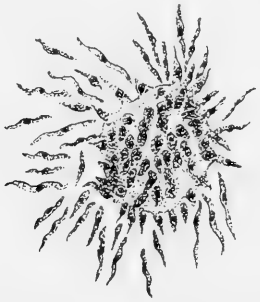


FIG. 31.—Liberation of young indifferent Trypanosomes from the coiled ookinete in T. ziemanni. (After Schaudinn.)

give rise at length to numbers of male and female individuals. When fully organised, the young Trypanosomes, be they male, female, or indifferent, liberate themselves and move away, leaving behind a large residual mass of unused cytoplasm (fig. 31).

When first set free, the young trypaniform parasites are very small, the males being indeed, according to Schaudinn, hardly visible.[2] The indifferent ones become greatly extended in length, and somewhat spirally twisted, soon attaining the adult form. In all fundamental respects the organization of the different types agrees completely with that of other Trypano-

[1] The number varies according to the type of ookinete.

[2] This is also true of the indifferent forms after repeated multiplication, which can then only be made out when moving or agglomerated in clusters. Schaudinn puts forward the interesting suggestion that there may possibly be Protozoan parasites which, at certain periods of the life-cycle, can no longer be optically resolved. Such may perhaps be the case in yellow fever.

somata; differences in detail have been commented upon in the section on Morphology.

Longitudinal fission is peculiar because of the fact that the two resulting daughter individuals do not at once separate, but remain united by the non-flagellate ends and take up a position of alignment one with the other (fig. 32 B), either end of the thread being formed by the flagellar extremity of one of the two parasites. These double individuals or "couples" constitute very thin, corkscrew-like, spirochætiform threads.[1] Moreover, further division takes place while the individuals of a couple remain thus joined. Fig. 32 C and D shows successive stages in the longitudinal

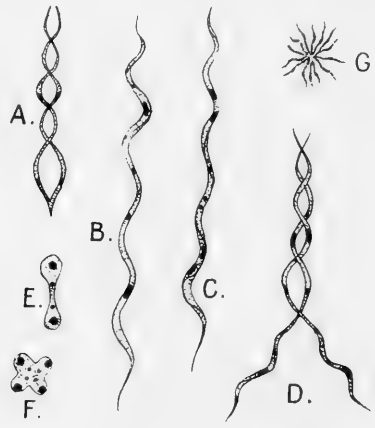


FIG. 32.—A—D, formation and multiplication of "couples" ("Spirochæta"-threads) in T. ziemanni; E, F, resting-phases of same; G, agglomerated cluster of very minute forms. (After Schaudinn.)

fission of a couple, and the separation of the two daughter couples from each other.[2] These couples move indifferently in either direction, now one member leading, now the other. There is at present no evidence as to the particular biological signification, if any, of this occurrence.

With the assumption by the parasites of a resting phase, the body becomes pear-shaped in form.[3] In fig. 32 E is seen a couple in the gregariniform phase, its two members being joined by their kinetonuclear ends. The

[1] The question of the "Spirochætæ" is discussed below, p. 314, et seq.

[2] The writer is not able to gather from Schaudinn's account whether the two members of a daughter-couple then separate before again dividing, or whether further multiplication goes on in a similar manner.

[3] The striking resemblance between such a stage and "Piroplasma" donovani (see below, p. 258, et seq.) hardly needs pointing out.

opposite ends are rounded and have lost the flagella. Multiplication also goes on during this phase; fig. 32 F shows four individuals, i. e. two couples not yet separated.

The behaviour of the indifferent forms, on entering the blood of the owl, is very much the same as in the case of Trypanomorpha. After alternation of resting, attached phases with multiplication periods has proceeded for some time, sexual individuals are developed in increasing numbers from the young indifferent forms. Sexual forms (gametocytes), whether male

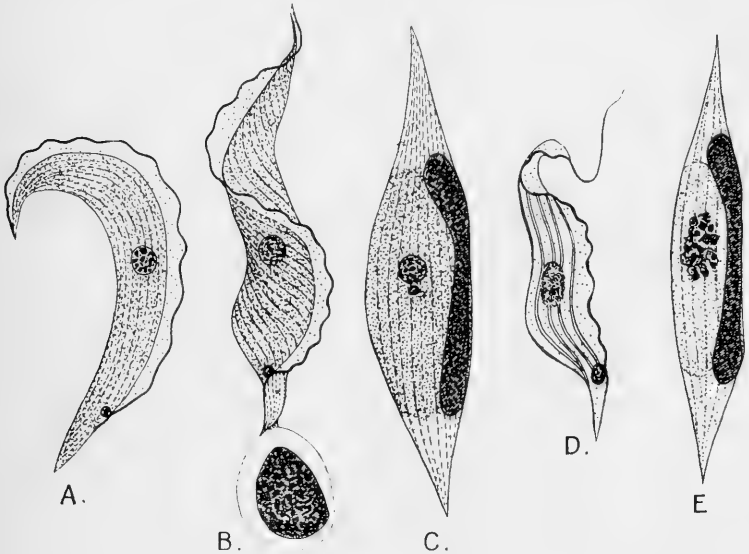


FIG. 33.—Active and resting phases of the gametocytes of T. ziemanni. A—C, megagametocyte (female Trypanosome); D, E, microgametocyte (male Trypanosome). (After Schaudinn.) [In D, the myonemes are rather too accentuated.]

or female, are easily distinguishable by the remarkable size to which they grow, becoming, as they do, very much larger than the leucocytes. An adult fully-grown male individual, or microgametocyte, in the trypaniform phase is seen in fig. 33 D. Both trophonucleus and kinetonucleus are very prominent, and the undulating membrane and flagellum are well-developed; the latter extends some distance beyond the posterior end of the body. The sixteen myonemes are arranged in four double rows or pairs on each side. A female Trypanosome (megagametocyte) in the same phase (fig. 33 A) is even larger than a male form, but its nuclei are relatively smaller, and there is no free prolongation of the flagellum. The myonemes of each side are not arranged in pairs.

Resting, intracellular phases (the "Leucocytozoon" of Danilewsky and the "Hæmamœba"-stage of Laveran) of these sexual forms are shown in fig. 33 c (male) and E (female). In both gametocytes the greater part of the body (endoplasm) becomes retracted into an ovoid mass; the ectoplasm with its myonemes, in conjunction with the cytoplasmic envelope of the host-cell, alone retains the original spindle-shaped outline of the parasite.[1] No trace of the flagellar apparatus is left, and the kinetonucleus takes up an internal position, close to the trophonucleus (fig. 33 c).

Fig. 34. Fig. 35.

Fig. 34.—Formation of the eight microgametes from the microgametocyte in T. ziemanni. The unused cytoplasm breaks up into three or four residual masses.

Fig. 35.—Fertilisation of a megagamete by a microgamete. The trophic and kinetic female pronuclei are seen on the left. Near the centre lie the two reduction-nuclei.

In both figures the remains of the host-cell, together with the cast-off ectoplasmic envelope of the parasite, are seen on the right. (After Schaudinn.)

The gametocytes in this stage are, of course, to be met with in the peripheral circulation, and when introduced (with the blood) into a gnat during the act of biting they at once proceed to gamete-formation. A fully grown microgametocyte may undergo the requisite nuclear changes and multiplication while still in the blood of the owl, and it is this process actually which is beginning in the individual seen in fig. 33 E.[2] Eight double nuclei

[1] Laveran (37), it may be noted, figures also rounded or spherical forms of the gametocytes and their host-cells, but Schaudinn does not mention the occurrence of such.

[2] In some cases apparently the microgametes may even be formed and liberated from the parent-cell while in the blood. Laveran (l. c.) figures

are formed, and the reduction of the chromosomes of the trophonuclear parts, from sixteen to eight, occurs at this time. The formation and liberation of the microgametes (fig. 34), the maturation of the megagametocyte and the fertilisation of the megagamete (fig. 35), all take place, according to the author, in the same manner as in Trypanomorpha (Halteridium) noctuæ. The microgametes themselves are constituted on the same Trypanosome plan, allowance being made for the different number of chromosomes. With fertilisation[1] and the subsequent formation of the vermiform, motile ookinete, the life-cycle of Trypanosoma ("Hæmamœba") ziemanni is completed.

It only remains to add that "recurrence" is produced in the usual way, by parthenogenesis of female forms remaining over in the blood of the bird. The only point requiring notice is that the rejuvenated parasite, instead of giving rise to a single individual of any type, undergoes multiple division like an ordinary ookinete and produces many little Trypanosomes.

(B) Evidence in favour of a corresponding Digenetic Life-cycle in other Trypanosomes.

Since Schaudinn's work was published, evidence has been accumulating which tends to show that the two examples so brilliantly investigated by this author are not isolated cases, but are rather to be regarded as, if not in every way typical,

four such in the act of being set free in this same parasite. MacCallum (**72**) also observed the same number liberated in a species of Halteridium, and, moreover, the actual fertilisation of a megagamete. Compare also the "Polymitus"-forms of earlier authors, which were simply detached "flagella" or gametes. Whether the occurrence is due to unfavourable circumstances (e. g. removal from the body) is not certain, but it does not seem to be quite normal, the number of gametes, for instance, being, at any rate in the first named case, only half the normal number produced in the gnat. Is this possibly consequent on the non-completion of the reduction phenomena?

[1] Details of the process are not given, but the writer would point out that the penetration of the microgamete into the megagamete is, most probably, by the opposite end to that which moves first in Trypanomorpha, namely, the non-flagellate end. This would be in accordance with the behaviour of the parasites as regards attachment, agglomeration, etc. Probably also in this case (it appears so, indeed, from Schaudinn's figure) there is a true flagellar prolongation posteriorly to act as a steerage organ.

at least indicative of the general course of events in the usual life of a Trypanosome. For one thing, there can be now little or no doubt that most, if not all, Trypanosomes possess an alternate, Invertebrate host, in which a definite part of the life-cycle, including sexual conjugation, is undergone. Again, it is very probable that many are capable of entering upon a resting, attached phase, at different periods of the life-history, during which the parasites lose, for the time being, their trypaniform nature, and become gregariniform. In certain cases, indeed, they have gone even farther, and acquired a completely intra-cellular, or Hæmosporidian phase.[1]

The facts in support of the above-mentioned propositions are most conveniently treated more or less separately. Apart from the many Invertebrates which are known, or with good reason suspected, to be, at least, "carriers" of Trypanosome parasites (see below in the Systematic section), there are certain other Trypanosomes, from different Vertebrate hosts, for which the possession of an alternate Invertebrate host has been, practically speaking, proved. Perhaps the most important instance, since it relates to a Mammalian form, is that lately described by Prowazek (**88**).[2] This author finds that T. lewisi undergoes an essential part of its life-cycle in a louse, Hæmatopinus.

Soon after their arrival in the mid-gut the parasites undergo reduction of the nuclear apparatus, preparatory to conjugation. By this means the number of chromosomes is reduced from sixteen to four. The differences between the gametocytes of different sex (male and female Trypanosomes) are not well marked. A noteworthy distinction from the instances above described is that the male form (comparable to a microgametocyte) does not give rise to several micro-

[1] The derivation of the Hæmatozoa as a whole, and the direction in which evolution appears to have tended, are considered in Section XI.

[2] As the writer has only had access to Prowazek's work since this article was sent to the publishers, it is impossible to do more than summarise the principal features.

gametes, but becomes itself a single one, in the same way that a female form becomes a megagamete after maturation. The body diminishes in size, and the nucleus (trophonucleus), becomes elongated and band-like (fig. 36 A) ; also the cytoplasm stains differently from that of a female gamete. Hence, when fully formed, the two kinds of sexual element are readily distinguishable, although there is not any pronounced dissimilarity in type. Actual conjugation stages are shown in fig. 36 B and C; and it is, in the writer's

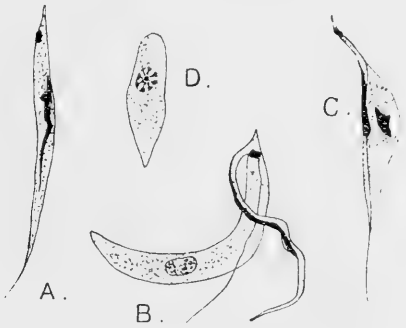


FIG. 36.—A, microgamete of T. lewisi; B and C, stages in conjugation; D, zygote (ookinete). (After Prowazek.)

opinion, a point of great importance that the gametes come into contact by their non-flagellate, kinetonuclear ends. The zygote becomes an ookinete (fig. 36 D), quite similar in constitution to those of Trypanomorpha and Trypanosoma ziemanni; and this gives rise to a single Trypanosome by the separation of kinetonucleus and locomotor apparatus, also in the same manner as in those parasites.

Prowazek has also endeavoured to ascertain more of the life-cycle of T. brucii. He was unable, however, to obtain the Tsetse-fly (Glossina morsitans) in which, as he remarks, the sexual phases normally occur, and only very rarely were maturation-processes, comparable with those described for T. lewisi, to be seen in the blood. In one case, where the parasites were in a guinea-pig which had just died, he was able to observe what was undoubtedly an actual conjugation, although probably not altogether typical (cf. footnote [2], p. 246).

In addition the author describes various nuclear changes and divisions undergone by the parasites (both forms) while in the blood of the Vertebrate host. These include a regulatory process, characterised as autosynthesis of the karyosome (karyocentrosome) and parthenogenesis.[1]

Equally interesting is the evidence already to hand, which tends to prove that the rôle played by an Insect in connection with the Trypanosomes of warm-blooded Vertebrates is performed by a leech in the case of those of cold-blooded Vertebrates.

To Léger (**66** and **67**) we owe certain instructive observations relating to Trypanoplasma varium and Trypanosoma barbatulæ from the loach.[2] This investigator distinguishes indifferent and female forms of Trypanoplasma varium in the blood of the fish. When a leech (Hemiclepsis marginata) sucks blood containing the parasites, which thereupon pass into its stomach, the indifferent forms degenerate and perish, while the female ones become massive and show nuclear changes (division of both tropho- and kinetonucleus), preparatory, Léger thinks, to a sexual process. At any rate, after some days the intestine of the leech contains little, narrow Trypanoplasmata, of which certain, very filiform, ones represent, perhaps, male forms.[3] Other stages were also noticed whose interpretation remains at present doubtful. In the case of Trypanosoma barbatulæ the resemblance between the development of the parasites in another leech (a Piscicola) and that of Schaudinn's Avian forms in the gnat is still more pronounced. Eighteen hours

[1] It may be pointed out that certain of the appearances depicted as representing different stages in these processes do not suggest, quite so readily as might be desired, the interpretation given of them. In one or two cases, at any rate, the figures recall those of Bosc (**5**), who describes some wonderful phases in the development of a Trypanosome from the rabbit which the writer, however, does not for a moment consider are really normal.

[2] Only a short preliminary note without figures is as yet available.

[3] Brumpt (**10**) has also noticed small, very motile Trypanosomes in this leech.

after infection Léger observed, in the intestinal contents, pyriform ookinetes without a flagellum; some of these possessed a single large nucleus (i. e. a "compound" nucleus), either at rest or in process of heteropolic division, while others had two nuclei, of which one was smaller than the other. Four days later the intestine contained numerous Trypanosomes which could be distinguished as belonging to one of the three types described by Schaudinn in Trypanomorpha, namely, indifferent, male and female. The indifferent forms, it may be noted, multiply actively by longitudinal fission (see p. 238). Other details of these various types are given, but their further evolution and the manner of their passage back into the loach was not followed. The above-stated facts, however, hardly leave room for doubt that both these piscine Trypanosomes have a true, alternating, Hirudinean host.

Billet's work (3) on Trypanosoma inopinatum is also very important, and goes far towards proving both propositions for this form. The author brings forward evidence to show that (1) the alternate, Invertebrate host of this parasite is another leech, Helobdella algira, and (2) that it has an actual ontogenetic relationship with a Drepanidium or Lankesterella parasitic in the same Vertebrate, and which is, most probably, its Hæmosporidian phase. The investigation was somewhat complicated by the presence in the same frogs of the common T. rotatorium, but, on the other hand, the coincidence was instructive, since the result obtained tends to show that that particular leech is not the alternate host of the latter parasite.

The principal facts brought out by Billet's experiments are as follows:— (a) After the examination of a number of leeches which had been ectoparasitic upon frogs containing in their blood either Lankesterella (Drepanidium) plus T. inopinatum plus T. rotatorium, or only Lankesterella, it was found that, in either case, the Helobdellæ contained in their intestine only T. inopinatum. Neither Lankesterella, as such, nor T. rotatorium was met with in this host. (b) Moreover, Billet several times observed in the intestine of the leeches, within twenty-four hours after the time of infection, parasites which were more or less rounded

and mobile, and with nucleus and centrosome (i. e. tropho- and kineto-nucleus) distinct—stages intermediate, that is, between a Hæmogregarine and a Trypanosome.[1] (c) Frogs quite free from all Hæmatozoa and then infected by placing Helobdellæ, whose digestive tube contained T. inopinatum, upon them were found afterwards to contain only Lankesterella.

Billet considers the Trypanosome phase to be very uncommon in the frog, but of general occurrence in the leech, and, conversely, the Hæmogregarine phase to be absent, as such, in the Invertebrate host, but common in the Vertebrate. T. inopinatum does of course occur in the frog—Sergent (**101**) first described it in that host—but apparently it is only at rare intervals that the parasites lose the Hæmogregarine condition and become trypaniform. It may be pointed out in this connection that Sergent (**103**), who has corroborated Schaudinn's researches, says that Trypanomorpha in the Trypanosome-form is comparatively rare in the blood of the owl, but common as Halteridium, and, vice versâ, in the gnat the latter phase is not represented.

Further, Billet in a previous communication (**4**) has described forms which he regards as intermediate between merozoites of a Lankesterella and the typical trypaniform phases of T. inopinatum. He also observed the latter penetrate into the red blood-corpuscles, losing the flagellum in so doing, and then multiply in this endo-globular situation, either by binary longitudinal fission or by schizogony.

The study of this important question of the life-history of a Hæmatozoan, with regard to its bearing upon the relationship between Trypanosomes and Hæmosporidia, may also be approached from exactly the opposite direction. In other words, it is sometimes quite as easy, or even easier, to work from the latter to the former, to look for a trypaniform phase in a recognised and well-known Hæmosporidian. Some very interesting instances of such a discovery are now to hand, foremost among them being one for which we are again indebted to that marvellous investigator, Schaudinn.

This author states that he has observed the development of a motile trypaniform phase at two points in the life-cycle of

[1] Brumpt (**10**), it is important to note, has also observed in the ookinetes of Hæmogregarina bagensis (from Emys leprosa) which he found in the œsophageal and stomach diverticula of Placobdella catenigera, two nuclear bodies, a large nucleus of the ordinary type, and a smaller, highly-staining body much resembling the centrosome (i e. the kinetonucleus) of a Trypanosome.

the tertian parasite (Plasmodium vivax), both the sporozoites and merozoites evincing in their construction characteristic Trypanosome features.[1] He further considers, and hopes later to completely demonstrate, that this malarial parasite agrees with Trypanosoma ("Hæmamœba") ziemanni in other fundamental respects, as follows:—(*a*) That the ookinetes of Plasmodium are really formed of a compact coil: and (*b*) that the "sporozoites" are not all of the same character, but that indifferent and female ones can be distinguished; those corresponding to the male forms, however, perish prematurely while still in the ookinete of that type. Moreover, while the indifferent sporozoites are typical, actively-motile Trypanosomes, the female ones no longer appear able to assume the trypaniform condition, but remain gregariniform.

Schaudinn concludes his remarkable work with an appendix in which he brings forward certain facts noticed by Kossel and Weber, which led these investigators to consider that there is a similar close relationship between Piroplasma and the Hæmoflagellates. With this view Schaudinn, who has examined the preparations, expresses himself in complete agreement; and (it may be here mentioned) it has recently received strong confirmation from the work of Rogers, on a new human parasite, Piroplasma donovani.[2] So long ago as 1900, Weber observed in preparations[3] of the blood of a cow suffering from hæmoglobinuria, besides the typical Texas-fever parasites (P. bigeminum), Trypanosome-like forms, much smaller than those of Surra (Nagana). These parasites showed the general characteristics (shape, nuclear dimorphism, etc.)

[1] In this connection the writer would call attention to the markedly spirochætiform nature of the sporozoites of Hæmogregarina stepanovi, as described by Siegel (**105**) in Placobdella.

[2] A full résumé of present knowledge relating to this remarkable parasite is given in the next section.

[3] It may be noted that the blood was drawn towards evening, and the animal was confined in a stall into which scarcely any light came. Cf. the time when "Halteridium" parasites leave the blood-corpuscles and become free in the blood, as Trypanomorpha.

of a Trypanosome. Besides this, Schaudinn has since also noticed nuclear dimorphism (i. e. the presence of a larger and a smaller nuclear body) in typical endoglobular individuals of P. canis (cp. P. donovani below, p. 260); and Kossel and Weber on re-staining and re-examining smears of the intestinal contents of ticks which had fed upon cattle suffering from piroplasmosis came across similar forms.

Lastly, the instances where authors mention the association, concurrently, of Trypanosomes with either Avian malarial parasites, Piroplasmata, or Hæmogregarines are too numerous to specify (see below in Systematic). Of course, in many cases, this may reasonably be set down as a mere coincidence; it would be unduly straining that explanation, however, to suppose that it is of universal application.

Section X. The "Leishman-Donovan-Wright"[1] Bodies.

We may now consider, in some detail, the peculiar parasites which are generally held to be the cause of certain tropical fevers, particularly prevalent throughout Indo-Burmah, though not, apparently, by any means restricted to that region. These diseases, characterised by irregular pyrexia, splenomegaly and cachexia,[2] are known by various names (e.g., Dum-dum fever, Kala-azar, tropical splenomegaly, etc.) according to the slightly different features and circumstances attending their occurrence in different cases. These varieties are, however, most likely, all due to one and the same specific form of parasite. Moreover, organisms very similar to these parasites (morphologically, indeed, the two sorts appear hardly distinguishable) are found in certain superficial sores or ulcers, to which people in various

[1] In order not to injure the delicate susceptibilities of medical investigators, where priority rights are concerned (vide the pages of the 'Lancet' and 'B. M. J.' during the last few years with reference to the discovery of parasites in tropical diseases!), the fullest possible title is conferred for the nonce upon these unhappy parasites.

[2] Other prominent symptoms in different cases are ulceration of the intestine, œdema of the feet, and increase in pigmentation of the skin.

parts of the East are liable, and which are known by such names as Delhi boil, "bouton d'Alep," oriental sore, tropical ulcer, etc. The latter type of disease is one of localised infection, the organisms being restricted to the neighbourhood of the sore or ulcer, whereas in the former there is a general infection, the parasites spreading to all parts of the body, and being met with in the liver, spleen, bone-marrow, etc., and (rarely) in the peripheral circulation. No actual connection has yet been established between the parasites of local and general infectivity.[1]

Medical opinion on the whole is at present inclined to regard both these types of disease as being due to different manifestations of the same kind of parasite, the differences in symptomatology being largely explainable by the different habitat of the parasites in the two cases.[2] In view, however, of this different habitat and behaviour it is very uncertain whether the organisms, notwithstanding their apparent similarity, really belong to one and the same species. Bearing in mind the very slight morphological differences to be found with any constancy among many Mammalian Trypanosomes, which are, on other grounds (habitat, behaviour towards immunising sera, etc.), regarded as belonging to distinct species, it is not unlikely that the same holds good here also. But the question is still far from being settled.[3]

[1] In other words, the parasites, when limited to the neighbourhood of an ulcer or sore, never seem to become generally distributed throughout the body, and produce the febrile type of disease; although persons suffering from the latter may have skin eruptions or papulæ, leading to the formation of small ulcers which somewhat resemble "oriental sores."

[2] James (**126**) is a little inclined to doubt whether the organisms are really the cause of such a severe illness as Kala-azar, although recognising the constancy of their occurrence. He hesitates because of the great resemblance which they bear to the parasites of the other type of disease (oriental sore, for example), and the very different effects to be accounted for (see next footnote, however).

[3] Manson (**132**) puts forward the interesting hypothesis that the two types of disease, of such different gravity, may bear to one another the same relation that variola and vaccinia do. He suggests that the germ of Delhi boil may be that of Kala-azar, which has become attenuated by passing

The history of our knowledge of these organisms is soon stated. The parasite of cachexial fever and splenomegaly was first discovered by Leishman in 1900 in a splenic puncture taken, post-mortem, from a soldier who had contracted Dum-dum fever. His first account of it (**129**) was published in the spring of 1903. About this time Donovan, in Madras, found these bodies in the same situation, but he appears to have been dubious about their nature until he learnt of Leishman's discovery. Since then many investigators have examined the parasites and published their views concerning them, among others being Laveran and Mesnil (**127**), Christophers (**123**), Donovan (**124**), and Marchand and Ledingham (**136**). Progress in this direction has culminated, for the time being, in the very important discovery of Rogers (**138**) already mentioned. With regard to the other type of disease, Wright (**142**) first published, at the end of 1903, an account of tropical ulcer in which similar parasites were clearly recognised and definitely described as such. Earlier writers on the Delhi boil malady (e. g. Cunningham, Firth) may or may not have actually seen the same organisms,[1] as distinct from altered leucocyte cells, etc., but from their descriptions it is quite impossible to say. Therefore the credit of the discovery can, logically, be no more given them than can, say, that of first finding a Trypanosome in the human blood be assigned to Barron, on the strength of his loose description of a Flagellate met with in an anæmic woman—which may have been anything. Quite independently of Wright, and before seeing his paper, two Russian workers, Martzinowsky and Bogroff, also found the parasites in cases of "bouton d'Alep," but did not publish their discovery till later.

After these preliminaries, we may pass to the parasites themselves and their relation to the host. Considering first Leishman's form, which may be termed the splenic variety, since it is always present in spleen punctures or smears; this is either free or intracellular. In the latter case the organisms are usually parasitic in large uninuclear leucocytes (fig. 37 11) or (and perhaps chiefly) in cells of the vascular endothelium, particularly of the spleen (fig. 37 I *M*), which are often packed with the little parasitic elements, becoming greatly enlarged and distended (macrophages). Parasite-containing cells, both leucocytes and macrophages, are also

through camels (cf. footnote, p. 266), just as the smallpox germ is deprived of its virulence by passing through the cow.

[1] It seems very improbable that Cunningham saw the real parasites; the "nucleoid" bodies he describes averaged nearly three times the lineal diameters of those below described.

to be found, in greater or less number, in the other organs, —liver, kidneys, mesenteric glands,—and in the granulation tissue of intestinal ulcers and skin lesions.

In films or smears made from "spleen-pulp" many of the parasites seem to be "free"—i. e. not definitely intracellular. They are, however, embedded in a zooglœa-like matrix or stroma, and frequently clustered together in groups. This matrix is composed of rounded or irregular elements, of a finely-granular or reticular nature, and varying greatly in size, which have more or less run together to give the stroma-like appearance. The generally-accepted explanation of this structure (which is not evident in sections of the same material) is that of Christophers, who considers it to be mechanically produced during the preparation of the film by the rupture or fragmentation of the large macrophages. These often possess cytoplasmic buds or outgrowths, easily detachable from the main cell, and each usually containing a larger or smaller number of the parasites.

There can be no doubt that a similar process goes on normally. The organisms appear to be quite uninjured by the leucocytic cells; but these, on the contrary, when strongly infected, or after endogenous multiplication has gone on for some time, become vacuolated and gradually used up, and reduced to a mere skin or envelope, which at length ruptures and liberates the enclosed parasites, just as in the case of an ordinary Hæmosporidian. It is not known if the parasites remain free in the general circulation for any length of time before invading a fresh host-cell; the life-history has not yet been sufficiently ascertained for us to do more than conjecture. It appears not unlikely, however, that multiplication also goes on in this condition. Certain workers (e. g. Laveran and Mesnil, Donovan, and Rogers) figure multiplication forms free in the blood, and from analogy, either with Piroplasma or a Trypanosome, such an occurrence might be expected. It is most likely that, here also, time and circumstance are largely responsible for the behaviour of the organisms when liberated.

It is also uncertain whether the infection of the leucocytes is an active or a passive one. The opinion has been expressed that the host-cells, in their phagocytic capacity, ingest the parasites—if so, verily a case of the "biter bit." On the other hand, if the parasites enter the cells when in a Flagellate phase (as e.g. T. ziemanni penetrates the leucocytes of the owl) it is more likely an instance of active infection, the organisms being especially adapted to that kind of cell—"Leucocytozoa" in fact. Nevertheless, it appears highly probable that the parasites are not exclusively limited to such a habitat, but that they also attack red blood-corpuscles as well, being also, therefore, in that respect, true Hæmosporidia. Both Laveran and Mesnil and Donovan describe and figure them as endoglobular, and great weight must be assigned to the view of the first-named investigators. Moreover, the figures of Donovan (l. c.) are sufficiently convincing; and, having regard to the unmistakably Piroplasma-like facies which the organisms at times possess, the rod or comma-forms depicted (fig. 37 I *e*) strongly recall the "bacillary" type of P. bigeminum in a similar situation (see Laveran [**34**]). Lastly, Donovan has observed the parasites in the peripheral circulation, although only rarely and during periods of high fever; and Laveran, who has examined his preparations, confirms this observation.

There is little additional to be noted concerning the habitat of the localised parasite (Wright's ulcer form). This also is either free or intracellular; in the latter case it is parasitic in the ulcer cells and in the large migratory corpuscles (phagocytes), which doubtless correspond, in part, to the macrophageal cells of the other type. Mesnil, Nicolle, and Remlinger (**137**) have seen, in sections, multiplication forms, both free and intracellular.

The parasites themselves are very minute, and appear rounded, ovoid, or pyriform in shape (fig. 37); the typical form may very well be that of a slightly flattened pear. The splenic form is somewhat smaller than Wright's parasite, and this is about the only visible distinction between the

two. The former is, when round or oval, from 2—3½ μ in diameter,[1] when pyriform from 3½—4 μ in length by 1½ μ or

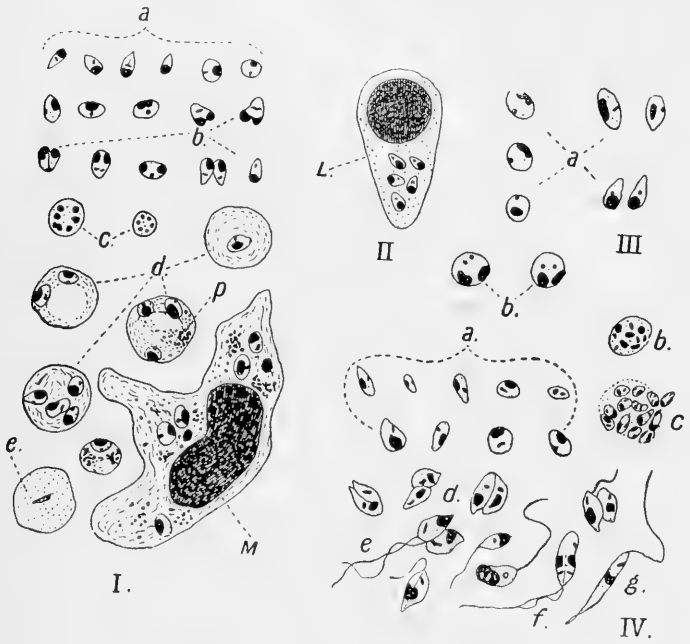


Fig. 37.—I. Piroplasma donovani (Lav. and Mesn.): *a*, typical pear-shaped or oval forms; *b*, various stages in longitudinal division; *c*, nuclear division preparatory to multiple fission; *d*, endoglobular forms, in red blood-corpuscles (*p* = pigment grains); *e*, bacillary form of the parasite in a corpuscle; *M* = large macrophageal cell with many parasites. (After Donovan.)

II. Uninuclear leucocyte (*L*) containing several parasites. (After L. and M.)

III. P. (Helcosoma) tropicum (Wright). *a*, single individuals; *b*, dividing forms. (From Mesnil, mostly after Wright.)

IV. P. donovani in cultures of different ages. *a*, ordinary forms of varying size; *b*, *c*, stages in multiple division; *d*, binary fission; *e*, *f*, and *g*, flagellate forms. (After Rogers.)

slightly more in breadth. The forms from tropical ulcers average about 4 μ by 3 μ (fig. 37 III). The body is, most pro-

[1] Laveran and Mesnil describe those occurring in the peripheral blood as being much smaller, and probably young forms; this may account for their being frequently overlooked.

bably, not limited by any distinct cuticle or membrane.[1] The cytoplasm is finely granular and fairly uniform in character (Rogers, Laveran and Mesnil); in some of Donovan's figures there is a faintly stained or clear area of varying size, more or less centrally situated, which possibly represents a vacuole.[2] The most interesting point in the morphology of these bodies is the fact that two chromatic masses, of very unequal size, are invariably to be recognised, except in very young forms. The larger nuclear mass, which, it may be at once said, is in all likelihood homologous with the trophonucleus of a Trypanosome, is usually round or oval,[3] and varies in position; in the pyriform parasites it is generally situated near the basal end, and in the oval ones about the middle of one side. The smaller nuclear body (representing, probably, a kinetonucleus) stains more intensely, and takes the form either of a little rod (sometimes curved) or of a round dot or grain. It is generally quite separate from the larger nucleus. In the round or oval parasites it is on the opposite side of the body and at the periphery; in the pear-shaped forms it is about the middle of the length or nearer the narrow end (fig. 37 I).[4] In some

[1] Ross (**140, 141**) considers that the free forms, at any rate, possess a distinct and resistant cell-wall or cuticle, comparable to a spore membrane. Christophers at first thought so too, but in his later Report appears to be doubtful, in view of the non-resistance of the bodies and their rapid break up after the death of the host.

[2] Both Christophers and Wright represent the bodies as having only a narrow peripheral border of stainable cytoplasm, all the central part remaining unstained, and constituting (according to Christophers) one or two huge vacuoles. These authors have probably been misled by deceptive appearances due to staining peculiarities, which have, unfortunately, led them to an incorrect interpretation of the bodies (see below, p. 263).

[3] In some cases it is heart-shaped or bilobed, probably indicative of approaching division.

[4] These different forms and appearances are explainable, as Christophers points out, by regarding the parasite as viewed from different aspects, e. g. end-on or sideways. This may, very likely, often be the case, the typical form being that of a slightly flattened pear. There is no necessity, however, to consider the body as having a fixed and unchangeable shape,

cases, however, the smaller nucleus is in contact with, or attached by a delicate thread to, the larger one.

The parasites multiply in two ways—(*a*) by binary fission, and (*b*) by multiple division or segmentation. The principal stages in the first mode are well known—at least, their general outlines—and certainly offer strong resemblances to the process in Piroplasma. In the pyriform parasites the division is evidently longitudinal (fig. 37 I *b*), and, all things considered, it is most probable that binary fission usually takes place in the long axis (compare the figures of Rogers, Laveran and Mesnil, and Donovan), the apparently transverse division of oval forms being due to their being seen more or less end-on. The large nucleus becomes bilobed and finally constricted into two; the smaller nucleus becomes elongated transversely, and also cut into two halves; lastly, the cytoplasm splits up, the cleavage furrow commencing either, usually, at the broad or basal end, or, occasionally, at the narrow, pointed extremity. Daughter-individuals, which have evidently just separated, are seen lying side by side in the figure.

The other form of multiplication—multiple division—is probably largely responsible for the numbers of parasites with which the host-cells are often packed and distended. This process, however, has not yet been so satisfactorily made out as that of binary fission. It appears to conform more or less to the radial or rosette type, enlarged, rounded parasites, with a varying number of nuclei (up to about eight), equally arranged near the periphery, having been frequently noticed (fig. 37 I *c*). Different writers, however, describe and figure this nuclear division somewhat differently. While, according to Laveran and Mesnil and Donovan, the nuclei are all of one size (the two kinds of nuclear element having apparently united), according to Christophers and

as would be the case if it were rigidly limited by a spore membrane; delicate, endocellular parasites are usually capable of change in form to a certain extent, and the rod-like or bacillary form well instances such a change here. Compare also other Hæmosporidia.

Rogers the larger and smaller chromatic elements (in other words, the trophic and kinetic parts) remain distinct and divide up independently (fig. 37 IV *b*). Laveran and Mesnil consider that the very young forms observed, with only one nuclear body, result from the segmentation up of such a stage with several similar nuclei. Rogers (l. c.) has followed the process in parasites kept in citrated blood, maintained at a temperature considerably below blood heat. Fig. 37 IV *c* represents, according to this author, practically the end stage in the multiple division of a parasite, a group of young daughter forms, each with two chromatic masses, being seen embedded in a faintly-staining ground substance, which, there can be little doubt, is of the same nature as the zooglœal matrix above discussed.[1] Further investigation is necessary to ascertain the particular circumstances which bring about the occurrence of these two varieties—if they are really distinct.

The above is a summary of all that was known concerning the morphology and development of this parasite prior to the quite recent announcements of Rogers, and many and various have been the views expressed with regard to its nature. Leishman, the discoverer of the splenic form, at

[1] Rogers considers that this ground substance is derived from the organism itself, and consists of residual cytoplasm not used up in forming the daughter individuals. In other words, the author, to judge from his description and figures, regards the daughter parasites as being formed within the body of the parent; i. e. this is an instance of endogenous multiplication. The writer does not agree with this conclusion, as all our knowledge, both of Trypanosomes and Hæmosporidia—as well as of other Sporozoa derived from a Flagellate ancestor—goes to show that multiple division is uniformly exogenous or peripheral, and quite distinct in character from the endogenous type (cf. the preceding pages and see Minchin, l. c.). Rogers figures another similar group of young forms—still clustered together—in fresh blood from the spleen; in these there is no sign of residual matrix. It is much more probable that Christophers' explanation holds good here also. Rogers mentions that the parasites (in the cultures) are in a slimy or zooglœa-like matrix, which develops in the course of a day or two, and this is doubtless due to the alteration and breakdown of splenic cells, blood corpuscles, etc., in which the parasites are developing.

first considered the organisms as representing involution or degeneration appearances of Trypanosomes, being largely influenced by the two unequal chromatin masses; in this view he has been supported by Marchand and Ledingham. Later, Leishman has gone somewhat farther, and regards the parasites as perhaps representing an actual stage in the life-cycle of a Trypanosome. Laveran and Mesnil, taking more particularly into account the general form and very suggestive binary fission, considered the parasite to be a new species of Piroplasma, which they called P. donovani; donovani is, therefore, the correct specific name of this form. Donovan concurred in this view, and Mesnil, Mouton and Remlinger (l. c.), who have studied Wright's form in a case of "bouton d'Alep" also consider this as a Piroplasma,—probably, however, a distinct species.[1]

Other authorities (e.g. Christophers, Ross and Wright) have gone somewhat wide of the mark, and have seen in this parasite an entirely different kind of Sporozoan; or, rather, they (with the exception of Wright) have regarded the parasitic bodies as being, themselves, only the spores of a new Myxosporidian, the parent body or plasma of which has not yet been satisfactorily made out.[2] This view is at once put out of court by the fact that Sporozoan spores never divide up in the way these bodies admittedly do; a valve or lid may open, liberating enclosed germs, after which the spore-case is cast empty aside, but the whole spore, membrane and all, cannot possibly divide up into two or more "daughter-spores!"

The above description of the parasites sufficiently justifies, we think, Laveran and Mesnil's opinion that they agree closely enough with the known stages of Piroplasma to be considered as belonging to that type of organism.[3] This does not, of course, prejudice, in any way, the view that these parasites represent, nevertheless, only a phase or part of a complete life-cycle. Now, as mentioned above, there is evidence that P. bigeminum is closely associated with

[1] The specific name, in that case, will be tropicum, as Wright termed his form Helcosoma tropicum.

[2] Ross, who has created the genus Leishmania for the parasites, thinks the matrices above discussed represent "relics of the parent organism."

[3] Since Schaudinn has observed nuclear dimorphism in a typical Piroplasma (namely, P. canis, see above, p. 254), any objection based upon this feature of P. donovani is removed. The only difference of any importance, in fact, appears to be that of habitat, and, granting this (though the new parasites do not appear to be, by any means, exclusively leucocytic), at least one Hæmosporidian, namely "Hæmamœba" (Trypanosoma) ziemanni, also has a leucocytic habitat.

a Trypanosome, and the connection between these new Piroplasma-like forms and a Trypanosome—or, at any rate, a Hæmoflagellate—has been definitely established by the work of Rogers, to which allusion has several times been made.

The parasites with which Rogers experimented were splenic forms taken from cases of cachexial fever and Kala-azar. As the author himself admits, the artificial conditions in which the organisms were cultivated cannot be supposed to have been as favourable to their further development as the natural conditions, whatever these may be, which bring about the same changes. Hence there must be, for the present, more or less uncertainty as to how far the forms described accurately represent typical evolutive stages of the parasites. Some of Rogers' figures certainly suggest the idea that the parasites were unhappy at the time he portrayed them.[1]

However, the great fact remains, that what were unmistakably Flagellate forms developed in the cultures at different intervals. Fig. 37 IV shows two pear-shaped forms, lying side-by-side after binary division, one of which has developed a flagellum near one end. This was in a culture of the third day. Another pair of pyriform stages with longer flagella, belonging to a fourth day culture, is seen at IV *e*. The most convincing stages (IV *g*) developed suddenly in a one-day culture from another patient. Rogers accounts for this by the condition of the blood being less altered than after three or four days' incubation. Probably, also, the organisms, when they left the host, were in a more favourable condition or phase for further development than in the other cases. In nearly all instances, the flagellum originated from that side or end of the body, near which the smaller nucleus

[1] Among these are one or two rather indefinite forms which Rogers considers represent stages in fusion (comparable to conjugation); earlier stages in the process are, the author thinks, exemplified by many of the pairs or couples (e.g. those in fig. 37 IV *d*). The writer does not consider this very likely; the couples much more probably represent individuals which have not yet separated after division (cf. the figs. of Donovan, and Laveran and Mesnil), the somewhat atypical form and arrangement being readily accounted for by the medium in which the parasites are.

(kinetonuclear element) was situated, and the author mentions that he was occasionally able to trace a connection between the two. From the figures it certainly appears as if the parasites by successive divisions, became more fusiform and less pear-shaped, that seen at *g*, being, perhaps, derived from a form like that of *f*, which is in the act of dividing. Even in the most slender and Trypanosome-like stage observed, however, Rogers could not distinguish any indications of an undulating membrane, and the kinetonucleus was never far from the insertion of the flagellum.

These results have since been fully corroborated by Chatterjee (**122**), Christophers (**123** [3rd Rep.]), and Leishman and Statham (**131**). The general appearance of the Flagellate stages figured by these workers quite agrees with that seen in fig. 37 IV, *f*. and *g*. Leishman and Statham bring forward interesting additional observations, and the illustrations given are particularly good. The cytoplasm of the parasites is usually very vacuolated; this is most probably due to the effects of the artificial medium upon the metabolism. Leishman and Statham (and also Christophers) describe the actual formation of the flagellum, which is developed very suddenly, in a remarkable manner, from a distinctive, vacuole-like structure, termed the "flagellar vacuole"; this arises at the anterior (?) end, in close connection with the kinetonucleus ("micronucleus"). Some of the contents of this vacuole are expelled to the exterior in the form of a tuft or branched process, and, at the same time, the flagellum appears. At present it seems impossible to say exactly what occurs. Another remarkable process described is unequal longitudinal fission. Very thin, sickle-like ("spirillar") portions of the body are split off from one side of the parent-individual. More than one thread-like form may be thus separated off. The strange feature about the process is that neither of the two principal nuclear elements appears to be concerned. In a few of the parasites chromatin grains are noticeable in the cytoplasm, and in one of the peculiar fission-forms two of these grains are contained in the portion being cut off. Whether these would become the definitive nuclear organellæ of the daughter-parasite is not certain. Anyhow, later on, when the sickle-like form has developed a flagellum, two chromatic elements are present, apparently corresponding to those in the ordinary (adult) forms.

All these accounts agree with that of Rogers with regard to the entire absence of an undulating-membrane.

Nevertheless, bearing in mind the fact that cultural forms of many Trypanosomes have either a very small membrane

or none at all (cf. above), the possibility of these organisms possessing an undulating membrane during certain phases of the life-history, when this is undergone in normal conditions, is by no means excluded. On the contrary, indeed, the markedly Herpetomonad-like facies of the parasites, which greatly resembles that of "cultivated" Hæmoflagellates, strongly points to their being closely related to this group. If so, there can be little doubt that Piroplasma (Leishmania) donovani (and, by inference, P. (L.) tropicum) also has, at some period or other, a typical trypaniform phase.

Nothing is known with regard to the transmission of the parasites, and the possible occurrence of an alternate host. The superficial position of the localised form strongly points to infection in this case by the bite of some blood-sucking Insect.[1] That being so, it seems most natural to infer that the same holds for the splenic type, although what determines the limitation of the parasites in the one case, and their dissemination throughout the system in the other, remains a mystery. It has been suggested that, in the case of the latter type, the organisms leave the host by way of the alimentary canal, since they have been found in ulcers of the large intestine. At any rate, it is very likely that an important part of the life-cycle is passed through outside the human host, though whether in the free condition or in an Insectan host, or in both combined, has still to be learnt.[2]

[1] Crombie ('Brit. Med. Journ.,' 1904, ii, p. 658) points out that persons who attend upon camels are very liable to a form of oriental sore. The camel is the host of a Trypanosome and, possibly, the "camel-fly" transmits the parasites to man. There is, indeed, a close parallelism between the distribution of camels and oriental sore.

[2] Rogers' experiments showing that the parasites live and develop in cold solutions outside the body, but rapidly degenerate when kept at blood heat, point to the first part of this sentence being correct. With regard to the second part, this same worker, in a more recent note (**139**), finds that cultures of the parasites (Leishman's form) develop most rapidly and successfully in an acid medium. In his opinion, this indicates the acid-containing contents of the stomach of some blood-sucking Insect as the place in which the extra-corporeal stages of the parasite's existence are

Section XI. Phylogeny and Evolution.

The subject of the derivation of the Trypanosomes is one of much difficulty, owing to our very insufficient knowledge of the majority of the parasites. The views put forward below, which involve an apparently different interpretation of the orientation of the body in different cases, are, to a large extent, based upon Léger's important researches, on the one hand, upon Trypanoplasma (**64** and **65**), and, on the other, upon certain Herpetomonadine forms (**61**—**63**, **68**, **69**). The Trypanosomes, as a whole, are to be regarded as including two entirely distinct families, in one of which the attached flagellum becomes free at the true anterior end, and in the other at the true posterior end. Before discussing the reasons for this division of the Hæmoflagellates into two groups, however, we may consider the principal features in the structure of the Herpetomonadine parasites to which reference has just been made.

In a typical Herpetomonas (e. g., H. muscæ-domesticæ,[1] H. jaculum, or H. gracilis [fig. 38 B]), the kinetonucleus is situated near the anterior end; the flagellum is not attached to the side of the body at all but straightway

undergone; and, in this connection, he is inclined to suspect fleas or bugs. The difficulty in the way of this view is the rarity of the occurrence of the organisms in the peripheral circulation; the skin papulæ which sometimes occur are suggested as furnishing a source of infection for the Insectan host.

[1] H. muscæ-domesticæ is here included as a typical uniflagellate Herpetomonad. Léger has observed no signs of two flagella, either in this species or in others of the genus (not considering, of course, individuals about to divide by longitudinal fission). Prowazek (**87**) interpreted this form as a bipolar Flagellate in which the body has been bent up so that the two ends have come together and united, the flagella alone remaining distinct. This view is the less tenable since Schaudinn's conception of a primitive, bipolar "Urhæmoflagellate," based upon Laveran and Mesnil's unfortunate description of Trypanoplasma borreli, appears to have no prospect of being realised; for, up to the present, there is no evidence of this bi-polarity in any known Flagellate.

becomes free, and, correlated with this, there is no undulating membrane. These forms are, for the most part, parasites of Insects which do not suck blood. A stage in advance is seen in H. subulata (fig. 38 E, F), parasitic in the digestive tube of Tabanus glaucopis and Hæmatopota italica, which are predatory on cattle and horses. This parasite, when in the Monadine form, has still the usual acicular shape. The kinetonucleus, however, lies much farther from the anterior

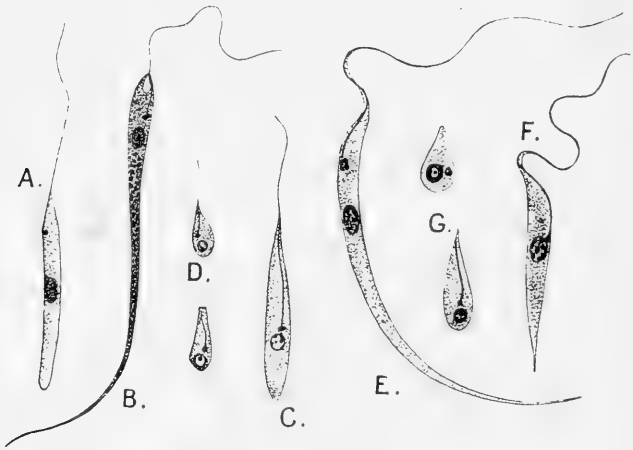


FIG. 38.—A, C, Herpetomonas (Crithidia) minuta, Léger; D, attached (gregariniform) stages of same; B, H. gracilis, Lég.; E, F, H. subulata, Lég.; G, attached stages of same. (All after Léger, × 1800.)

end, and may, in fact, be almost opposite the trophonucleus. The flagellum, which has been, as it were, drawn back with it, is, in the majority of individuals, attached, for the proximal part of its length, to the anterior part of the body by means of a delicate cytoplasmic border, which constitutes a rudimentary undulating membrane. The flagellum, it will be seen, has its root in a diplosome (probably of centrosomic nature) just in front of the kinetonucleus.

H. (Crithidia) minuta, Léger, parasitic in Tabanus tergestinus, differs from the last-mentioned type in having the posterior end thicker and more rounded (fig. 36 A and C);

it is intermediate, in short, between the acicular forms and the genus Crithidia, characterised by its pyriform shape. The kinetonucleus may apparently be either near the anterior end or near the trophonucleus; but L. does not mention the occurrence, in the latter case, of any rudimentary membrane. We come next to Crithidia fasciculata, which Léger found in the intestine of Anopheles maculipennis (females). A striking resemblance in form is offered by certain phases of this parasite[1] to those of Trypanomorpha noctuæ when in the gnat. One side of the body appears more delicate than the other, possesses a distinctly wavy border, and is prolonged anteriorly, attached to the flagellum, gradually tapering away however before the latter terminates.

There are one or two other important morphological points to note in connection with these Herpetomonadine forms. In many a vacuole can be easily demonstrated situated in the cytoplasm near the anterior flagellate end. In H. muscæ-domesticæ and H. gracilis (fig. 36 B) this structure is contractile or pulsatile, but in H. subulata (fig. 36 E), a more specialised type, it appears constant (non-contractile), though probably still retaining its original excretory function. A feature in H. gracilis is the occurrence of a number of deeply-staining grains in the cytoplasm, doubtless comparable to the chromatoid grains described in many Trypanosomes. In this parasite they are all in the non-flagellate part of the body behind the nucleus.

Lastly, it is to be remembered (1) that all the parasites mentioned have also gregariniform resting-phases, in which the locomotor apparatus may become reduced to a short rostrum serving for attachment to a host-cell, or may entirely vanish (see fig. 36 D and G); and (2) that H. subulata, as well as certain other species, becomes united in rosettes in which the parasites are all attached by the anterior end.

From these considerations it appears scarcely open to doubt that an intimate degree of relationship exists between

[1] Unfortunately the only figures at present available are not very good.

Trypanomorpha (Trypanosoma) noctuæ and Herpetomonas. In all known respects there is either entire agreement between the two genera, or the condition found in the former is easily derivable from that existing in the latter. Certain species of Herpetomonas may well represent a phylogenetic stage in the evolution of Trypanomorpha; indeed, in some cases—especially where they have been described from blood-sucking Insects—parasites identified as Herpetomonads probably themselves constitute, in reality, only a phase in the life-cycle of a particular Hæmoflagellate. As Léger points out, Crithidia fasciculata itself is very likely a Hæmoflagellate, and closely allied to Trypanomorpha, though exactly how closely cannot be said until its life-history in the Vertebrate host has been investigated.

Many, perhaps most, authorities regard all uniflagellate Trypanosomes as descended in this manner, and as agreeing with Trypanomorpha in having the flagellum at the anterior pole of the body. There are, however, in the writer's opinion, several cogent reasons against accepting this view, reasons which, on the contrary, lend considerable support to the alternative theory that, at any rate, most Trypanosomes (sensu stricto) are derived from a type like Trypanoplasma by the loss of the anterior free flagellum, so that the non-flagellate end is really the anterior one.[1] These reasons are best discussed under two headings, dealing, respectively (*a*) with biological or physiological considerations, and (*b*) with certain morphological points. To the former particular weight must be assigned, and the indications which they afford are borne out and, in fact, strengthened by a consideration of the latter.

(*a*) It may, we think, be fairly assumed that the sensory extremity of a Hæmoflagellate is, as in non-parasitic Flagellates, the anterior end. By the sensory extremity is here meant that region of the body which is most in touch with the environment; the region specially concerned with the adjustment of the voluntary relations of the parasite to the

[1] This view was first outlined by Léger (65).

immediate surroundings and conditions. There can be no doubt that such is the case in those forms for which the correct orientation of the body is known (cf. on the one hand Trypanomorpha, and on the other the delicate tactile and sensory beak of Trypanoplasma and the allied Trypanophis), and there is no reason to suppose that the same is not true for the numerous uniflagellate forms. In other words, we may regard the sensitive or physiological end of the body as homologous throughout the group.

Among the biological processes in which the sensitive extremity may be expected to take the lead are the following:

(1) Attachment.—Schaudinn's discovery that Trypanosoma ("Hæmamœba") ziemanni, when about to enter upon a resting phase, attaches itself to and penetrates a leucocyte by its non-flagellate extremity, is of the greatest importance in this connection. This manner of fixation is in sharp contrast to that met with in Trypanomorpha and other Herpetomonadine forms. Although the mode of attachment in Trypanoplasma is not yet known, there can be no doubt that, if it occurs, fixation takes place by the anterior end, as Keysselitz (l. c.) has found is the case in Trypanophis, the short active beak or rostrum of which itself probably serves this purpose. In the case of most Trypanosomes attachment has not, so far, been described. There are, however, one or two observations on record with regard to certain species of Trypanosoma, which tend to show that fixation occurs in these also by the non-flagellate end (see above, p. 173).

(2) Agglomeration.—Whatever its exact meaning, it is most likely that this peculiar phenomenon is a biological feature of definite import. This seems indicated, for instance, by the fact that—in what may be regarded as normal circumstances, if not, indeed, under all conditions [1]—the union, in any particular case, occurs in a constant manner. Moreover, as already described, the mode of agglomeration and that of attachment differ in exactly the same way in

[1] See Section VII (B), with reference to "rosette-formation" in cultures.

Trypanomorpha noctuæ and Trypanosoma ziemanni. Hence, it may be taken for granted that agglomeration normally occurs by the sensitive pole. It cannot but be regarded as highly significant, therefore, that, in all species of Trypanosoma for which this property has been ascertained, the union is by the non-flagellate end; in the writer's opinion, this evidence to a large extent compensates for the paucity of observations relative to attachment. Up till now agglomeration has not been observed in Amphibian or Piscine forms.

(3) Conjugation.—It is difficult to imagine a microgamete, which exhibits definite cell-polarity, coming into contact with the female element, prior to union or penetration, by any other than the sensitive, anterior pole. Hence, in the case of the Hæmoflagellates, the end by which a microgamete (a male Trypanosome or its homologue, it is to be remembered) conjugates with a megagamete is of great interest. Unfortunately, very few instances of fertilisation are, so far, to hand. From Schaudinn's account, the process in Trypanomorpha noctuæ fully bears out the evidence afforded by other considerations, and the same is probably true of Trypanosoma ziemanni[1]; a noteworthy point in the former case is that the flagellum of the microgamete, being at the anterior, penetrative end, is not developed. The only other case known is that of T. lewisi (Prowazek [l. c.]), to which reference has been made. Granting, as is very probable, that this author's description and figures (see above, p. 249) represent the manner in which true conjugation occurs, the question of the correct orientation of the body in this form appears conclusively settled in favour of the view here taken.

(4) Movements of Investigation.—In this category are to be placed the slow creeping or crawling movements which have been observed in certain Trypanosomes (see Section VII (A)). Both the apparently tentative or investigatory character of the movement, and the fact that it is

[1] See footnote, p. 247.

essentially comparable in manner and origin to gregarinoid or euglenoid movement, would naturally lead us to expect that the sensitive or anterior extremity goes first. The fact, therefore, that this mode of progression is with the non-flagellate end in front in all the instances hitherto recorded, is of considerable importance in the present argument. It entirely negatives—in the writer's opinion it distinctly outweighs—the opposing fact that the movement during rapid displacement usually occurs with the flagellate end leading. For, in these energetic movements, where the principal object of the parasite is to change its vicinity as quickly as may be, it obviously cannot much matter which end goes first, the determining factor being, doubtless, mechanical considerations (cf. also the account of movement).

As a corollary may be mentioned the plastic or "amœboid" nature of the non-flagellate end in Trypanosoma—i. e. its power of retraction and extension, commented upon by Laveran and Mesnil (cf. fig. 42 D, of T. equiperdum). In short, all the physiological data we possess tend to show that this non-flagellate end in Trypanosoma is the sensitive extremity, and homologous with the anterior end of Herpetomonas, Trypanomorpha, and Trypanoplasma.

(*b*) Morphological Points.—Reference has above been made to the frequent presence of a vacuole near the non-flagellate end in different species of Trypanosoma (e. g. T. brucii, T. gambiense, Hanna's Trypanosoma of Indian birds, etc.), here considered to be a normal cell-constituent (though not necessarily one of invariable occurrence), which probably represents the (originally) contractile vacuole of an ancestral Hæmoflagellate. For there is no reason to doubt that this vacuole is homologous with that described both in Trypanoplasma (fig. 17 F) and in certain Herpetomonadine forms.[1] The important point of distinction is that

[1] In Trypanomorpha, it will be remembered, Schaudinn describes one or more cytoplasmic vacuoles of varying size, in the anterior part of the body (cf. fig. 8); this perhaps represents a more advanced condition, in which the formation of a distinctive organella is tending to be lost.

in the last-named instances this structure is near the anterior, flagellate extremity of the cell. If, therefore, Trypanosoma sensu stricto is a Herpetomonadine form, why is the vacuole, where known to occur, always near the non-flagellate, apparently opposite end of the body?

(1) Conversely the chromatoid grains, so frequently noticeable in the cytoplasm (see above, p. 211), are, in Trypanosoma,[1] mainly or entirely in the flagellate half of the body, or, at any rate, on that side of the nucleus (figs. 16, 17, 42, 47, and 57). On the other hand, in Herpetomonas gracilis, which also possesses a number of similar grains, they are all in the non-flagellate part of the body, that is to say, behind the nucleus (fig. 58 B). Compare the cytological details of this parasite with those of, say, T. nelspruitense (fig. 17 E), and it appears unquestionable that the flagellate end of the former corresponds to the non-flagellate one of the latter. Again, in Trypanomorpha, pigment grains and other effete material collect near the non-flagellate (posterior) end, and the cytoplasm near this extremity is frequently more granular and deeply staining (figs. 8, 10, and 13) than it is near the flagellate end, or just the opposite to what is generally the case in Trypanosoma.

It is surely needless to suppose the position of these two cytological constituents in the body of Trypanosoma to have become, for no obvious reason, absolutely reversed (which one is bound to do if the Herpetomonadine view be adhered to), when, on the one hand, no stage from Herpetomonas to Trypanomorpha inclusive shows any indication of such a change, and, on the other hand, in Trypanoplasma borreli, they have exactly the same position relative to the attached (posterior) flagellum that they have in Trypanosoma (cf. fig. 17 F and G, and especially Laveran and Mesnil's amended Fig. LVI, 5, [56], in which the chromatoid grains are entirely behind the nucleus). Granted the loss of the anterior flagellum, and we

[1] In T. granulosum (fig. 17 K) they are uniformly distributed throughout the cytoplasm, but this is, apparently, the only exception.

have a body which is in complete agreement with that of Trypanosoma.

3. Lastly, what undoubtedly seems to be a transitional stage in this disappearance of the anterior flagellum is seen in Trypanoplasma cyprini (fig. 17 H), where this organella is short and delicate, and evidently undergoing reduction.

While the evidence thus adduced points, in our opinion, unmistakably to the derivation of at least some of the forms included in the genus Trypanosoma from a Heteromastigine ancestor, there is, it may at once be admitted, one morphological feature which does, at first sight, seem to oppose this theory. The character in question is the variation in position of the kinetonucleus, especially during certain developmental phases.

In most species of Trypanosoma, the kinetonucleus occupies, in adult parasites, a position near the non-flagellate end. Its position here, therefore, compares with that in the simple Herpetomonadine forms (e. g. H. gracilis, jaculum, and muscæ-domesticæ) and in Trypanoplasma in exactly the same way as does that of the other cell-constituents referred to above (where noticed) in the two sets of cases.

Were the approximately terminal position of this organella constant, therefore, this character would be in complete agreement with the other morphological ones, and would be, undoubtedly, a very strong point in favour of the view here taken. As it is, however, the kinetonucleus varies considerably with respect to its situation in the body. On the one hand, in Herpetomonas subulata, some individuals of H. minuta and Trypanomorpha noctuæ, it has passed backwards almost as far as, or sometimes even behind (posterior to), the trophonucleus; and, on the other hand, in certain species of Trypanosoma (e. g. T. inopinatum, T. rotatorium, T. transvaaliense) it occupies a similar position on one side or other of the trophonucleus, in more or less contiguity to the same. Hence, having regard to these facts alone, it might reason-

ably be inferred that, in the latter forms also, the result is attained in the same manner, by the passage backwards of the kinetonucleus from the flagellate (anterior) end; and that in the other species of Trypanosoma, in which the kinetonucleus is practically terminal, this body has merely passed still further back, right to the non-flagellate (posterior) end,[1] thus bringing about the great development of the undulating membrane.

This is, in fact, the explanation given by the adherents of the Herpetomonadine theory. They consider that the condition observed in young parasites of T. lewisi, formed by "budding" or multiple division (see above, p. 238 et seq., and, figs. 27—29), furnishes strong support to this view; and, indeed, they look upon the manner in which the young forms become adult in this parasite as indicating the actual course of morphological evolution in Trypanosoma. Did these facts constitute the only evidence available, there could be, it is needless to say, little doubt as to the correctness of the monophyletic theory. When, however, we bear in mind the many opposing facts already discussed, including certain biological considerations of fundamental importance which it is difficult, if not impossible, to reconcile with this view, we are bound to conclude that the migration of the kinetonucleus cannot possess the significance attributed to it.

There is one point which may be suggested as perhaps accounting, at any rate in part, for this feature. It may be regarded as proved beyond question that the kinetonucleus is of nuclear origin. Hence its wandering propensity in general and frequent contiguity to the trophonucleus are not difficult to understand. This association of the two organellæ is particularly marked in cases of multiple division, whether occurring in normal (figs. 27, 28) or in cultural conditions

[1] Admitting, for the moment, that this explanation of the position of the kinetonucleus in Trypanosoma is possible, it must be emphasized that the argument relative to the other morphological characters is not thereby invalidated; for no intermediate stages in the "reversal" of their position are known (cf. H. subulata, and especially Trypanomorpha).

(fig. 29). It is not found, on the contrary, in binary longitudinal fission,[1] which, it must be remembered, is by far the most prevalent and most typical mode of division in natural circumstances. In such cases there is no indication whatever of a Herpetomonadine facies (cf. figs. 23, 25). Hence it seems to the writer that this association of the nuclear elements bears some relation to their rapid multiple division during segmentation, perhaps facilitating the complex mechanical processes which apparently occur.

Now the juxtaposition of the two nuclear bodies of itself confers a semi-Herpetomonadine aspect on an individual exhibiting it. When further, as frequently in this rosette type of multiplication, the daughter-nuclei travel towards the periphery, doubtless to assist in the segregation of the general cytoplasm into the individual portions,[2] the resemblance to a Herpetomonad is much increased. We have, in short, what may be termed a "pseudo-Herpetomonadine" form developed (cf. figs. 28, 29). The absence or rudimentary character of the undulating membrane is sufficiently explained by the position of the kinetonucleus and the very short portion (if any) of attached flagellum present in consequence, and need not be further considered.

To sum up, therefore, this difficulty with regard to the variation in position of the kinetonucleus does not seem insuperable; on the other hand the biological considerations, as well as the other morphological characters above mentioned appear to offer far greater objections in the way of the Herpetomonadine theory. Nevertheless, this does not necessarily imply that all the species included below under Trypanosoma are derived from a Trypanoplasmatine ancestor. Some almost certainly are, e.g. T. brucii and T.

[1] This refers, of course, to adult Trypanosomes as such; parasites exhibiting the "pseudo-Herpetomonadine" form may divide while retaining it (see above, p. 240).

[2] With reference to the arrangement of the parasites in the multiplication-rosettes found in cultures, which cannot be regarded as entirely normal see above, p. 241.

lewisi among Mammalian forms, T. ziemanni among Avian parasites, and T. nelspruitense among Amphibian types. And the same is probably true of the majority of the Piscine forms; at any rate, their morphology—the only evidence available at present—agrees more closely with that of Mammalian parasites than with that of Trypanomorpha. On the other hand, in the case of one or two Amphibian forms, it is much more doubtful which interpretation of their morphology is the correct one. One of these, most unfortunately, happens to be Trypanosoma rotatorium, the type-species of this genus. It can at least be said, however that this parasite is quite as probably a Trypanoplasmatine form as a Monadine one (see below, p. 288).

Evolution.

In conclusion, one may, perhaps, venture to indicate what appears to have been the general course followed in the evolution of the Hæmatozoa, although the tentative character of any hypothesis which can be at present advanced must be fully recognised.

The Hæmoflagellates are here considered to be, in general, descended from forms originally parasitic only in Invertebrate hosts (probably non-predatory), in which, as intestinal or entero-cœlomic parasites, all their life-cycle was undergone. Upon their becoming associated with a blood-sucking host, either by the acquirement by the original one of this mode of life, or by the adaptation of a particular parasite to a closely allied predatory host, the Flagellates gradually became specialised for life in the blood. In other words the Invertebrate is here regarded as the primary host, and the Vertebrate as the secondary or intermediate one.

There can be no doubt that this is the case in Trypanomorpha, at any rate. In this form the development of the characteristic undulating membrane appears to have been brought about by this change in habitat. Transitional stages are beautifully illustrated by Léger's observations (l.c.) on Her-

petomonadine forms, of which mention has above been made. Moreover, in this parasite the primary host is also the definitive one, or that in which the sexual process takes place.

Turning next to the Heteromastigine forms we are led to the same conclusion, though on more general grounds, for we lack, at present, an illustrative series like that just referred to. For instance, with regard to the origin of the undulating membrane. In Trypanoplasma, which is most probably to be derived from a Bodo-like ancestor, the undulating membrane very likely arose, as Doflein (**19**) points out, as the result of the contiguity of the trailing flagellum to the side of the body, which led to fusion without loss of motility and thus brought about the development of a membranous expansion. Now there are one or two forms which possess an undulating membrane, but which are not, however (so far as is known), Hæmatozoan parasites, namely, Trypanophis grobbeni, which inhabits the cœlenteric cavity of Siphonophores, and an enteric form parasite in Box boops, which Léger (**70**) regards as a Trypanoplasma (intestinalis), but which appears to the writer, if anything, nearer Trypanophis (see below, in Systematic). Hence it would seem that the presence of an undulating membrane in a Trypanoplasmatine form does not necessarily imply a hæmal habitat.

In connection with this derivation of the Heteromastigine forms it is interesting to note the gradual change in the degree of development of the flagella which can be traced. Starting with Bodo lacertæ, both flagella are of equal length, and the trailing one does not reach the posterior limit of the body. In Trypanophis grobbeni (fig. 41) the posterior flagellum is more developed than the anterior one, and, of course, attached to the side of the body. Its free termination, however, is very short. In T. (Trypanoplasma) intestinalis this is longer and more strongly developed (fig. 40), though still shorter than the anterior flagellum itself. In Trypanoplasma borreli (fig. 17 F, G) the anterior flagellum and the free portion of the hinder one are of equal

length. In T. cyprini (fig. 17 H) the former is much shorter than the latter, and shows signs of reduction, while in Trypanosoma it is entirely lost.

Hence, while it is very probable that the condition of the locomotor apparatus in Trypanophis represents a phylogenetic stage in the development of the condition found in Trypanoplasma, this form itself is not of much assistance in determining the original kind of host of the Heteromastigine Hæmoflagellates.

The reasons for thinking that this section of the Hæmoflagellates is also derived from a primitively Invertebrate type are, as a matter of fact, the considerations fully discussed in Section IX, which point, on the one hand, to the possession by the parasites of an alternate, Invertebrate host, in which conjugation takes place, and, on the other hand, to a relationship between the Trypanosomes and the Hæmosporidia. For, on à priori grounds, we may reasonably infer that the definitive host is the primary, original one. Thus would be explained the necessity which exists in most, if not all, Hæmatozoa for the return of the parasites to the alternate host. Again, both sections of Hæmosporidia, those of warm- and of cold-blooded Vertebrates, have recently been brought into line in respect of the occurrence of conjugation only in the Invertebrate (see Introduction, p. 161). It follows, therefore, that all evidence pointing to a connection between the two "groups" of Hæmatozoa at the same time favours the view here put forward.

While, of course, the possibility of a Hæmatozoan being derived from a form primarily parasitic in the intestine of a Vertebrate must not be ignored, the writer agrees, nevertheless, with Léger, who aptly remarks that one is not justified in so deriving any Hæmoflagellate until a cœlomic form, known with certainty to undergo sexual conjugation in the Vertebrate, has been found.[1]

[1] Even in the case of T. equiperdum, the fact that the parasites are transmitted directly from one Vertebrate to another, during the act of coitus, does not imply that this form has never possessed a definitive Insectan

The course of evolution of the Hæmatozoa (as a whole) in the blood of the Vertebrate appears to have been characterised by the acquisition of an intra-cellular phase, together with the capacity for multiplication while in that condition. In such cases the subsequent tendency has evidently been to develop the gregariniform or Hæmosporidian phase at the expense of the trypaniform or Hæmoflagellate one. For, on carefully reviewing the evidence set forth in Section IX, it seems scarcely open to doubt that there is a very close phylogenetic relationship between the Hæmoflagellates and the Hæmosporidia, and that, moreover, in some cases there is an actual ontogenetic connection between the two types of form at the present day.[1]

host, or that it is not still obliged to return, if only at rare intervals, to such a habitat, in order that, by sexual conjugation, the stock may be reinvigorated. The direct mode of transmission, most probably a secondary acquirement, is readily accounted for by the peculiar superficial distribution of the organisms (see above, p. 176). It must be borne in mind that this is the only species which is known to be able to traverse healthy mucous membranes, and this itself is against the derivation of Trypanosoma from an intestinal or entero-cœlomic, Vertebrate form.

[1] Novy and McNeal (**81**), basing their view upon the results of their cultural methods of work, conclude that there is no connection between Cytozoa (Hæmosporidia) and Trypanosomes. They disbelieve in Schaudinn's work, and think that this author was misled by working with "impure cultures," as they regard the conditions in which the parasites occur in their natural hosts. But, as Mesnil says, the presence of the characteristic Hæmosporidian pigment in the trypaniform phase is a fact very difficult to explain away. Again, Novy and McNeal consider that the appearance of Flagellate forms in Rogers' culture of "P." donovani was merely a coincidence, indicating the existence of a variety of human trypanosomosis hitherto unknown, the two parasites being quite distinct! The ample confirmation which both these important researches have already received sufficiently disposes, we think, of the above view.

It is, of course, very probable that there are Trypanosomes of birds (as of other Vertebrates) which have no Hæmosporidian phase. It is not safe, however, to argue that there is no connection between a given Hæmoflagellate and a given Hæmosporidian from the negative evidence obtained by cultures and their injection. For it is most unlikely that the further development of the parasites can go on in an artificial medium as successfully and as normally as in the Insectan host, to which their biology is specially

It is, indeed, possible to construct what may be regarded as stages in the direction of evolution thus outlined. The first step is shown by those forms which possess resting, attached phases (Trypanoplasma almost certainly, and probably some species, at any rate, of Trypanosoma, e.g. T. equinum and T. equiperdum). A stage in advance is exemplified by Trypanomorpha noctuæ and Trypanosoma ziemanni; these parasites become, for a portion of their life, intracellular, but do not multiply asexually in this condition. On the other hand, it appears very probable from Billet's work (p. 251) that certain Amphibian parasites possess (as Hæmogregarines) typical schizogony, and this may be also the case in some Piscine forms. Here the trypaniform phase is very likely much less in evidence, and assumed (in the blood) only at rare intervals. Probably about this level, too, come the Piroplasmata, which are very interesting in that some of them retain the nuclear dimorphism while in the intracellular phase. Lastly, a trypaniform phase in the life-cycle can only be recognised with difficulty, in the sporozoites or merozoites (Plasmodium vivax, possibly other malarial parasites and certain Hæmogregarines [cf. p. 253]); or, in other cases, may be quite lost.

It will be gathered from the above remarks that, with the advance of our knowledge, it may well become extremely difficult to decide whether a given parasite should be looked upon as a Hæmoflagellate or a Hæmosporidian, and, if so, where to draw the dividing line. The writer is truly thankful that he does not feel himself called upon, at present, to attempt to answer such momentous questions.

adapted; or that the "cultivated forms" when injected into the Vertebrate host, can give rise to the same phases as those which may develop after natural infection (see also below, p. 301).

Section XII. Systematic Enumeration.

The reasons for the division of the Trypanosomes into two distinct and entirely independent families have been fully discussed in the preceding section. Apart from the fundamental diagnostic characters, it is quite likely that other important features in which the parasites differ in the two cases will become known as our knowledge of the complete life-cycle increases.

Sub-order.—Monadina.

Family.—Trypanomorphidæ, n. fam.[1]

Hæmoflagellates derived from a uniflagellate, Herpetomonadine form, in which the point of insertion of the flagellum into the body has travelled backwards from the anterior end for a greater or less distance, the flagellum itself having become, concurrently, attached to the body for a portion of its length by means of an undulating membrane. At present only one genus can be said to be known with certainty.

Genus Trypanomorpha, n. g. With the characters of the family. The only species yet known is the type species, T. noctuæ (Celli and San Felice). [Syn. Trypanosoma n. (C. and S. F.), Schaud. = Halteridium n. (C. and S. F.)].[2] The full life-cycle of this parasite has been described above (Section V). Vertebrate host: Athene noctua (Little Owl); Invertebrate host: Culex pipiens.

[1] It is considered best to remove Trypanomorpha, and other allied Monadine forms which are true Hæmoflagellates, from the old family of the Cercomonadidæ or Oicomonadaceæ, in the same way that Doflein (19) has separated the Heteromastigine section (his inclusive genus Trypanosoma) from the Bodonidæ.

[2] Schaudinn places this form in the genus Trypanosoma. The writer, however, inclines to the view that the type-species of that genus (T. rotatorium) is a Heteromastigine form (see below, p. 288), in which case this Avian parasite cannot be included therein. Moreover, it is altogether uncertain whether the type-sp. of Halteridium (H. danilewskyi) agrees generically with H. noctuæ. Quite possibly it does not, since, for one thing, it possesses typical schizogony. Hence the writer thinks it best to place H. noctuæ in a distinct genus, Trypanomorpha.

There are, in addition, two or three forms, which are most probably to be placed in this family, but which are not yet sufficiently characterised for their generic position to be settled. It is, for instance, quite likely that Léger's parasite Crithidia fasciculata (see above, p. 269) from females of Anopheles maculipennis is sufficiently allied with Trypanomorpha for the two forms to be united in the same genus, in which case, of course, the name Crithidia will take priority.

Sub-order.—Heteromastigina.

Family.—Trypanosomatidæ, Dofl., emend.

Flagellates, in the great majority of instances hæmal parasites, derived from a biflagellate, Bodo-like type, in which the posteriorly-directed flagellum (the so-called trailing flagellum) is always present and attached to the body by means of an undulating membrane, of which it constitutes the thickened edge. The other, the anterior, flagellum may or may not persist. Three genera so far distinguished.

Genus Trypanoplasma, Lav. and Mesn., 1902. The anterior flagellum is present. Both flagella are inserted close together at or near the anterior end of the body. Three species certainly known, which can be arranged in two groups:

(A). The anterior flagellum is well developed, and the free portion of both flagella are of about equal length.

T. borreli, L. and M., 1902. Length[1] from 20–22 μ, of free flagella 13–15 μ; breadth $3\frac{1}{2}$–$4\frac{1}{2}$ μ (figs. 17 F, G, 18). Hosts: (V.)[2] Leuciscus (Scardinius) erythrophthalmus, rudd, and Phoxinus lævis, minnow; (I.) not yet known.

T. varium, Lég., 1904. Length (medium) about 25 μ, of free flagella 18–20 μ. Hosts: (V) Cobitis barbatula, loach; (I) Hemiclepsis marginata, perhaps also Piscicola sp., leeches. This parasite differs from T. borreli by the rather longer flagella, and by its not having the pronounced cytoplasmic granules of the latter form. Léger considers specific distinction[3] is also shown by the fact that, in streams containing both loach and minnows, only the former are infected with this parasite.

(B). The anterior flagellum is much shorter than the free portion of the posterior one, and apparently tending to disappear.

T. cyprini, Plehn, 1903. Medium length about 20 μ (fig. 17 H). Host: (V) Cyprinus carpio, carp.

[1] Of the body alone, independent of the flagella.

[2] (V.) signifies vertebrate hosts; (I.) invertebrate ones.

[3] See remarks on specific distinctions, pp. 288—9.

It is uncertain whether the Flagellate organism described by Labbé (**29**) from the medicinal leech (Hirudo), which had probably sucked the blood of a horse or ass, should be placed here or not. This parasite (fig. 39), to which Labbé gave the name of Trypanomonas danilewskyi,[1] was elongated almost filiform (15–20 μ by 1 μ), with apparently a long, thin, more or less coiled flagellum at either end. It also possessed a delicate undulating membrane. Labbé considered one of the flagella to be a very attenuated prolongation of the body and membrane, on the analogy of the spindle-like forms figured by Danilewsky (cf. fig. 16 G of Hanna's Trypanosome from Indian pigeons). In that case, the parasite would really be a spirochætiform Trypanosoma, but the figures, so far as they go, do not convey that impression. Re-investigation of it is necessary in order to settle its position.

Fig. 40.

Fig. 39.

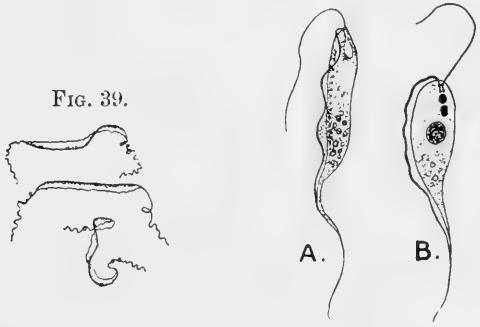


Fig. 39.—"Trypanomonas" danilewskyi, Labbé, × 1200. (After Labbé.)

Fig. 40.—Trypanophis (Trypanoplasma) intestinalis (Léger). In A, note the row of spherules down the side near the undulating membrane; in B the kinetonucleus is in two parts, probably resulting from division. (After an unpublished drawing kindly lent by Prof. Léger.)

Genus Trypanophis, Keysselitz, 1904. The body resembles that of Trypanoplasma in form and general appearance. The locomotor apparatus does not appear to be so well developed, however, especially in T. grobbeni. The

[1] Even if this form is found to agree generically with Trypanoplasma, Labbé's name Trypanomonas could not be used, since this designation was originally employed by Danilewsky for the young forms of a Trypanosoma, with which, therefore, it is synonymous.

anterior flagellum is longer than the free portion of the posterior one. The species included are not, so far as is known, hæmal parasites.

T. grobbeni (Poche), 1903. Average length 60—65 μ; width about 4 μ. The undulating-membrane is relatively narrow and not expanded into wider folds at intervals (fig. 41). The anterior flagellum is fairly long, but the free part of the posterior one is short. This species is parasitic in certain Siphonophora, Cucubalus kochii, Halistemma tergestinum, Monophyes gracilis, of common occurrence in the Gulf of Trieste. Apparently the same parasite has also been observed in Abyla

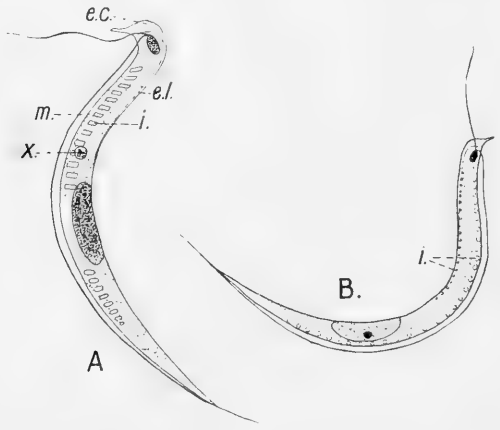


Fig. 41.—Trypanophis grobbeni (Poche). *e.c.*, ectoplasmic cap; *e.l.*, delicate ectoplasmic layer, thinning out posteriorly; *m.*, undulating-membrane; *i.*, inclusions in the cytoplasm; *x.*, nuclear body of uncertain origin and significance. (After Keysselitz.)

pentagona, from the Gulf of Naples. The organisms are to be met with in all the ramifications of the cœlenteron, from the digestive cavity of the gastrozoids to the radial canals of the medusoid buds.

Nothing is known regarding the transmission of the parasite from one Siphonophoran colony to another. That is to say, we are quite in the dark as to whether they have any alternate host or not. Of the two suppositions, the latter seems much the more probable.

T. (Trypanoplasma) intestinalis (Léger), 1905. A much smaller form than the last (fig. 40). The dimensions given are: Length of body (without "tail") 14 μ; of anterior flagellum, 16 μ [judging from the

figures it does not seem quite so long]; of tail and posterior flagellum (free part) 16 μ. Habitat: œsophagus and anterior part of stomach of Box boops.

This form is manifestly closely allied to T. grobbeni. At the same time it also exhibits great resemblance to Trypanoplasma borreli, in which genus Léger has included it. In the writer's opinion, it appears to be, as regards its morphology, intermediate between these two parasites, and it is not easy to decide where to place it. The fact that the free part of the posterior flagellum is not so long as the anterior one, the occurrence of a row of spherules on that side along which runs the undulating membrane, and, lastly, the fact that this form is not a hæmal parasite point to its association with Trypanophis. Léger is inclined to do away with this latter genus altogether, but this seems premature, until the life-cycle is better known.

Genus Trypanosoma, Gruby, 1843. Principal synonyms[1]: Undulina, Lank., 1871; Herpetomonas, Kent, 1880 (only in part, since the type sp. is H. muscæ-domesticæ); Paramœcioides, Grassi, 1881; Hæmatomonas, Mitrophan, 1883; Trypanomonas, Danil, 1885 (for young forms). There is no anterior flagellum. The point of insertion of the attached (posterior) flagellum into the body, and, consequently, the commencement of the undulating membrane may be

[1] The synonymy of this genus and of its different species is well discussed by Laveran and Mesnil (**47, 56**), and Salmon and Stiles (**96**), from whom most of the information here given is compiled. An explanatory note may be added with regard to Herpetomonas, since until recently the parasite of rats (T. lewisi) was called by this name. This parasite was referred by Kent (**27**) to his new genus Herpetomonas, founded for H. muscæ-domesticæ (Burnett), the genus Trypanosoma being reserved for the frog parasite and for Eberth's form from the cæcum of birds, which Kent termed T. eberthi. Later, when H. lewisi became better known, Senn (**100**) revised the diagnostic characters of the genus. As a result, the principal feature left by which to distinguish between Herpetomonas and Trypanosoma was the presence in the former of a thickened external border to the undulating membrane, of flagellar nature, which was supposed to be absent in Trypanosoma. With Laveran and Mesnil's reinvestigation (**45**) of T. rotatorium (T. sanguinis), this difference was found not to exist, and therefore the older name prevails. The name Herpetomonas is retained for the original type (muscæ-domesticæ), which has no undulating membrane.

almost anywhere in the anterior half of the body, but is usually near the extremity.[1]

The question of the sub-classification of this genus is one of much difficulty. One is confronted by a great number of forms, several of which have no very definite or constant differential feature by which to characterise them, such as in the case of the Gregarines and Coccidia, for example, is naturally afforded by the spores. This is largely owing to the fact that so little is yet known of the life-history of most

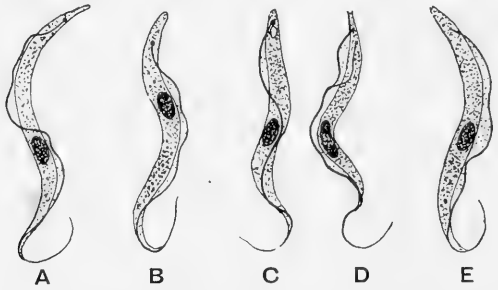


Fig. 42.—Different Mammalian Trypanosomes to show uniformity in size and shape. A, Trypanosoma evansi; B, T. brucii; C, D, T. equiperdum; E, T. equinum, × 1500. (After L. and M.)

that reliance has to be placed almost entirely upon the adult size and form in any endeavour to classify the parasites on a morphological basis. Now it has been already stated that, in many cases, the variation in this respect is very slight, and, in addition, in several instances a particular form may

[1] The type-species is T. rotatorium (Mayer) of frogs. At present, unfortunately, this parasite cannot with certainty be included in the above diagnosis, owing to its unusual shape, position of kinetonucleus, etc. The occurrence, however, of an allied form in Hyla, which is evidently intermediate between T. rotatorium and the more typical, fusiform species of the genus, strongly points to the agreement of the former (T. rotatorium) with the majority of Trypanosomes in belonging to the Heteromastigine section. At any rate, it would be premature to separate the Mammalian and Piscine forms under a new generic name; the confusion which this course would entail is to be avoided until it is shown to be necessary.

itself vary almost as much at different times and under different conditions (see under Morphology). For the present, at any rate, a very useful aid towards distinguishing different species is furnished by the biological relations of the parasites. Speaking generally, it may be assumed that here, as is known to be usually the case in the Sporozoa, a particular species is restricted either to one particular host or, at most, to a few closely allied ones. The greatest difficulty arises in considering the Mammalian forms, many of which have never been observed in the true natural hosts, but only in various "foreign" animals, for which they are all more or less pathogenic. The immunisation experiments of Laveran and Mesnil (see above, p. 176) render it most likely, however, that such forms are, at all events, distinct varieties, and, quite probably, distinct species.

On the other hand, having regard to the gross or extreme differences in form which are met with (cf. T. rotatorium and T. inopinatum) one might be tempted to arrange the different species into sub-genera, grouping together, for example, parasites with a blunt or rounded anterior extremity, or those with a filiform or attenuated one, and, again, separating forms with no free prolongation of the flagellum posteriorly, from those which have one. Bearing in mind, however, the great polymorphism which is known to occur, any such arrangement would be quite arbitrary and certainly premature in the existing state of our knowledge. It may very well be that when more life-histories come to be revealed some of the forms at present included in the genus Trypanosoma will have to be transferred to one of the other genera of Hæmoflagellates or placed in a new one.

In this article the writer considers it best to arrange the different species under the different classes of Vertebrate hosts in which they are parasitic; this will, at any rate, facilitate reference to any particular form.

Mammalian Forms.

(A) Non-pathogenic.—T. lewisi, (Kent), 1879. (Syn. Herpetomonas l., Kent.) Length 24–25 μ, breadth $1\frac{1}{2}$–$1\frac{3}{4}$ μ.[1] This species (figs. 16 A, 27) is characterised by its thin, drawn out, and pointed anterior extremity, and also by the position of the trophonucleus in the posterior half or third of the body. The cytoplasm is very clear and free from granules. Hosts: (V.) Mus decumanus, M. rattus, and M. rufescens; (I.) the life-cycle can certainly be undergone in the rat-louse, Hæmatopinus spinulosus, which is therefore a true alternate host. Prowazek thinks, however, that fleas may also serve the purpose of transmission. A closely allied species (by some considered as only a race or variety of T. lewisi) is found in Cricetus arvalis (frumentarius?), the hamster; this form is not inoculable into rats, and, conversely, T. lewisi is not capable of living in the hamster.

T. duttoni,[2] Thiroux, 1905. Length 25—30 μ, of free flagellum $6\frac{1}{2}$—10 μ; breadth $2\frac{1}{2}$ μ. This parasite resembles T. lewisi, and also, in a general fashion, the Trypanosomes of other small Rodents mentioned below. (V.) host: Mus musculus. This form is not inoculable into rats, and is therefore distinct from T. lewisi. It was found in mice in Senegal, and Thiroux wonders whether it is identical with the parasite described as a Herpetomonad by Dutton and Todd (**21**), also from Senegambian mice. In the latter, however, no undulating membrane was observed, and hence it is doubtful whether it was really a Trypanosome.

In addition to the above forms, Trypanosomes have been casually observed in various other Rodents, but as yet they are unnamed and not much is known about them.

Petrie (**82**) recently found three rabbits spontaneously infected with a Trypanosome (fig. 43 A). The parasites were quite numerous in the blood, and, in one case, present for six months at least, without causing any ill-effects. This form appears to be similar, as regards size and morphology, to T. lewisi, but most likely belongs to a distinct species, since the latter form is not inoculable to rabbits.

Another Trypanosome (fig. 43 B) has been recently observed by Donovan in an Indian squirrel (Sciurus palmarum). Its total length was 18—20 μ,

[1] The dimensions given are intended to indicate the average size of the adult parasite, but, as above said, they can only be considered approximate. Unless otherwise stated, the length is inclusive of the flagellum.

[2] The writer commenced this article with the intention of having a figure of every species; the number of those known has increased so greatly, however, while it has been in progress, that this has proved impracticable, and hence some of the most recently described species are unfigured.

distinctly smaller, that is, than T. lewisi. This is probably also a distinct species.

Galli-Valerio (**24**), again, has remarked upon the presence of a Trypanosome-like Flagellate, about 22 μ in length, in the blood of a dormouse Myoxus avellanarius).[1]

An earlier observation is that of Chalachnikow (**15 a**) of an elongated form, 30—40 μ by little more than 1 μ, from Russian marmots (Spermophilus guttatus and S. musivus).

T. pestanai, Bettencourt and França, 1905. Length given as 30—32 (somewhat uncertainly because the parasites are generally rolled up); of free flagellum 4·5—5 μ. This is a relatively wide form, the breadth being 5—6½ μ. Anterior end long and fine; kinetonucleus some distance from the extremity. (V.) host: Meles taxus, badger.

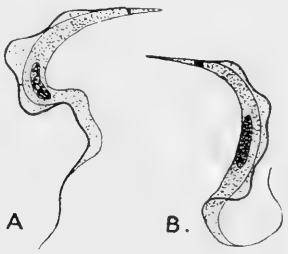


Fig. 43.—A, Petrie's Trypanosome from a rabbit; B, Donovan's Trypanosome from a squirrel. × 2000. (After L. and M.)

Trypanosomes appear to be not uncommon in bats. Dionisi[2] first reported the occurrence of the parasites in Miniopterus schreibersii; Durham[3] noticed some in a Phyllostoma in Brazil—to be exact, in the blood contained in the stomach of a Stegomyia which had fed on the bat; and Donovan (vide L. and M. [**56**]) mentions having observed a large form in Pteropus medius, in Madras. These were probably all distinct species, but no account of them has yet been given. During the last year or two, however, somewhat brief descriptions of certain species are to hand.

T. nicolleorum, Sergent E. and E., 1905. Length 20—24 μ, of free flagellum 4—5 μ; breadth 1½ μ. Anterior end tapering and pointed. The

[1] Brumpt appears to have named a Trypanosome from the garden dormouse (Myoxus nitela), T. blanchardi (vide Brumpt and Lebailly [**11**]); the writer can, however, find no reference to the paper describing this form.

[2] Dionisi, 'Atti Soc. Studi Malaria,' i, p. 145, 1899.

[3] Durham, 'Rep. Yellow Fever Expedition, Para,' Liverpool Sch. Trop Med., mem. 7, 1902, p. 79.

kinetonucleus is some distance from the extremity. (V.) hosts: Myotis muruius, and Vespertilio kuhli, Algeria and Tunis. In certain of the Vespertilio kuhli examined much larger forms were seen, 25—30 μ by 6 μ, for which the name T. vespertilionis is proposed provisionally. The possibility is recognised, however, that these forms may be individuals about to divide [or perhaps sexual (female) individuals?]. Petrie (**83**) has recently observed Trypanosomes in Pipistrellus pipistrellus, in Hertfordshire, which he thinks may have been T. nicolleorum, although they were shorter (apparently only about 16 μ long).

T. dionisii, Bettencourt and Franca, 1905. Full dimensions not given; length of free flagellum 6·5 μ. Kinetonucleus quite at anterior extremity. Hosts: Vesperugo pipistrellus, V. serotinus, and V. nattereri, Portugal.

B. Pathogenic forms.—We come next to the so-called pathogenic group. These parasites have been "successfully" inoculated into many and various Mammalia, which cannot, however, in the majority of cases be regarded as natural, tolerant hosts. In dealing with these disease-causing forms, it is obvious that the more narrowly the original source of the parasite is defined the closer do we get to the true host or hosts. Similarly with the Invertebrate hosts, it is sometimes rather difficult to be certain which is the natural one for the species concerned, for experiment has shown that a biting-fly, other, in all probability than the true host, can, as it were, accidentally convey the parasites, if, after feeding on an infected animal, it is allowed to bite a fresh one within a limited time. One helpful factor in this determination is the coincidence of the zone of a particular Insect[1] with that of any disease.

T. brucii, Plimmer and Bradford, 1899. (Syn. T. brucei, Buff. and Sch., followed by L. and M. and others.) Length 28–30 μ, breadth $1\frac{1}{2}$–$2\frac{1}{2}$ μ. The anterior end is usually bluntly rounded or truncated (figs. 42 B, 44). The cytoplasm often contains in the posterior half large deeply-staining granules. Hosts: (V.) probably Antilopidæ, such as Catoblepas gnu, Strepsiceros capensis ("Koodoo"), and Tragelaphus scriptus sylvaticus ("Bushbuck"), perhaps also buffaloes; (I.) Glossina morsitans and G. pallidipes, Tsetse-flies. The cause of Nagana or Tsetse-fly disease in South and South-east Africa among cattle, horses, etc. Most domestic animals are susceptible.

Other trypanosomoses, more or less allied to Nagana, and perhaps caused by different varieties or races of the same parasite, have been observed in German East Africa and Togoland among cattle, horses, and other animals.

Again, the disease known as "Aino," which occurs in Somaliland among

[1] A very useful map showing the zones of distribution of the different species of Glossina is given by Austen, E., Rep. S. S. Comm. Roy. Soc., 6, p. 278, 1905.

dromedaries, and which appears to be transmitted by another Tsetse-fly, namely, G. longipennis (locally termed the "Aino"), is probably also a variety of Nagana (see Brumpt [**9 a**]).

T. evansi (Steel, 1885). (Syn. Spirochæta evansi, Steel.) Length about 25 μ, breadth about $1\frac{1}{2}$ μ. This parasite (figs. 42 A, 45) is, morphologically, very like T. brucii. It is generally rather more slender than that form, and the anterior end rather more tapering and usually acutely conical. Moreover, the free part of the flagellum is slightly longer in T. evansi, and the cytoplasm lacks the prominent granules of T. brucii. T. evansi also performs greater and more rapid movements of displacement than the Nagana parasite. Natural hosts uncertain; (V.) perhaps to

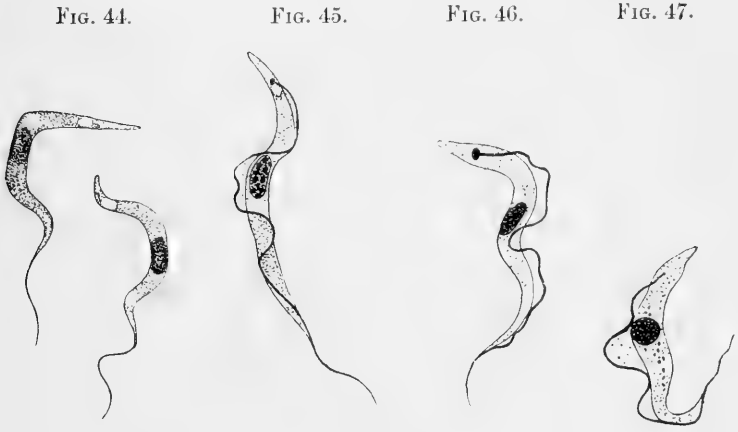


FIG. 44. FIG. 45. FIG. 46. FIG. 47.

FIG. 44.—T. brucii (after Bradf. and Plim.).
FIG. 45.—T. evansi. × 2000. (Original, from a preparation of the blood of a mule, kindly lent by Mr. Plimmer.)
FIG. 46.—T. equiperdum. (After Lignières.)
FIG. 47.—T. equinum. (After Lignières.)

be found among indigenous Bovidæ, and (I.) is probably a Tabanus, T. tropicus and T. lineola having been suggested (Rogers [**92, 92 a**]); in Mauritius the epidemic is thought to have been spread by Stomoxys nigra. The cause of Surra in Indo-Burmah, which is particularly dangerous to Equidæ. The disease is less fatal to cattle than Nagana is. Various other animals also liable. The malady has been recently imported into Mauritius and the Philippines.

The illness known as "Mbori," occurring among dromedaries coming from the Sahara into the Soudan (Timbuctoo, etc.), which is apparently also conveyed by a Tabanus, is considered both by Vallée and Panisset

(**117 a**) and Laveran and Mesnil (**54 a**) to be a milder form of Surra, the parasite which causes it being a "race" of T. evansi.

T. equiperdum, Doflein, 1901. (Syn. T. rougeti, L. and M.) Length 25–28 μ, breadth $1\frac{1}{2}$–2 μ; slightly smaller than T. brucii. Also differs from that species in not having prominent grains in the cytoplasm. The kinetonucleus is relatively large and well-developed (figs. 42 C and D, 46). This form has been the least studied of the better known pathogenic ones. The cause of Dourine in horses in Algeria and in certain parts of Southern Europe (chiefly the Mediterranean littoral). The infection is transmitted (invariably?) during the act of coitus, and this explains why mules and geldings are exempt. In the case of roving (wild) asses the illness is usually slight, and the parasites are apparently more or less latent, but whether these animals constitute the true Vertebrate host or not cannot at present be said. Moreover, it is not yet certain whether the parasites in the natural conditions have any alternate Insectan host into which they must pass at intervals in order to complete the life-cycle. (See also p. 280, footnote.)

There appear to be one or two other varieties of trypanosomosis in Northern Africa (Algeria). Thus, Sergent (**101 a**) announces a malady of dromedaries, which is very similar to Mbori; and Rennes (**91**), and Rouget (**93, 93 a**) and others seem unable to decide whether there is a trypanosomosis of horses, distinct from Dourine.

T. equinum, Voges corr., 1902. (Syn. T. elmassiani, Lignières.) Length 22–25 μ, breadth $1\frac{1}{2}$–2 μ. A morphological character which sharply distinguishes this species from the rest—and which may ultimately prove to be of more than specific importance—is the very minute size of the kinetonucleus (figs. 42 E, 47). Cytoplasmic grains present, but not so numerous as in T. brucii. In shape and details of form this parasite much resembles T. evansi. The Vertebrate host is most probably Hydrochœrus capybara; the transmitting Insect perhaps a Tabanid (many workers have considered a Stomoxys, either S. nebulosa or S. calcitrans, to be the carrier, but Lignières and Elmassian and Migone think otherwise). T. equinum causes the destructive disease of horses known as Mal de Caderas in Brazil, Argentina, and Central South America.

T. gambiense, Dutt., 1902. (Syn. T. ugandense,[1] Castellani, T. castellanii, Kruse.) Length 21–23 μ, breadth $1\frac{1}{2}$–2 μ. This species (fig. 48) is, according to its average size, one of the smallest yet found. The parasites in the blood frequently exhibit slight morphological differences from those in the cerebro-spinal fluid. The former are somewhat longer and more slender,

[1] The specific name ugandense was the one first given to the parasite found in sleeping-sickness cases, Dutton's name having been previously conferred on the form originally found in cases of human trypanosomosis (Trypanosoma fever). See next page.

and, correlated with this, the kinetonucleus is situated farther from the anterior end than it is in the more stumpy forms. Investigators are, however, at one in considering that these differences are due merely to the different habitat, since both varieties, when inoculated into other animals, give rise to the same kind of form. The cerebro-spinal fluid would appear to be less favourable a medium than the blood. T. gambiense is the cause of human trypanosomosis in West and Central Africa, the earlier stages of which, when the parasites are confined to the blood, are known as Trypanosoma-fever, the later ones, after they have penetrated into the cerebro-spinal canal,[1] constituting the deadly malady of sleeping sickness.

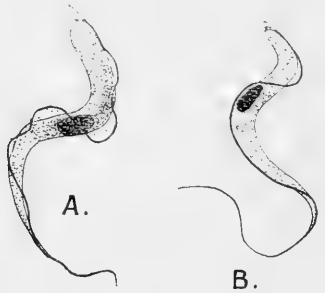


FIG. 48.—T. gambiense, from the blood. (A, after Bruce and Nabarro; B, after Castellani.)

It seems most probable that the original Vertebrate source or "reservoir" of this parasite is some indigenous tribe or race of natives in whose blood—and in all probability only in their blood—the Trypanosomes live, as it were, normally, parasite and host having become mutually tolerant.[2] Whether any animal other than man is also a natural host, is quite unknown. If

[1] Plimmer (**85**) has lately expressed the view that the forms met with in Trypanosoma-fever and sleeping-sickness are distinct species, basing his opinion on the behaviour and appearance of the parasites after being inoculated into the same host (rat), and also on the symptoms presented by the latter in the two cases. The weight of evidence at present, however, is decidedly against this view; see the Reports of the S. S. Comm., also Brumpt and Wurtz (**13**), Laveran (**38**), Thomas and Linton (**116**), and several other workers.

[2] For various factors which have helped to bring unaccustomed, unadapted tribes and individuals into the zone of the parasite and have thus led to the spread of the infection and its invasion of fresh regions in the character of a fatal disease, the reader is referred to Lankester's instructive article (**31**).

there is no other host among the Primates it is improbable that there is any other Mammalian one for this species. The Invertebrate host is, undoubtedly, Glossina palpalis; the possibility of this being so appears to have been first suggested by Brumpt (**9**). The distribution of this Tsetse-fly narrowly coincides with that of the disease, and where there is no fly, sleeping-sickness is not prevalent. It has not yet been proved whether or no other species of Glossina also naturally transmit the parasites.

Nabarro and Grieg (S. S. Rep., No. 5) mention, in addition, the occurrence of three or four cases of trypanosomosis among diseased animals which came under their observation in Uganda. The parasites occurred as follows:—(a) in sick transport oxen in Entebbe, which came originally from East Africa (the illness caused being locally known as "Mukebi"); (b) in a herd of diseased cattle at Jinga, Busoga ("Sutoko"); (c) in an English dog which had contracted the disease while with the Abyssinian Boundary Commission; and (d) in a sick mule at Entebbe. From the observations and animal experiments by these workers, and subsequently by Grieg and Gray (**24 b**), the conclusions arrived at are as follows:—the Trypanosomes concerned are probably all distinct from T. gambiense; (a) the parasite in sick oxen at Entebbe ("Mukebi") is a distinct species; (b) the "Jinga" Trypanosome is most probably a variety of T. brucii, the illness being an acute form of Nagana; (c) and (d) the "Abyssinian Boundary" parasites and the Trypanosomes found in a sick mule appear to be identical, and constitute probably another distinct species.

T. dimorphon, Dutt. and Todd, 1904. As implied by the specific name, more than one "type" of this parasite is usually distinguished. One is small and tadpole-like (fig. 49, I *b*, II *a*), the other long, fusiform, and more resembling an ordinary Trypanosome (I *a*, II *d*). A third variety, wide and stumpy (II *b* and *c*) is also distinguished by Dutton and Todd, but Laveran and Mesnil (**54**) consider that this only represents an enlarged tadpole form about to divide. The average dimensions are: of the tadpole variety, length 10–15 μ by ·7–1·5 μ; of the elongated one, length about 25 μ by $1\frac{1}{2}$–2 μ. The exact relations of these two kinds of form to one another have not yet been ascertained. Although Laveran and Mesnil describe and figure a series of intermediate stages (I), they do not think that the smaller type grows or passes into the longer one, basing this view on the ground that each is capable of reproducing its like by equal binary fission.[1] In one important respect the accounts of Laveran and Mesnil and of Dutton and Todd are at variance. The first-named authors maintain that none of the parasites have any free continuation of the flagellum, this terminating, in all cases, at the posterior end of the body, which is here

[1] It is quite possible, however, that these large parasites possess some other mode of division in addition, by means of which they give rise to a fresh succession of tadpole forms.

attenuated and filiform (fig. 49, I *a*). On their part, Dutton and Todd figure a distinct free portion of the flagellum in all their types (II), it being short in the tadpole-like and stumpy forms, but long and well-developed in the large (adult?) ones. Reconciliation between these two views must await further research. T. dimorphon causes marked trypanosomosis of horses in Senegambia; these animals are few in number in that colony and mostly imported. The short forms are met with in the earlier stages

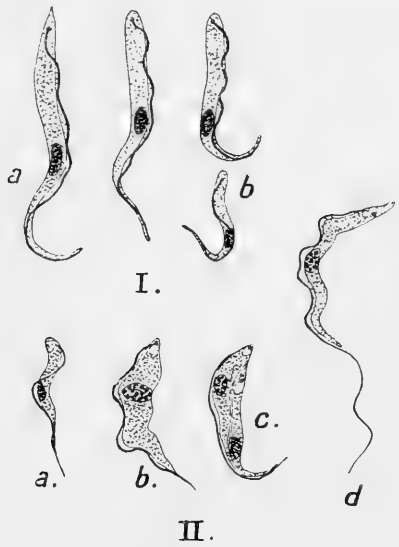


Fig. 49.—T. dimorphon. I, after Lav. and Mesn.; *a*, long form, *b*, short form. II, after Dutton and Todd; *a*, tadpole-form, *b*, stumpy form (from a rat), *c*, stumpy form dividing, *d*, long form. × 1500.

of the malady, the long ones occurring later. The "natural" Vertebrate host is not known. The transmitting Insect is, possibly, Glossina palpalis, which is abundant in the district, but it should be added that Dutton and Todd's endeavours to artificially transmit the parasites to rats by this means were unsuccessful.

T. theileri, Laveran,[1] and T. transvaaliense, Laveran, 1902. These two forms are considered together, since it is uncertain whether they do not really both belong to one species, which would bear the former name. T. theileri (fig. 50 *a* and *b*) is the largest known Mammalian Trypano-

[1] Laveran (32) named the parasite on the 3rd March, 1902, and Bruce (7) on the 8th of the same month.

some; medium length about 50 μ, breadth $3\frac{1}{2}$—4 μ. It is thus readily distinguished by its size from the other Mammalian forms, although it agrees with most of them in being of the typical fusiform shape. It appears to be confined to Bovidæ, occurring especially in the Transvaal, and causing the disease of cattle known as "Galziekté" or bile-sickness. Herds of cattle imported from Argentina, Texas, etc., are particularly liable to suffer from it. Theiler, who discovered the parasites, thinks that they are transmitted by a biting fly, Hippobosca rufipes. Another species, H. maculata, recently imported with cavalry from India, may also aid in spreading the disease. T. transvaaliense (fig. 50 *c*—*e*) averages about 30 μ in length by 4 μ in width. From the dimensions given (though not, apparently, from the figures) it would appear to be rather wider than T. theileri. Its distinguishing morphological feature, however, is the position of the kineto-

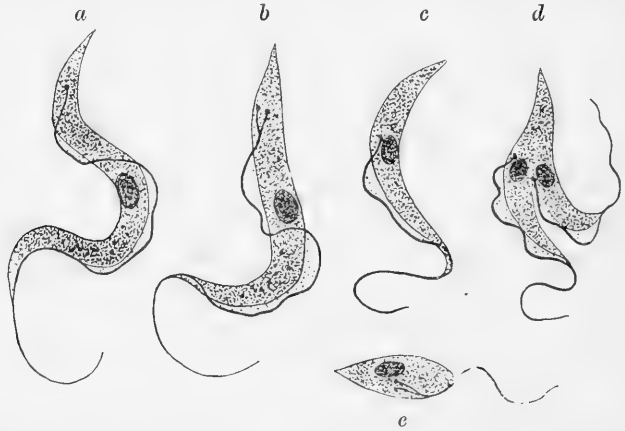


Fig. 50.—*a* and *b*, T. theileri; *c*—*e*, T. transvaaliense. *e* is a small form dividing. × 1250. (After L. and M.)

nucleus; this organella is rod-like and situated in contact with the tropho-nucleus. Correlated with this, the undulating membrane is short and poorly-developed. Moreover, this variety is quite capable of division (fig. 50 *d*), even in the case of very young individuals (*e*). Nevertheless, Theiler has recently observed transitional forms, intermediate between T. theileri and T. transvaaliense, with a varying distance between the tropho- and kineto-nucleus. This author has also found that, on inoculating the latter variety into Bovidæ, the former (T. theileri) is produced in time as well. These facts would seem to show that the two forms are not really independent and distinct species.

Forms Parasitic in Avian Hosts.

T. ziemanni (Lav.). (Synn. Spirochæta z. [Lav.], Schaud., "Hæmamœba" z. Lav.; the "Leucocytozoon" of Danil.). Schaudinn, who has fully described the life-history of this form (see above, p. 242), does not give the actual dimensions. According to Laveran (**37**) the size of the female gametocytes in the resting, intra-cellular condition, varies from 12—21 μ by 4—7 μ, the male ones being slightly less. The length of the microgametes ("flagella") is from 20—25 μ. The ordinary indifferent individuals are characterised by their extremely Spirochæta-like facies and their habit of remaining united together in pairs after division. Hosts: (V.) Athene noctua, Little Owl, also Syrnium aluco (see below, under

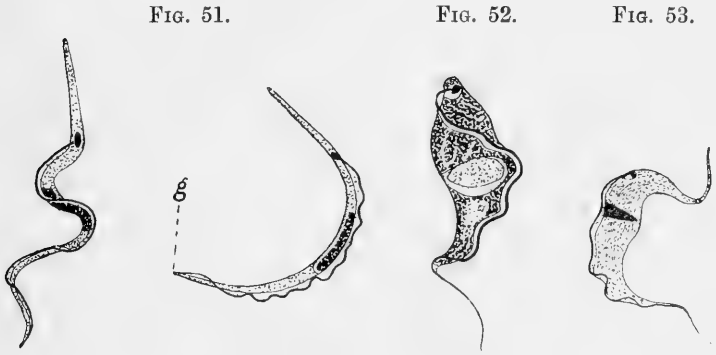


Fig. 51. Fig. 52. Fig. 53.

Fig. 51.—T. johnstoni. g = deeply-staining granule at distal extremity of flagellum. × 1500. (After Dutton and Todd.)

Fig. 52.—T. sp., from Senegambian birds. × 1500. (After D. and T.)

Fig. 53.—T. sp., from Indian birds (Hanna's T.). (After Hanna.)

T. avium) and "a little white owl from Cameroun" (Ziemann [**121**]), in which sexual forms were seen; (I.) Culex pipiens.

T. johnstoni, Dutton and Todd, 1903. Length 36—38 μ, width 1·4 to 1·6 μ. This parasite resembles T. ziemanni in shape, being also markedly spirochætiform (fig. 51). The undulating membrane is narrow and poorly developed. The most interesting morphological point is the absence of any free continuation of the flagellum. This terminates abruptly, at the end of the body, in a distinct, deeply-staining granule (g), which is probably of centrosomic nature. This species was found in small birds

"millet-eaters" (Estrelda estreld), in Senegambia. (I.) host probably (for this and other Avian forms) some species of gnat or mosquito.

Another form, not named, is described by the same authors from the same host and also from another (Crithagra). The birds were more frequently infected with this parasite than with T. johnstoni. This Trypanosome represents the other extreme of type, being relatively very wide and stumpy (fig. 52). Its total length is about 32 μ, and its greatest width 8 μ. The free portion of the flagellum is from 10—12 μ long. The trophonucleus is placed transversely across the body. The kinetonucleus is very close to the anterior end, and immediately behind it is a vacuole.

Trypanosomes from Indian Birds.—Hanna (**26**) has recently noted two forms, one parasitic in the crow, the other in the pigeon. The former

Fig. 54. Fig. 55.

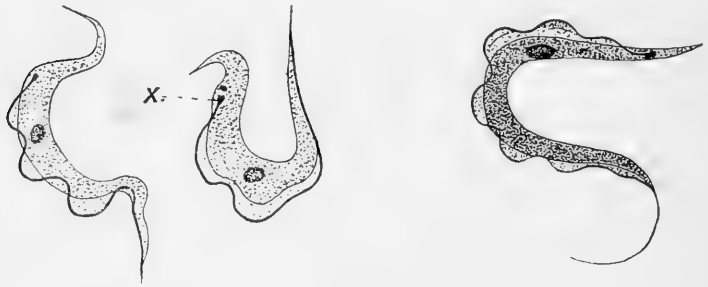


Fig. 54.—T. paddæ, Thiroux. At *x* the base of the flagellum is separate from the kinetonucleus, and thickened prior to division. × 1200. (After Thiroux.)

Fig. 55.—T. avium. Here the kinetonucleus is itself divided. (After L. and M.)

is only briefly mentioned, since, owing to the preparation having lost its staining colour, little beyond the general form could be made out. The parasite appears to conform to the usual spindle-like shape. Its size is given as from 40—56 μ by 3—4·8 μ. The other parasite (fig. 53), from the pigeon, is more fully described, and possesses several points of interest. It also is relatively very wide, but not at all stumpy. Its length is from 45—60 μ, breadth 6—8 μ. The posterior part tapers away finely, and there is a free prolongation of the flagellum. The characteristic feature, however, is the long, drawn-out and extremely thread-like anterior end. The kinetonucleus is, correspondingly, a considerable distance from the anterior end, and behind it is a vacuole. In this case, also, the trophonucleus stretches transversely across the body, and is of a curious triangular shape. The surface of the body exhibits longitudinal striations. This Trypanosome is most probably quite distinct from Dutton and Todd's parasite.

T. paddæ, Lav. and Mesn., 1904. Length 30–40 μ, breadth 5–7 μ. From the description this form (fig. 54) appears to be of a very similar type to the last. The undulating membrane is perhaps rather better developed, however, and the free part of the flagellum is shorter. The anterior end is usually very attenuated, sometimes so much so that "it appears as though there was a flagellum at each extremity." The cytoplasm shows fine longitudinal striations. (V.) Host: Padda oryzivora (from the Paris markets), in whose blood also Halteridium danilewskyi is often found.[1]

T. polyplectrum, Vassal, 1905. Length 46 μ, of free flagellum 12 μ; breadth 5 μ. Anterior end extremely attenuated and flagelliform, even more pronouncedly than in T. paddae. From a pheasant (Polyplectrum germani) in Annam.

T. avium, Danil., 1885 (Laveran emend., 1903). Length 35—45 μ, inclusive of flagellum. Body elongated and fusiform in shape (figs. 16 F, 55); anterior end tapering. Undulating membrane well-developed, with a longitudinal striation or fold running in it. Laveran found the parasites in Syrnium aluco, Tawny Owl. Danilewsky observed Trypanosomes in various phases (fig. 3) in owls (sp. indet.) and also in "roller-birds" (Coracias garrula). Their size is given as from 20—60 μ by 7—8 μ. Laveran (**36**) thinks it not unlikely that the Trypanosome in the rollers is a distinct species. It is interesting to note that Laveran mentions the occurrence, in the Tawny Owl which contained T. avium, of "Hæmamœba" ziemanni and "H." (Halteridium) danilewskyi. Is it possible that the latter Hæmatozoan represents the corresponding phase of T. avium which the former does of T. ziemanni?

Novy and McNeal's work (**81**) on the Trypanosomes of birds is most difficult of exact estimation from a systematic standpoint for the reasons already given (pp. 281, 282). In only a single instance were their numerous cultures of the parasites successful in giving rise to Trypanosomes, when injected into the blood. This, certainly, does not point to the organisms having been at the time in a very healthy condition; on the contrary, some of the authors' excellent photomicrographs strongly suggest abnormal and involuted phases (cf. their pl. 7)

Novy and McNeal consider they have investigated at least four different species. A parasite identified as Danilewsky's T. avium is most frequent. Two types are recognised (corresponding to that worker's majus and minus). One is of less frequent occurrence, and measures 50 μ by 6 μ, the flagellum being 15—20 μ in addition; the other (more common) is 20 μ by 3—5 μ, the flagellum 10 μ extra. This smaller variety is thought

[1] According to Thiroux this association is purely accidental, there being apparently no connection between these two parasites (see, however, under T. avium.

to be the same as Laveran's form in Syrnium aluco. In this species, moreover, the authors would include both Dutton and Todd's Senegambian Trypanosome and Hanna's Indian one, although keeping the very similar type T. paddæ distinct. Cultures produced two forms of the parasites, wide spindle-like ones and thin spirochætiform ones with very short flagellum.[1] The hosts from which this parasite was obtained were: Agelaius phœniceus, Colaptus auratus, Cyanocitta cristata, Icterus galbula, Melospiza fasciata, Merula migratoria, Passer domesticus, Sialia sialis, and Zenaidura macroura.

T. mesnili, Novy and McNeal, 1905. Length 50 μ; breadth 8 μ. Considered to be distinct by reason of its large size and peculiar shape and behaviour in cultures. The latter grow very rapidly, and show two types of cells: small ones (multiplication rosettes) very short and wide (10—12 μ by 6 μ): and larger ones (20—25 μ × 4—6 μ) corresponding to the spirochætiform type above, with, however, a very long flagellum. It is the latter parasites which form the typical agglomeration rosettes. This Trypanosome was obtained from Buteo lineatus.

T. laverani, Novy and McNeal, 1905. In size this parasite agrees with the smaller variety of T. avium, being only a trifle wider. Its specific distinction is based on cultural forms, which grow very slowly, and do not give rise to such markedly diverse types as in the above cases. Host: Spinus tristis.

A form from Cyanocitta cristata and Scolephagus carolinus is regarded as distinct, and others from Dryobates crilosus, Harporhynchus rufus and Troglodytis ædon gave rise to various subtypes and strains, which might or might not be distinct.

It only remains to add that Trypanosomes have also been observed in other Avian hosts, but, for the most part, their occurrence only is mentioned. Thus, Sergent, E. and E. (**102**), have noticed the parasites in various Algerian birds, e. g. goldfinch (Fringilla (Carduelis) carduelis), linnet (Sylvia atricapilla), and swallows. Donovan informs Laveran and Mesnil (**56**) that he has seen Trypanosomes in the blood of an owl (Athene brama) in Madras; and the same worker has also observed them in Milvus govinda (Indian Kite), the parasites being 34 μ × 3—3½ μ, free flagellum 16 μ (see Thiroux [**114**]). Lastly, Ziemann[2] has found a Trypanosome in a chaffinch (Fringilla cœlebs).

---

[1] These forms strongly recall T. johnstoni; although Novy and McNeal think that parasite, again, is distinct.

[2] 'Ueber Malaria und andere Blutparasiten,' Jena, 1898 (p. 106).

Reptilian Forms.

Trypanosomes have been but rarely found, so far, in Reptiles. The only one described and figured is—

T. damoniæ, Lav. and Mesn., 1902. Length 32 μ, breadth 4 μ. The body (fig. 16 J) is fusiform and fairly wide in the middle, but in general structure presents nothing remarkable. The parasite often appears rolled up on itself. The chromatic grains in the cytoplasm are more or less uniformly distributed throughout the body, the posterior end being, if anything, freer.

In addition, Dutton and Todd (21) mention having observed Trypanosomes at rare intervals in the blood of tortoises, and Gehrke [1] has noticed one in a gecko.

Forms parasitic in Amphibian hosts.

T. rotatorium (Mayer). (Synn. Amœba rotatoria and Paramœcium costatum or loricatum,[2] Mayer, July, 1843; Trypanosoma sanguinis, Gruby, Nov., 1843; Undulina ranarum, Lankester, 1871). —The great variation in form exhibited by this parasite has been already discussed. Two principal types are distinguished, one having the surface of the body thrown into parallel ridges, which run either longitudinally or with a slightly spiral course, the other having a smooth regular surface. These two types and the manifold varieties of shape are best realised by a comparison of figs. 17 A and B, 56.

In size, the parasites vary from 40—60 μ in length, by from 5—40 μ in breadth; the two dimensions vary more or less inversely, the width being greatest when the parasites are relatively short, which gives them often an extremely broad and stumpy appearance. Correspondingly, the anterior end may be either drawn-out and finely pointed, in the comparatively narrow forms, or conical, obtuse, or even blunt and rounded, in the stumpy ones. The undulating membrane is very well developed and thrown into numerous folds. The free portion of the flagellum is usually comparatively short. The kinetonucleus is generally situated some distance from the non-flagellate or anterior extremity,[3] and may be quite close to the trophonucleus (figs. 17 B, 56 A); sometimes, however, it is fairly

[1] 'Deutsche med. Wochenschrift,' 1903, p. 402.

[2] From the drawings given, "Amœba rotatoria" is almost certainly a Trypanosome, the other organism much more doubtfully so; hence the first-named specific designation.

[3] With regard to the bearing of this point upon the correct generic position of this form see above, p. 288.

near the anterior end. (V) hosts: Rana esculenta, R. temporaria, R. trinodis (?), and Bufo viridis. (I) host, probably a leech (possibly an Ixodes?[1]).

The Trypanosome (fig. 57), unnamed, but perhaps a distinct species, which Laveran and Mesnil (56) figure from Hyla arborea, is of interest, since, while of the voluminous T. rotatorium type, it has the kinetonucleus close to the anterior end, and this occupies, in respect of its shape, a position midway between the tapering and the bluntly-obtuse forms of that parasite. The length of this Trypanosome is given as 75 μ, its breadth 7 μ.

Dutton and Todd (l. c.) describe two Trypanosomes, both characterised by

FIG. 56. FIG. 57.

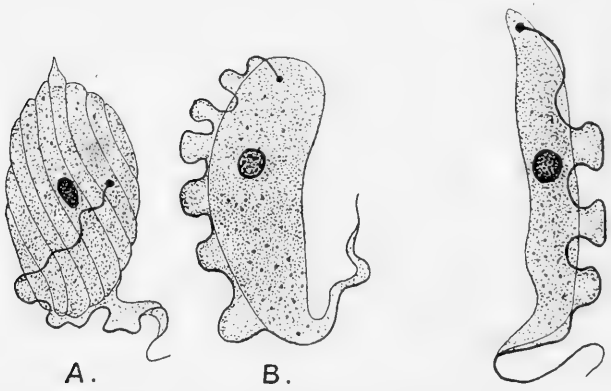


FIG. 56.—T. rotatorium (Mayer). A, ribbed form; B, smooth form. × 1000 (about). (After L. and M.)
FIG. 57.—T. sp., from Hyla arborea. (After L. and M.)

their great length, from frogs (sp. incert.) in Gambia. The authors give each form provisionally a new specific name, T. mega and T. karyozeukton respectively. Both parasites strongly resemble the fusiform type of T. rotatorium (which was also encountered in the frogs of that district), so much so that Laveran and Mesnil consider them to be hardly specifically distinct. Although in these new forms the anterior end is very long and proboscis-like, these authors say that they have observed individuals of T. rotatorium with an equally thin and extended anterior extremity. While the parasites are undoubtedly closely allied to that

[1] Durham (l.c. [fn. 3, p. 291]) considers that an Ixodes is the Invertebrate host of a Lankesterella (Drepanidium) of toads; see, however, T. inopinatum, below.

species, they exhibit certain cytological differences, and T. karyozeukton possesses one uncommon morphological feature which well differentiates it. It is, therefore, considered preferable to retain the distinctive names for both, pending the further investigation of their exact degree of relationship to T. rotatorium.

T. mega, Dutton and Todd, 1903. Length from 82—87 μ, the free flagellum being from 10—15 μ; the breadth, in the widest part, is about 8 μ. The kinetonucleus is immediately in front of the trophonucleus, about one third of the length from the anterior end. The longitudinal ridges and furrows are well marked, but do not extend towards the anterior end of the body, as in T. rotatorium (cf. fig. 58), becoming indistinct about opposite the

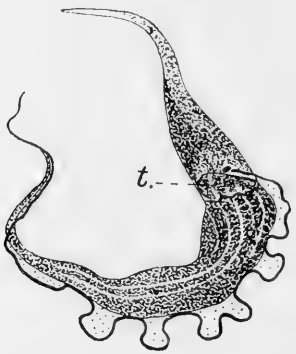


Fig. 58.—T. mega, Dutton and Todd. t = trophonucleus. × 1350. (After D. and T.)

nucleus. Thus, while the posterior two thirds of the cytoplasm appears composed of parallel, alternating darker and lighter bands, the anterior third seems of a spongy and alveolar nature.

T. karyozeukton, Dutt. and Todd, 1903. Length about 82·5 μ, that of the free flagellum being about 15 μ; breadth 6½ μ. The cytoplasm (fig. 17 D) shows the same general division into anterior and posterior portions which characterises the last form. In this species, however, the kinetonucleus is situated about midway between the trophonucleus[1] and the proboscis-like

[1] According to Dutton and Todd the trophonucleus itself is the pale, structureless-looking body seen on the right in the figure, the large chromatic grains in a compact mass being independent. It seems, however, equally probable that the latter form an integral part of the nucleus, the pale appearance being perhaps due to shrinkage.

anterior extremity. A noticeable feature is the presence of a chain of chromatic rodlets (perhaps really a chromatic thread) running from one nuclear body to the other. The loops of the undulating membrane are not so wide as in T. mega.

T. inopinatum, Sergent, 1904. Length 25–30 μ; breadth 3 μ. The shape of this parasite is that of a typical Mammalian or Piscine Trypanosome (fig. 17 c). It is very like T. remaki, being slightly wider than T. lewisi, which, however, it more resembles in having the anterior end finely tapering. The kinetonucleus is well developed, and often stretches transversely across the body, in a rod-like manner. It is usually situated about midway between the trophonucleus and the anterior extremity, but may be nearer either the former or the latter. (V) host, Rana esculenta; (I) host, a leech, Helobdella algira. Billet's very important work on the alternation of hosts of T. inopinatum and the parasite's relation to a Lankesterella is discussed above (p. 251).

T. nelspruitense, Laveran, 1904. This is another very distinct and well characterised form. Average length from 55—60 μ; breadth 3 μ. The free flagellum is extremely long, about 25 μ or more, or almost as long as the body itself. The body is slender and vermiform in shape. The posterior and is relatively short and blunt. The trophonucleus lies well in the anterior half. The cytoplasm in the hinder two thirds of the body is dense and uniformly filled with deeply-staining grains; that in the anterior third is much clearer and faintly-staining, with only a few small grains, and around the nucleus itself there is usually a quite pale zone. This Trypanosome somewhat resembles T. granulosum of fishes. The name of the frog in which the parasite was found is not given.

Forms Parasitic in Piscine Hosts.

These Trypanosomes are, on the whole, very uniform in shape, being typically fusiform and slender. The alternate, Invertebrate host is most probably, in all cases, a leech. This has already been proved by Léger (66) for T. barbatulæ (also for a Trypanoplasma, see above, p. 250); moreover, various workers have remarked on the general occurrence of Ichthyobdellids (Hemibdella, Piscicola, Pontobdella) on the skin of infected fish (vide e.g. L. and M. [51]).

T. remaki, Lav. and Mesn., 1901. This parasite occupies a corresponding position among Piscine forms to that of T. lewisi among Mammalian Trypanosomes. It is very slender, with tapering, pointed extremities, not quite so drawn-out, however, as in T. lewisi. The trophonucleus is in the posterior half of the body, and often shows a large, deeply-staining grain centrally (centrosome?). Laveran and Mesnil distinguish two varieties,

characterised by difference in size.[1] They consider the morphological agreement between the two kinds of form to be, otherwise, too close for them to be assigned to different species, especially since both occur in the same host, namely, Esox lucius, pike. T. remaki, var. parva (fig. 59). Length (medium) about 30 μ, of free flagellum alone 10—12 μ; breadth 1½ μ. Certain individuals may, however, reach a length of 42 μ. The cytoplasm is fairly homogeneous and faintly-staining. T. remaki, var. magna (fig. 17L). Minimum length 45 μ, of which 17—20 μ is for the flagellum; breadth 2—2½ μ. In the largest forms the length may attain 57 μ. The cytoplasm appears more deeply-staining than in the other variety, but, as Laveran and Mesnil point out, this may be partly due to the increased thickness.

Fig. 59. Fig. 60.

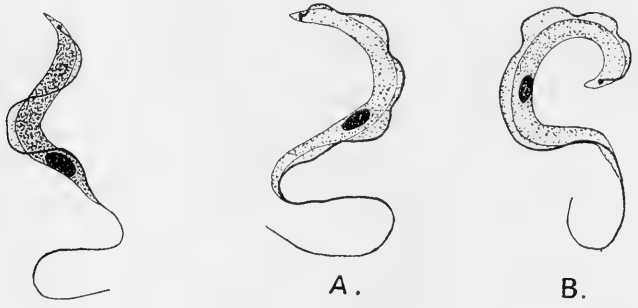


Fig. 59.—T. remaki, var. parva, Lav. and Mesn. × 2000. (After L. and M.)

Fig. 60.—A, T. danilewskyi; B, T. tincæ. × 1500. (After L. and M.)

We have, next, a series of forms, from closely allied hosts, many of which are, doubtless, the same as those noticed by earlier observers, e. g. Chalachnikow and Danilewsky, but which, for the most part, have been left for Laveran and Mesnil and Léger to rediscover and name.

T. danilewskyi, Lav. and Mesn., 1904. Length 35—45 μ by about 3 μ in width. Free portion of flagellum 15—17 μ. The anterior end (fig. 60 A) is shorter and more bluntly conical than in T. remaki. The undulating membrane is rather better developed, with wider folds. The nucleus is rather nearer the posterior than the anterior end. (V.) host, Cyprinus carpio, carp (probably also in the minnow [Phoxinus], Laveran [**39 a**]).

[1] Laveran and Mesnil say they have not noticed any stages which might be regarded as intermediate between the two varieties. Nevertheless, as is apparent from the dimensions given, the maximum size of the smaller variety closely approaches the minimum of the larger one!

T. tincæ, Lav. and Mesn., 1904. Average length 35 μ; width $2\frac{1}{2}$—3 μ. This form (fig. 60 B) is very similar to the last. The free part of the flagellum seems to be rather shorter—judging from the figures, its length not being stated. (V.) host, Tinca tinca, tench.

T. abramis, Lav. and Mesn., 1904. Named, but not diagnosed. The authors say the blood of the specimen in which this Trypanosome was seen was in too bad a condition to permit of examination. From a bream, Abramis brama.

T. barbatulæ Lég., 1904.—The body of this parasite is rather wider (relatively), and more stumpy than usual. Inclusive length 30—40 μ; width 4—6 μ. Free part of flagellum 11—12 μ. The undulating membrane possesses large and deep folds. The anterior end terminates in a small beak, about $1\frac{1}{2}$ μ in length. (V.) host, Cobitis barbatula, loach; (I.) host, Piscicola, sp., a leech, in the intestine of which Léger has observed

Fig. 61.—A, T. carassii, Mitr.; B, T. cobitis, Mitr. (After Mitrophanow, from Doflein.)

important evolutive stages of the parasite (see above, p. 250). Léger regards this form as being distinct from the following one.

T. cobitis, Mitrophanow, 1883. (Synn., Hæmatomonas c., Mitr., Trypanosoma piscium and T. fusiforme piscium, in part, Danil.).—This and the other Trypanosome originally described by Mitrophanow do not appear to have been studied since, hence the insufficient description and also the poor figures (fig. 61 B), which are the only ones available. The length is given as from 30—40 μ, and the breadth 1—$1\frac{1}{2}$ μ [?]. The flagellum alone is 10—15 μ. (V.) host, Cobitis fossilis, another loach.

T. carassii, Mitr. 1883. (Synn., Hæmatomonas c., Mitr., T. piscium and T. fusiforme piscium, in part, Danil.).—Larger, but more flattened, than the preceding form, which, otherwise, it much resembles. This very slight description is about all that one has upon which to rely for a diagnosis of this form from Carassius vulgaris, Prussian carp.

T. granulosum, Lav. and Mesn., 1902, is readily distinguishable from the preceding forms, being more sharply marked off from them by its morphological characteristics than they are from one another. There can be no

doubt that this parasite, at any rate, belongs to a distinct type. It is very long and narrow, typically eel-like (fig. 17 K), and attains a length of 70—80 μ, with a width of only $2\frac{1}{2}$—3 μ. The free flagellum is long, about 25 μ, and, indeed, the whole locomotor apparatus is well developed. The kinetonucleus is large and situated very near the anterior end, which ceases abruptly in a short but pointed cone. Practically the entire cytoplasm is filled with large deeply-staining grains, which may surround and obscure the nucleus. (V.) host, Anguilla vulgaris, eel. The discovery of this Trypanosome is due to Sabrazès and Muratet (**94**).

T. clariæ, Montel, 1905. Length 60 μ; breadth 4 μ. Ribbon-like. Anterior end short and sharply conical, with kinetonucleus close to the extremity. This parasite appears not unlike the last, differing in being somewhat wider. (V.) host Clarias (Silurus c.), Cochin China.

Flat-fish appear to have a fair share of these Hæmatozoa, but only one form has been figured. Moreover, in nearly every case, a Hæmosporidian has been often found associated with the Trypanosomes, the latter being usually much rarer than the former.

T. soleæ, Lav. and Mesn., 1901.—Length about 40 μ; width not given. The free flagellum is only about 8 μ long, or relatively short (fig. 17 J). The anterior end is fairly tapering, more so than the hinder one, which is somewhat blunt. The spherical kinetonucleus is very large, and occupies the entire width of the parasite. Fine longitudinal striations are noticeable in the cytoplasm. (V.) host, Solea vulgaris, sole, which also contained Hæmogregarina simondi. (I.) host, probably Hemibdella soleæ, a leech, which was common on all the fish examined.

T. platessæ, Lebailly, 1904.—Length 52 μ; width 3—$3\frac{1}{2}$ μ. Free flagellum 12 μ. Anterior end finely tapering. Trophonucleus in the posterior half of the body. (V.) host, Pleuronectes platessa (Platessa vulgaris), plaice. Associated with a new Hæmogregarina, H. platessæ.

T. flesi, Lebailly, 1904.—Length 55 μ; width 5 μ, or rather wider than T. platessæ. Free flagellum 10 μ long. Kinetonucleus rather nearer the anterior end than in the preceding species, and trophonucleus about in the middle. (V.) host, Pleuronectes flesus (Flesus vulgaris), flounder, which also contained a new Hæmogregarina, H. flesi.

T. laternæ, Lebailly, 1904.—Length 65 μ; breadth 5—6 μ. Flagellum very short, only about 8 μ long. (V.) host, Platophrys laternæ. Associated with Hæmogregarina laternæ, Lebailly.

These three forms of Lebailly, have, it will be gathered, much in common with each other, and also with T. soleæ. The relatively short flagellum is a feature in all.

T. bothi, Lebailly, 1905. Length 42 μ; of flagellum alone 13 μ; width 3 μ. Anterior end thin and tapering. Trophonucleus in the posterior half of the body. This parasite much resembles the next one. (V.)

host, Bothus rhombus (Rhombus lævis), brill. Associated with a new Hæmogregarina, H. bothi.

Brumpt and Lebailly (11) have briefly described a number of new Piscine Trypanosomes, and also, at the same time, several new Hæmogregarines, many from the same hosts as the Trypanosomes.

T. limandæ, Brumpt and Lebailly, 1904.—An extremely thin, mammalian like form, differing from T. platessæ in having a much longer flagellum. Length 45 μ, of flagellum alone 20 μ. Breadth only 2—2½ μ. Anterior extremity very pointed. (V.) host, Limanda platessoides.

T. delagei, Brumpt and Lebailly, 1904.—A shorter form than the foregoing, but also thin and fusiform. Length 33 μ, flagellum alone 12 μ. Breadth 2½ μ. Anterior part pointed and rectilinear (?). Host, Blennius pholis.

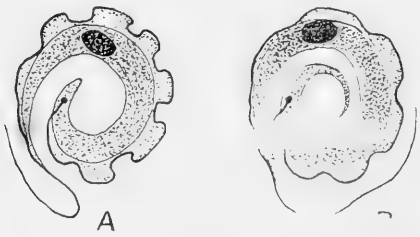


Fig. 62.—A, T. scyllii; B, T. rajæ. × 1200. (After L. and M.)

The remaining forms are larger, and relatively much broader, and agree with the majority of parasites from flat-fish above described in possessing a very short flagellum.

T. gobii, Brumpt and Lebailly, 1904.—Length 66 μ, of flagellum alone 10 μ. Width 5—5½ μ. Anterior extremity generally somewhat blunt or rounded. (V.) host, Gobius niger.

T. cotti, Brumpt and Lebailly, 1904.—Length 53 μ, of flagellum 8 μ. Width about 5 μ. Anterior end fairly short and rounded. This parasite, especially in the case of the largest individuals, resembles T. gobii. (V.) host, Cottus bubalis.

T. callionymi, Brumpt and Lebailly, 1904. Length 70 μ, of flagellum alone about 5 μ (in some cases up to 8 μ). Breadth 5 μ. Anterior extremity long and tapering. Correspondingly, the kinetonucleus is situated some distance from the end. (V.) host, Callionymus dracunculus.

Lastly, both divisions of the Elasmobranchs furnish hosts for these ubiquitous parasites.

T. scyllii, Laveran and Mesnil, 1902. This is a very large parasite, being from 70—75 μ long, by 5—6 μ broad; free flagellum about 14 μ long. The body is generally rolled up on itself (fig. 62 A), often forming a com-

plete circle. It is fusiform in shape, and the anterior end is shorter and rather blunter than in (say) T. soleæ, while the posterior extremity is drawn out and tapering. The undulating membrane has many well-developed folds. (V.) hosts, Scyllium canicula and S. stellare (catulus), dogfish.

T. rajæ, Laveran and Mesnil. This form is, if anything, even larger than T. scyllii, being from 75—80 μ long, by 6 μ wide. The free part of the flagellum is about 20 μ. The shape and appearance of the parasite agree in general with that of the last form, but the anterior extremity is, usually, more tapering, and may, indeed, be very attenuated and proboscis-like (fig. 62 B). (V.) hosts, Raja clavata, R. macrorhynchus, R. mosaica, and R. punctata. Laveran and Mesnil consider that the Trypanosomes found in these different rays all belong to the same species. (I.) host, probably Pontobdella muricata, of frequent occurrence on infected rays.

Trypanosomes, probably distinct species, have also been observed in other Piscine hosts, but not adequately described. Thus Valentin, in 1841, noticed a Hæmatozoan parasite in a trout (Salmo fario) which, to judge from his account, was in all likelihood a Trypanosome, this being the first recorded observation of such. In addition, Trypanosomes which have still to be identified have been mentioned at various times as occurring in the perch, gudgeon, and certain members of the Siluridæ (e.g. Macrones seenghala, M. tengara, Ophiocephalus striatus, and Trichogaster fasciatus).

APPENDIX.

(A) Doubtful Trypanosomes.

There are one or two parasites which have been relegated to this group of organisms which appear to be not really Trypanosomes. Thus, there is the form originally described by Eberth in 1861 from the cæcum and ileum of poultry (hens, geese, ducks, etc.), which was named by Kent (**27**) T. eberthi. This parasite is now generally thought to be a Trichomonas.

Much more important is the organism described by Certes (**143**) in 1882 as Trypanosoma balbianii. This parasite occurs in oysters and other bivalves (Ostræa edulis, O. angulata, Tapes decussata, and T. pullastra), where it inhabits the digestive tube, including the crystalline style (fig. 63 B). All who have written on this form have agreed that it has no free flagellum, but possesses, apparently, an undulating membrane (figs. 63 A and C). Its length may be relatively enormous, from 50 μ or less up to 150 μ, but it is extremely thin, only from 1—3 μ wide. The two most recent

accounts are those of Laveran and Mesnil (145 a) and Perrin (146). According to both, the nucleus consists of numerous small chromatic masses, having the form of grains or rodlets (c) transversely arranged, and extending almost from one end of the body to the other. These rodlets are about equidistant from one another and separated by faintly-staining spaces. Further, according to Perrin, these transverse bands are arranged in a single row upon a delicate, spirally-wound thread or axis, the spiral being very flat where the chromatin rodlets are.

The two accounts differ, however, concerning the other characteristic feature of this parasite. Laveran and Mesnil consider that what appears to

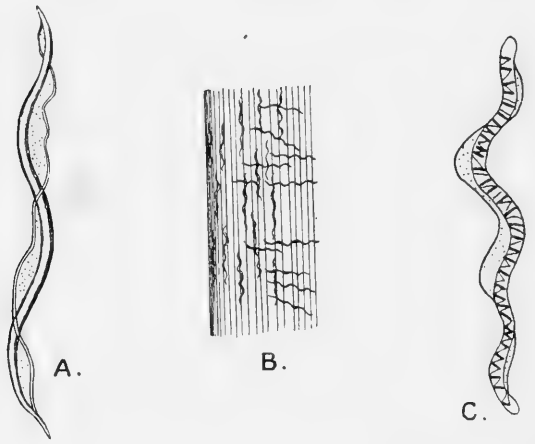


Fig. 63.—Spirochæta (Trypanosoma) balbianii (Certes). (B, a number of individuals associated with the crystalline style of a cockle.) A and B after Certes, C after Perrin.

be an undulating membrane is, in reality, a wide, "periplastic" sheath or investment, which may be attached only at the two ends, the greater part of the body of the organism being more or less free inside it. In certain circumstances, especially if flattened or ribbon-like, and having regard to the spiral form of the body, this sheath would simulate the appearance of an undulating membrane. Perrin, on the other hand, regards this structure as a true undulating membrane, comparable to that of a Trypanosome, and believes, in addition, that it possesses a thickened chromatic border, connected to one end of the nuclear spiral by a delicate thread.

It seems most likely that multiplication is by longitudinal, rather than by transverse fission. Perrin describes the process as commencing by the division of the undulating membrane; this is followed by the transformation

of the chromatic band into rounded granules (chromosomes) first arranged in one longitudinal row, and subsequently, by division, in two. The final division of the cytoplasm is slow, and the two halves may remain united at one end for some time before separating, thus giving the impression of transverse division.

Perrin regards the above form of the parasite as representing the "indifferent" type. In addition, he describes "female" forms and "male gametes;" the latter result after a kind of maturation-process, large hernias being formed at the side of the body, by means of which an expulsion of chromatic material takes place. Lastly, a process of encystment is described in the "indifferent" and "female" forms.

Laveran and Mesnil came to the conclusion that this parasite is not a

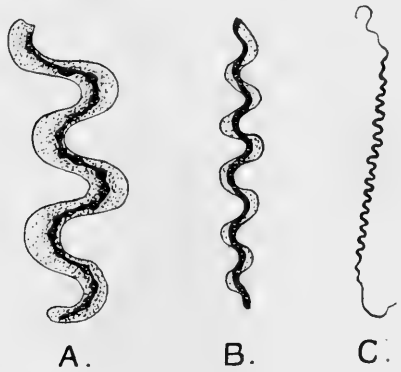


Fig. 64.—(A) Spirochæta plicatilis, Ehrenberg; (B) Spirochæta refringens, Schaudinn; (C) Spironema pallidum (Schaud.). (After Schaudinn.) [In B the central axis (drawn black) represents both endoplasm and nuelear core; in A, the spiral axis (also black) is the nucleus alone, the surrounding endoplasm not being distinctly indicated in Schaudinn's figure.]

Trypanosome but a Bacterium allied to Spirochæta,[1] in Ehrenberg's original sense of the term (145); and, it may be here mentioned, Léger, who has recently studied this organism, in a note to the writer expresses the same opinion. On the other hand, Perrin is confident of the essential Trypanosome nature of the parasite, and, while recognising its resemblance to Bacteria in nuclear structure, etc., sees in it a representative of the ancestral Hæmoflagellate, somewhat on the lines of Schaudinn's bipolar "Urhæmoflagellate" (see footnote, p. 267).

[1] The correct way of spelling this name is Spirochæta, not Spirochæte; vide Ehrenberg (l. c.).

Quite recently, Schaudinn (**148**) has published a brief note on certain "Spirochætæ," which is of great assistance in deciding between these two views. Part of a long individual of S. plicatilis, showing its form and structure, is reproduced in fig. 64 A; on comparing it with fig. 63 A and C, the general agreement between the two forms appears undoubted. Schaudinn describes a well-developed periplastic [ectoplasmic] undulating membrane enclosing the endoplasmic axis of the body; in the latter lies the nuclear apparatus, which, here also, has the form of a (fairly thick) thread on which is suspended a single row of large chromatic grains. The type of nuclear structure is manifestly the same as in "T." balbianii, but the spiral is much more condensed. When, in addition, the rounded termination of the body and the absence of any flagellum are noted, it seems obvious that in whatever group of organisms we place Spirochæta plicatilis we must also include "T." balbianii.

(B) Are the Spirochætæ Trypanosomes, i.e. Flagellates, or Bacteria?

This question has been much discussed during the last year or two. In Schaudinn's great memoir he regarded Trypanosoma ziemanni as possessing, in certain phases, the actual characteristics of a Spirochæta as then known; and, further, he was inclined to consider that other Spirochætæ (e.g. S. obermeieri of relapsing fever) were also really only phases in the life-cycle of a particular Hæmoflagellate. As a result of his more recent investigations on Spirochæta, however, he finds that this is not the case, but that the latter type of organism is essentially different in character from a Trypanosome; T. ziemanni, at certain periods of the life-history, merely simulates a Spirochæta to a remarkable degree (see above, p. 244).

The chief reasons for considering Spirochæta as a Bacterial form rather than a Flagellate may now be briefly discussed, since they bear upon the contrary view taken below with regard to Spironema pallidum.

(*a*) The bluntly-rounded ends, and the absence of any indication of a flagellum in Spirochæta are characters whose importance hardly needs further emphasis.

(*b*) The "undulating-membrane" of a Spirochæta,

although, doubtless, comparable functionally with the similarly-named organella of a Trypanosome, seems most probably not homologous with the latter as regards origin and structure. Although it is generally well-developed (cf. fig. 64 A, B), Schaudinn in no instance mentions the observation of any thickened edge comparable with the flagellar border of the membrane of a Hæmoflagellate.[1]

On the other hand, again, the more Spirochæta-like a Trypanosome is, the narrower and less developed is the undulating-membrane; indeed, in such cases the flagellar border is the most conspicuous part of this organella, the membrane being often recognisable only with difficulty (cf. T. ziemanni [fig. 32], T. johnstoni [fig. 51], other slender Trypanosomes, and, lastly, Spironema pallidum, below).

The writer is more inclined to Laveran and Mesnil's idea of the "membrane" of a Spirochæta as a general ectoplasmic investment surrounding the body (see above, p. 312); such a sheath might well appear, as in Schaudinn's figures.

(*c*) The character of the nucleus in both S. balbianii and S. plicatilis differs considerably in two respects from that of a Trypanosome. In the first place, it is (particularly in S. balbianii) very diffuse, more akin, in short, to the "distributed" type associated with Bacteria, and, in the second place, there is not the well-marked distinction between kinetic and trophic chromatic elements which is so characteristic of a Hæmoflagellate. The writer does not think, with Perrin and Schaudinn, that the axial thread represents the locomotor nucleus of a Trypanosome. It may be kinoplasmic, but it certainly appears to serve as a ground-work

[1] As mentioned above, Perrin considers there is such a chromatic border in "T." balbianii; but neither Laveran and Mesnil nor Léger noticed such a feature in their examination of this form. The writer cannot help thinking to some extent with Mesnil that Perrin has been rather too preoccupied with the idea of realising, in this parasite, the ancestral Trypanosome. Mesnil considers that some of the stages described by Perrin (e. g. the formation of "male gametes" and the encystment process) represent really involution forms.

or support (reticulum?) for the general chromatin, which is not the case with the kinetonucleus in a Trypanosome. In this connection we may refer to the nuclear structure of a microgamete of Trypanomorpha (see above, p. 197) where the trophonucleus is also greatly elongated, the chromosomes being similarly suspended on an axial thread. But the kinetonuclear elements are quite distinct and compactly arranged. Up till now no sign of such a kinetonuclear body has been observed in a true Spirochæta (cf. on the other hand, Spironema, below).

To sum up, we may, it seems to the writer, agree with Schaudinn (1) that the organisms exemplified by Spirochæta plicatilis are to be widely separated from Trypanosoma ziemanni (and equally, of course, from other Trypanosomes), with which they have, at most, only a very remote phylogenetic connection; and (2) that, at any rate, certain other spirilliform parasites, e.g. S. refringens (fig. 64 B) and S. ("T.") balbianii, agree fundamentally in structure with S. plicatilis, the type species. Where, exactly, among micro-organisms Spirochæta is to be placed, e.g. how close to, or distant from, Spirillum, need not be discussed here; it is sufficient to say that the available evidence appears to be against this form having anything to do with the parasitic Flagellates.

(c) Spironema pallidum, Schaudinn = Trypanosoma luis, Krzysztalowicz and Siedlecki.

In the spring of last year Schaudinn and Hoffmann published a note (**150**) on the discovery by the former of spirilliform organisms, greatly resembling Spirochætæ, in syphilitic lesions. Two principal kinds of parasite are distinguished. One, which Schaudinn called Spirochæta refringens, is strongly refractive in life, and of a somewhat coarse or rough appearance; the other, named by him S. pallida, is extremely delicate and very feebly refractive.

From this, and later papers (**149** and **151**) by the same authors, it appears that S. refringens is not met with in pure syphilitic lesions; S. pallida, on the other hand, occurs regularly both in the primary affections and in the secondary eruptive formations, as well as in the internal products and developments associated with the disease.

It can, therefore, be said that there is more probability of S. pallida having some causal relation to syphilis than there is of S. refringens.

Besides the above, other differences between the two parasites, with regard to behaviour and structure, are to be observed. The spiral[1] of S. pallida is much narrower, more acute, and composed of many more turns[2] than that of S. refringens (cf. fig. 64 B, C); it is, in short, more like a corkscrew than a series of curves, thus differing considerably from the usual type of spiral in a Spirochæta. Moreover, the former parasite is very difficult to stain by the methods used in other cases, while S. refringens stains readily.

In his later comparative note on Spirochætæ, Schaudinn (l. c.) shows that S. pallida differs also in more fundamental respects from other Spirochætæ. In fixed and stained preparations he was unable to demonstrate the presence of the undulating membrane which is so apparent in other forms when similarly stained (cf. figs. 63, 64); although from the movements of the parasites during life, he is inclined to suspect the existence of one, doubtless extremely thin. Again, the body tapers finely at both ends, and Schaudinn believes there is really a long delicate flagellum at each extremity (fig. 64c). At times he observed individuals with two flagella at one end, probably indicative of longitudinal division. For these reasons Schaudinn agrees with Vuillemin (**152**), who separates S. pallida both from Spirochæta on the one

[1] Its dimensions are as follows: length, from 4–14 μ; breadth extremely thin, $\frac{1}{4}$ μ; the number of turns is given as from 6–14, but in the later notes as from 10–26.

[2] When in its "spirochætiform" phase, ought perhaps to be added (see below).

hand and from Spirillum on the other, as the type of a new genus Spironema.

A few weeks subsequently to Schaudinn's paper Krzysztalowicz and Siedlecki published a remarkable account (147) of this same parasite. While agreeing as to its finely tapering and pointed appearance, which furnishes a ready means of distinguishing this organism from S. refringens, these authors consider that this is not due to the presence of flagella, but to the body itself being drawn out like a filament at both

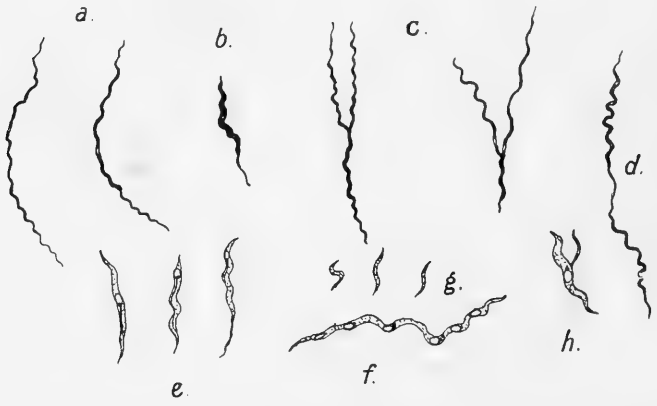


FIG. 65.—Spironema pallidum (Schaud.) = Trypanosoma luis, K. and S. *a*, indifferent (Spirochæta-) forms; *c* and *d*, stages in division of same; *b*, an indifferent form shortened and thickened, leading on to the "Trypanosome"-phase (*e*); *f*, elongated compound (multinuclear) form, which divides into several little sickle-shaped uninuclear individuals (*g*); *h*, conjugation of male and female elements. (After Krzysztalowicz and Siedlecki.)

ends. K. and S. find that, in the course of its movements, the parasite may contract itself considerably, and become much shorter and thicker (fig. 65 *b*.); even in this condition, however, one or both extremities remain attenuated and pointed. At such times Spironema greatly resembles a little Flagellate such as Micromonas. In both these phases complete longitudinal fission takes place (fig. 65 *c*); the parsites may remain for some time attached end-to-end

(fig. 65 *d*), and even divide again while so united [cf. Trypanosoma ziemanni].

So far these authors have been also unable to discern an undulating-membrane. They have observed, however, certain other interesting details of minute structure. In the most successful preparations of spirochætiform parasites the body appears almost severed in two near the middle, this being due to the occurrence of a small oval clear area (fig. 65 *a*, *c*, and *d*). This structure is regarded as the nucleus, apparently very poor in chromatin, and resembling an empty space; K. and S. compare it with the nucleus of a Coccidian, where the chromatin is contained mostly in the karyosome and the nuclear membrane.

In grave or neglected syphilitic lesions forms were encountered which resembled the contracted spirals, but which were relatively wider and more fusiform. In these individuals (fig. 65 *e*) one end of the body is drawn out as a long fine filament, while the other is short and sharply conical. In a varying position between the latter extremity and the middle of the body lies the relatively large ovoid vesicle, with well-defined contours, which is considered to be the nucleus. At one side of the nucleus is a distinct, deeply-staining grain or corpuscle. The whole of the body stains uniformly, but, nevertheless, in large "Spirilla," a deeply-staining filament can be recognised, starting at the corpuscle just alluded to, and running superficially in a somewhat sinuous manner (cf. fig.). This structure is regarded by the authors as entirely comparable with the flagellar border of the undulating-membrane of a Trypanosome, the corpuscle representing a kinetonucleus.

From these facts K. and S. conclude that, at certain periods of its existence, Spironema pallidum possesses a trypaniform phase. They consider that the "Trypanosome-forms" result from the growth of the spirochætiform parasites (in the retracted condition). Probably all the organellæ visible in the trypaniform phase are also present in the spirochætiform one, although mostly too fine to discern and study

They propose the name Trypanosoma luis[1] for the parasites when in the former condition.

K. and S. also bring forward observations pointing to the occurrence of sexual forms and conjugation. The wider trypaniform parasites are themselves considered to represent female elements. In the same preparations showing these are also to be found peculiar spirals differing from the ordinary ones in being much longer and relatively wider, and in possessing several "nuclei" each having a corpuscle in relation with it (fig. 65 *f.*). By the side of these elongated multinuclear forms may be seen also very fine and minute ones, about 3 μ long (*g.*). Each is falciform with pointed ends, possesses one "nucleus" and "corpuscle," and appears identical with one of the turns of the compound spiral. These tiny forms correspond to male elements and probably result from the fragmentation[2] of the others. Boths kinds of gamete may undergo longitudinal division, the males thus becoming extremely delicate and difficult to perceive [cf. T. ziemanni, p. 243].

Only in one case were K. and S. able to find stages which resembled the process of conjugation, in a preparation from a large primary ulceration which was commencing to cicatrise. A male gamete becomes attached to a female gamete by one of its extremities (fig. 65 *h.*), and the two gradually join together (laterally) and at length fuse completely. The subsequent development was not followed. The authors think that probably a resting-period ensues in which the parasite may become encysted, and this in turn gives rise to the indifferent

[1] Even if this parasite is ultimately shown to be a true Trypanosoma, and the generic name Spironema is, in consequence, retired, the specific name must still be pallidum. Pending further investigation, however, it seems best to retain the generic name also, as although probably a Trypanosome, this form may differ somewhat from the genus Trypanosoma.

[2] The authors consider that this apparent transverse division is easily derivable from the successive longitudinal fission of the "Spirochætæ," by supposing that the parasites remain all closely attached end-to-end, become somewhat modified (condensed), and ultimately separate into the constituent units.

("Spirochæta") type of form again. Various enigmatical, rounded, and kidney-shaped bodies were observed, which might possibly belong to the life-cycle, but as no intermediate stages were seen, this remains uncertain.

Brief comment may be made upon one or two points in the above description. The structure regarded by Krzysztalowicz and Siedlecki as a nucleus cannot be said to resemble greatly that of a Trypanosome. In the trypaniform parasites, at all events, it rather recalls the cytoplasmic vacuole of a Trypanosome, both by its appearance and its relation to the kinetonuclear grain.

Moreover, in one long irregular parasite figured (probably an abnormal "compound spiral") some of the "nuclei" certainly have a distinctly vacuolar appearance. However, Krzysztalowicz and Siedlecki think that in certain dividing forms, where the "nucleus" is not apparent as a vacuole, it is represented by a deeper-staining region of the body, its enhanced chromaticity at this time being due to nuclear changes. The solution of this point must await further research.

Again, with regard to the occurrence of a flagellum, Krzysztalowicz and Siedlecki do not think that the spirochætiform parasites have one. Schaudinn, on the other hand, thinks there is one at each end of the body. In some of the former authors' figures of retracted and trypaniform individuals, the appearance of the body at one end strongly suggests the presence of a flagellum (cf. fig. 65 *e*); and this, moreover, at the end towards which the sinuous, deeply-staining line runs, which is homologized with a flagellar border, and which may very likely be continued (in some phases, at any rate) as a free flagellum. It is more difficult to judge in the case of the "Spirilla." In one or two very spirilliform Trypanosomes (e. g. T. ziemanni, T. johnstoni) the free flagellum is very short or absent. Supposing, however, there is a flagellum at one end, it is hardly likely (if Spironema is a Trypanosome) that there is also one at the other; as a matter of fact, in certain indubitable Trypanosomes, the non-flagellate end of the body is often almost, if not quite, as fine as the flagellum itself (cf. T. ziemanni, Hanna's Trypanosome of Indian pigeons [fig. 53], and also T. polyplectrum).

However this may be, there can be little doubt, from the above account, that Spironema pallidum is allied, not to the Spirochætæ, but rather to the Flagellates, possessing, as it does, in certain phases at least, markedly trypaniform characters. It is clear, moreover, that the parasites hitherto grouped together under the general heading of "Spirochætæ" include, really, two quite distinct types of organism.

Further careful investigation of all (including, e.g. "S." obermeieri and "S." anserina) is necessary, with a view to their correct sorting and classification.

In conclusion, the occurrence of Spironema (Trypanosoma) pallidum in syphilis is extremely suggestive when the very similar biology of Trypanosoma equiperdum and its causal relation to Dourine (horse syphilis) are borne in mind (cf. above, p. 176). The former parasite has undoubtedly been evolved along similar lines to the latter. Probably, indeed, the two are not distantly connected, although Spironema is apparently more modified, and almost certainly now restricted to the Vertebrate host.

Bibliography.

[This list is intended to be a guide to the principal papers in connection with the zoological and biological study of the parasites published up to the end of January, 1906.]

A. Relating to the Trypanosomes.

1. Bettencourt and França, C.—"Sur un Trypanosome du blaireau (Meles taxus, Schreib.)," 'C. R. Soc. Biol.,' 59, p. 305, 1905.

2. ——— ——— "Trypanosome de la chauve-souris," t. c., p. 306, 1905.

3. Billet, A.—"Culture d'un Trypanosome de la grenouille chez une Hirudinée ; relation ontogénique possible de ce Trypanosome avec une Hémogregarine," 'C. R. Ac. Sci.,' 139, p. 574, 1904.

4. ——— "Sur le Trypanosoma inopinatum de la grenouille verte d'Algerie et sa relation possible avec les Drepanidium," 'C. R. Soc. Biol.,' 57, p. 161, 16 figs., 1904.

5. Bosc, F. J.—"Recherches sur la structure et l'appareil nucléaire des Trypanosomes (à propos d'un Trypanosome observé chez le lapin)," 'Arch. Protistenk.,' 5, p. 40, 68 figs., 1904.

6. Bradford, J. R., and Plimmer, H. G.—"The Trypanosoma brucii, the organism found in Nagana or the Tsetse-fly disease," 'Q. J. Micr. Sci.,' 45, p. 449, 2 pls., 1902. [This species was originally named and described in 'P. R. Soc.,' 65, p. 274, 1899.]

7. Bruce, D.—"Note on the discovery of a new Trypanosoma," 'Lancet,' 1902, i, p. 664, and 'Nature,' 65, p. 454, 1902.

8. Bruce, Nabarro, D., and Greig, E. D.—["Various Reports on sleeping-sickness and other trypanosomoses in Uganda"], 'Roy. Soc. Comm.,' Nos. 1, 4, and 5, 1903—1905.

9. Brumpt, E.—"Maladie du sommeil et mouche tsé-tsé," 'C. R. Soc. Biol.,' 55, p. 839, 1903.

9 a. ——— "La maladie désignée sous le nom d'Aino par les Somalis de l'Ogaden est une trypanosomose probablement identique au Nagana de l'Afrique orientale," op. cit., 56, p. 673, 1904.

10. ——— "Contribution à l'étude de l'évolution des Hémogregarines et des Trypanosomes," op cit., 57, p. 165, 1904.

11. ——— and Lebailly, C.—"Description de quelques nouvelles espèces de Trypanosomes et d'Hémogregarines parasites des Téléostéens marins," 'C. R. Ac. Sci.,' 139, p. 613, 1904.

12. ——— and Wurtz.—"Agglutination du Trypanosoma castellanii, Kruse, parasite de la maladie du sommeil," 'C. R. Soc. Biol.,' 55, p. 1555, 1903.

13. ——— ——— ["Experimental sleeping-sickness among various animals"], 'C. R. Soc. Biol.,' 56, pp. 567—573, 1904.

14. Buffard, M., and Schneider, G.—"Le Trypanosome de la Dourine," 'Arch. parasitol.,' 3, p. 124, with pls., 1900.

15. Castellani, A.—"Trypanosoma and sleeping-sickness," 'Reports S. S. Comm. Roy. Soc.,' Nos. 1 and 2, 1903.

15 a. Chalachnikow.—'Recherches sur les parasites du sang chez les animaux à sang froid et à sang chaud,' Kharkoff, 1888.

16. Crookshank.—"On T. (Trichomonas) sanguinis evansi," 'J. R. Micr. Soc.,' 6, 1886.

17. Danilewsky.—"Zur Parasitologie des Blutes," 'Biol. Centrbl.,' 5, p. 529, 1885.

18. ——— 'Recherches sur la parasitologie comparée du sang des oiseaux,' Kharkoff, 1888–89.

19. Doflein, F.—'Die Protozoen als Parasiten und Krankheitserreger nach biologischen Gesichtspunkten dargestellt,' Jena (G. Fischer), 1901.

20. Dutton, E.—"Note on a Trypanosoma occurring in the Blood of Man," 'Brit. Med. Journ.,' 1902, ii, p. 881, 1 fig.

21. ——— and Todd, J. L.—"First Report of the Trypanosomiasis Expedition to Senegambia (1902)," 'Mem. Livpl. Sch. Trop. Med.,' No. II ('Thompson-Yates Lab. Rep.' [5] 2), 1903.

22. Elmassian, M., and Migone, E.—"Sur le Mal de Caderas ou Flagellose parésiante des Equidés Sud-americains," 'Ann. Inst. Pasteur,' 17, p. 241, 1 pl., 1903.

23. Galli-Valerio, B.—"Untersuchungen über die Hämosporidien der Alpen-Vögel," 'Centrbl. Bakter.,' (I), 31, Orig., p. 162, 1902.

24. ——— "Notes de parasitologie," 'Centrlbl. Bakter.,' (I), 35, Orig., p. 81, 1903.

24a. Gray, A. C., and Tulloch, F. M.—"The Multiplication of the Trypanosoma gambiense in the Alimentary Canal of Glossina palpalis," 'Rep. S.S. Comm.,' No. 6, p. 282, 1 pl., 1905.

24b. Grieg, E. D., and Gray, A. C.—"Continuation Report on Sleeping-sickness in Uganda," t. c., p. 1, with plates, 1905.

25. Gruby.—"Recherches et observations sur une nouvelle espèce d'Hématozoaire (Trypanosoma sanguinis)," 'C. R. Ac. Sci.,' 17, p. 1134, 1843, and 'Ann. Sci. Nat.,' (3), i, p. 105, 7 figs., 1844.

26. Hanna, W.—"Trypanosoma in Birds in India," 'Q. J. Micr. Sci.,' 47, p. 433, 1 pl., 1903.

27. Kent, Saville.—'A Manual of the Infusoria,' vol. i, 1880–1.

28. Keysselitz, G.—"Über Trypanophis grobbeni (Trypanosoma grobbeni, Poche)," 'Arch. Protistenk.,' 3, p. 367, 3 figs., 1904.

29. Labbé, A.—"Note sur un nouveau parasite du sang (Trypanomonas danilewskyi)," 'Bull. Soc. zool. France,' 16, p. 229, 1 fig., 1891.

30. Lankester, E. R.—"On Undulina, the type of a New Group of Infusoria," 'Q. J. Micr. Sci.,' 11, p. 387, 4 figs., 1871.

31. ——— "The Sleeping-sickness," 'Quart. Rev.,' July, 1904, p. 113, 7 figs.

32. Laveran, A.—"Sur un nouveau Trypanosome des Bovidés," 'C. R. Ac. Sci.,' 134, p. 512, 1902.

33. ——— "Au sujet de deux Trypanosomes des Bovidés du Transvaal," op. cit., 135, p. 717, 5 figs., 1902.

34. ——— "Sur la piroplasmose bovine bacilliforme," op. cit., 136, p. 648, 18 figs., 1903.

35. ——— "Sur deux Hippobosques du Transvaal susceptibles de propager Trypanosoma theileri," 'C. R. Soc. Biol.,' 55, p. 242, 1903.

36. ——— "Sur un Trypanosome d'une chouette," t. c., p. 528, 2 figs., 1903.

37. ——— "Contribution à l'étude de Hæmamœba ziemanni," t. c., p. 620, 7 figs., 1903.

38. ——— "Sur l'agent pathogène de la trypanosomiase humaine, Tr. gambiense, Dutton," 'C. R. Ac. Sci.,' 138, p. 841, 1904.

39. ——— "Sur un nouveau Trypanosome d'une grenouille," 'C. R. Soc. Biol.,' 57, p. 158, 2 figs., 1904.

39 a. ——— "Trypanoplasme et Trypanosome du vairon," T. c., p. 250, 1904.

40. ——— and Mesnil, F.—"Recherches morphologiques et expérimentales sur le Trypanosome des rats, Tr. lewisi (Kent);" 'Ann. Inst. Pasteur,' 15, p. 673, 2 pls., 1901.

41. ——— ——— "Sur la morphologie et la systématique des Flagellés à membrane ondulante (genres Trypanosoma et Trichomonas, Donné)," 'C. R. Ac. Sci.,' 133, p. 131, 3 figs., 1901.

42. ——— ——— "Sur les Flagellés à membrane ondulante des poissons (genres Trypanosoma, Gruby, et Trypanoplasma, n. gen.)," t. c., p. 670, with figs., 1901 [fuller account in **47**].

43. ——— ——— "Sur le mode de multiplication du Trypanosome du Nagana," 'C. R. Soc. Biol.,' 53, p. 326, with figs., 1901 [see also **48**].

44. ——— ——— "Sur la nature centrosomique du corpuscle chromatique postérieur des Trypanosomes," t. c., p. 329, 1901.

45. ——— ——— "Sur la structure du Trypanosome des grenouilles et sur l'extension du genre Trypanosoma Gruby," t. c., p. 678, 3 figs., 1901.

47. ——— ——— "Des Trypanosomes des poissons," 'Arch. Protistenk.,' 1, p. 475, 15 figs., 1902. [For brief accounts see **42**, **49**, and **51**.]

48. ——— ——— "Recherches morphologiques et expérimentales sur le Trypanosome du Nagana ou maladie de la mouche tsé-tsé," 'Ann. Inst. Past.,' 16, p. 1, 13 figs., 1902.

49. ——— ——— "Sur le mode de multiplication des Trypanosomes des poissons," 'C. R. Ac. Sci.,' 134, p. 1405, 1902.

50. ——— ——— "Sur quelques Protozoaires parasites d'une tortue d'Asie (Damonia reevesii)," op. cit., 135, p. 609, 14 figs. 1902.

51. ——— ——— "Sur les Hématozoaires des poissons marins," t. c., p. 567, 1902.

52. ——— ——— "Le Nagana et le Mal de Caderas sont deux entités morbides bien distinctes," t. c., p. 838, 1902.

53. ——— ——— "Le Nagana le Surra et le Caderas constituent trois entités morbides distinctes," op. cit., 136, p. 1529, 1903.

54. ——— ——— "Sur un Trypanosome d'Afrique pathogène pour les Équidés, T. dimorphon, Dutton et Todd," op. cit., 138, p. 732, 7 figs., 1904.

54 a. ——— ——— "Sur le Surra et le différenciation des trypanosomiases," op. cit., 140, p. 831, 1905.

55. ——— ——— "Infections naturelles de rats blancs par Trypanosoma lewisi," 'C. R. Soc. Biol.,' 57, p. 247, 1904.

56. Laveran and Mesnil.—"Trypanosomes et trypanosomiases," Paris (Masson et Cie), 1904.

57. Lebailly, C.—"Sur quelques Hémoflagellés des Téléostéens marins," 'C. R. Ac. Sci.,' 139, p. 576, 1904.

58. ——— "Sur des Hématozaires nouveaux parasites de la barbue (Bothus rhombus L.)," 'C. R. Soc. Biol.,' 59, p. 304, 1905.

59. Léger, L.—"Sur la systématique des Cercomonadines aciculées san membrane ondulante," 'C. R. Ac. Sci.,' 134, p. 665, 1902.

60. ——— "Sur la structure et le mode de multiplication des Flagellés du genre Herpetomonas, Kent," t. c., p. 781, 7 figs., and 'C. R. Soc. Biol.,' 54, p. 398, 7 figs., 1902.

61. ——— "Sur un Flagellé parasite de l'Anopheles maculipennis," 'C. R. Soc. Biol.,' 54, p. 354, 10 figs., 1902.

62. ——— "Sur la forme gregarinienne des Herpetomonas," t. c., p. 400, 1902. [See **60** for figs.]

63. ——— "Sur quelques Cercomonadines nouvelles ou peu connues parasites de l'intestin des Insectes," 'Arch. Protistenk.,' 2, p. 180, 4, figs., 1903.

64. ——— "Sur la morphologie du Trypanoplasma des vairons," 'C. R. Ac. Sci.,' 138, p. 834, 1904. [For figs. see **65**.]

65. ——— "Sur la structure et les affinities des Trypanoplasmes," t. c., p. 856, 5 figs., 1904.

66. ——— "Sur les Hémoflagellés du Cobitis barbatula, L. I., Trypanosoma barbatulæ, n. sp.," 'C. R. Soc. Biol.,' 57, p. 344, 1904.

67. ——— "Trypanoplasma varium, n. sp., parasite du sang de Cobitis barbatula, L., t. c., p. 345, 1904.

68. ——— "Sur un nouveau Flagellé parasite des Tabanides," t. c., p. 613, 6 figs., 1904.

69. ——— "Sur les affinités de l'Herpetomonas subulata et la phylogenie des Trypanosomes," t. c., p. 615, 1904.

70. ——— "Sur la presence d'un Trypanoplasma intestinal chez les poissons," op. cit., 58, p. 511, 1905.

71. Lignières, J.—"Contribution à l'étude de la trypanosomose des Équidés Sud-americains connue sous le nom de Mal de Caderas (Trypanosoma elmassiani)," 'Rec. Med. vet., Bull. et Mem.' (8), 10, pp. 51, 109, 164, 2 pls., 1903.

72. Mac Callum, W. G.—"On the Hæmosporidian Infection of Birds," 'J. Exp. Med.,' 3, p. 117, 1 pl., 1898.

73. Martini, E.—"Über die Entwickelung der Tsetseparasiten in Säugethieren," 'Zeitschr. Hygiene,' 42, p. 341, 1 pl., 2 figs. 1903.

73 a. Mayer, A. F.—"Spicilegium observationum anatomicarum de organo electrico in Raiis anelectrias et de Hæmatozois," Bonn, 1873, 18 pp., 3 pls.

74. McNeal, W. J.—"On the Life-history of T. lewisi and T. brucii," 'J. Inf. Diseases,' 1, Nov., 1904.

75. Minchin, E. A.—"The Sporozoa," Section K in 'Lankester's Treatise on Zoology,' 1, p. 150, 127 figs., 1903.

76. Mitrophanow.—Beiträge zur Kenntniss der Hämatozoen," 'Biol. Centrbl.,' 3, p. 35, 2 figs., 1883.

77. Montel, R.—"Trypanosome d'un poisson de Cochinchine," 'C. R. Soc. Biol.,' 58, p. 1016, 1 fig., 1905.

78. Nepveu, G.—"Sur un Trypanosome dans le sang de l'homme," op. cit., 50, p. 1172, 1898.

79. Novy, F. G., and McNeal, W. J.—"On the Cultivation of Trypanosoma lewisi," 'Contrib. Med. Research, Michigan,' p. 549, 1903.

80. ——— ——— "On the Cultivation of Trypanosoma brucii," 'J. Inf. Diseases,' 1, part 1, 3 tables, 1904.

81. ——— ——— "On the Trypanosomes of Birds," op. cit., 2, p. 256, 11 pls., 1905.

82. Petrie, G. F.—"A Note on the Occurrence of a Trypanosome in the Rabbit," 'Centrbl. Bakter.,' (I), 35, Orig., p. 484, 1904.

83. ——— "Observations relating to the Structure and Geographical Distribution of Certain Trypanosomes," 'J. Hygiene,' 5, p. 195, 1905.

84. Plehn, M.—"Trypanoplasma cyprini, n. sp.," 'Arch. Protistenk,' 2, p. 175, 1 pl., 1903.

85. Plimmer, H. G.—"Note on the Effects produced on Rats by the Trypanosomata of Gambia-fever and Sleeping-sickness," 'P. Roy. Soc.,' 74, p. 388, 1905.

86. Poche, F.—"Über zwei neue in Siphonophoren vorkommende Flagellaten," 'Arb. Zool. Inst. Wien,' 14, p. 307, 1 pl., 1903.

87. Prowazek, S.—"Die Entwickelung von Herpetomonas, einen mit den Trypanosomen verwandten Flagellaten," 'Arb. kaiserl. Gesundtheitsamte,' 20, p. 440, many figs., 1904.

88. ——— "Studien über Säugethiertrypanosomen," op. cit., 22, 45 pp., 6 pls., 1905.

89. Rabinowitsch, L., and Kempner, W.—"Beitrag zur Kenntniss der Blutparasiten, speciell der Rattentrypanosomen," 'Zeitschr. Hyg.,' 30, p. 251, 1 pl., 1899.

90. ——— ——— "Die Trypanosomen in der Menschen- und Thierpathologie, so wie vergleichende Trypanosomenuntersuchungen," 'Centrbl. Bakter.,' (I), 34, Orig., p. 804, 1903.

91. Rennes.—"Contribution à l'étude de la Dourine," 'Rec. med. vet.,' (8), 10, p. 424, 1903.

92. Rogers, L.—"The Transmission of the Trypanosoma evansi by Horse-flies, and other Experiments pointing to the Probable Identity of Surra of India and Nagana or Tsetse-fly Disease of Africa," 'P. Roy. Soc.,' 68, p. 163, 1901.

92 a. ——— "On the Rôle of the Horse-fly in Trypanosoma-infection," 'Brit. Med. J.,' 1904, ii, p. 1454.

93. Rouget, J.—"Contribution à l'étude de la Dourine," 'Rec. med. vet.,' (8), 10, p, 81, 1903.

93 a.— ——— "Trypanosome de la Dourine; son inoculation aux souris et aux rats," 'C. R. Soc. Biol.,' 56, p. 744, 1904.

94. Sabrazès, J., and Muratet, L.—"Trypanosome de l'anguille (Anguilla vulgaris)," 'Pr.-verb. Soc. Bordeaux,' 57, pp. 82 and 124, 1 pl., 1903.

95. ——— ——— "Trypanosome de l'anguille. Processus de division," 'C. R. Soc. Biol.,' 56, p. 66, 1904.

96. Salmon, D., and Stiles, C.—"Emergency Report on Surra," 'Bull. U.S. Dep. Agric. and Animal Ind.,' No. 42, 152 pp., 112 figs., 1902.

97. Schaudinn, F.—"Studien über krankheitserregende Protozoen, II, Plasmodium vivax (Gr. and Fel.)," 'Arb. kaiserl. Gesundheitsamte,' 19, p. 169, 3 pls., 1902.

98. ——— "Generations- und Wirthswechsel bei Trypanosoma und Spirochæte (Vorl. Mittheilung)," op. cit., 20, p. 387, 20 figs., 1904.

99. Senn, G.—"Der gegenwärtige Stand unserer Kenntnisse von den flagellaten Blutparasiten," 'Arch. Protistenk, 1, p. 344, 1902.

100. ——— "Flagellata," in 'Engler und Prantl's natürlichen Pflanzenfamilien,' I, i a, 1903.

101. Sergent, E. and E.—"Sur un Trypanosome nouveau parasite de la grenouille verte," 'C. R. Soc. Biol.,' 56, p. 123, 1 fig., 1904.

101 a. ——— "Notes sur une trypanosomiase des dromadaires d'Algérie," t. c., pp. 120, 914, 1904.

102. ——— "Sur les Hématozoaires des oiseaux d'Algérie," t. c., p. 132, 1904.

103. ——— "Hémamibes des oiseaux et moustiques. 'Generations alternantes' de Schaudinn," op. cit., 58, p. 57, 1905.

104. ——— "Sur des Trypanosomes des chauves-souris," t. c., p. 53, 2 figs., 1905.

105. Siegel.—"Die geschlechtliche Entwickelung von Hæmogregarina stepanowi im Rüsselegel Placobdella catenigera," 'Arch. Protistenk.,' 2, p. 339, 7 figs., 1903.

106. Sivori, F., and Lecler, E.—"Le Surra americain ou Mal de Caderas," 'Ann. Min. Agric. Argentina,' 1, p. 1, 7 pls., 1902.

107. Smedley, R. D.—"The cultivation of Trypanosomata," 'J. Hygiene,' 5, p. 24, 2 pls., 1905.

108. Stassano, H.—"Contribution à l'étude du Trypanosome," 'C. R. Soc. Biol.,' 53, p. 14, 1901.

109. ——— "Sur la fonction et relation du petit noyau des Trypanosomes," t. c., p. 468, 1901.

110. Steel, J. H.—"Report on his Investigation into an Obscure and Fatal Disease among Transport Mules in British Burmah," 1885.

111. Thiroux.—"Sur un nouveau Trypanosome des oiseaux," 'C. R. Ac. Sci.,' 139, p. 145, 5 figs., 1904.

112. ——— "Sur un nouveau Trypanosome de la souris domestique (Mus musculus)," 'C. R. Soc. Biol.,' 58, p. 885, 2 figs., 1905.

113. ——— "L'infection du Padda oryzivora par le Trypanosoma paddæ n'a pas de rapport avec l'infection de cet oiseau par l'Halteridium danilewskyi," 'C. R. Ac. Sci.,' 140, p. 109, 1905.

114. ——— "Recherches morphologiques et expérimentales sur les Trypanosoma paddæ," 'Ann. Inst. Past.,' 19, p. 65, 1 pl., 1905.

115. ——— "Recherches morphologiques et expérimentales sur Trypanosoma duttoni (Thiroux)," t. c., p. 564, 1 pl., 1905.

116. Thomas, H., and Linton, S.—"A Comparison of the Animal Reactions of the Trypanosomes of Uganda and Congo Free State Sleeping-sickness with those of Trypanosoma gambiense, Dutton," 'Lancet,' 1904, i, p. 1337.

117. Valentin.—Über ein Entozoon im Blute von Salmo fario," 'Muller's Arch.,' 1841, p. 435.

117 a. Vallée and Panisset.—"Sur les rapports du Surra et de la Mbori," 'C. R. Ac. Sci.,' 139, p. 901, 1904.

118. Vassal, J.—"Sur un nouveau Trypanosome aviare," 'C. R. Soc. Biol.,' 58, p. 1014, 1 fig., 1905.

119. Voges, O.—"Mal de Caderas," 'Zeitschr. Hygiene,' 39, p. 323, 1 pl., 1902.

120. Wasielewsky and Senn, G.—"Beiträge zur Kenntniss der Flagellaten des Rattenblutes," op. cit., 33, p. 444, 3 pls., 1900.

121. Ziemann, H.—"Über ein neues Halteridium und ein Trypanosoma bei einer kleinen weissen Eule in Kamerun," 'Arch. Schiffs. u. Tropenhyg.,' 6, p. 389, 1902.

B. Relating to the "Leishman-Donovan-Wright" bodies.

122. Chatterjee.—["On the Cultivation of Flagellate Forms from the Leishman-Donovan bodies]," 'Lancet,' 1905, i, p. 16.

123. Christophers, S. R.—"Reports on a Parasite found in Persons suffering from Enlargement of the Spleen in India," 'Sci. Mem. India,' Nos. 8, 11, and 15, 1904–5.

124. Donovan, C.—"Human Piroplasmosis," 'Lancet,' 1904, ii, p. 744, 1 pl.

125. James, S. P.—"Oriental or Delhi Sore," 'Sci. Mem. India,' No. 13, 1905.

126. ——— "On Kala-Azar, Malaria, and Malarial Cachexia," op. cit., No. 19, 1905.

127. Laveran, A., and Mesnil, F.—"Sur un Protozoaire nouveau (Piroplasma donovani, Lav. et Mesn.) parasite d'une fievre de l'Inde," 'C. R. Ac. Sci.,' 137, p. 957, 17 figs., 1903.

128. ——— ——— "Nouvelles observations sur Piroplasma donovani, Lav. et Mesn.," op. cit., 138, p. 187, 1904.

129. Leishman, W.—"On the Possibility of the Occurrence of Trypanosomiasis in India," 'Brit. Med. J.,' 1903, i, p. 1252, 2 figs.

130. ——— "Note on the Nature of the Parasitic Bodies found in Tropical Splenomegaly," op. cit., 1904, i, p. 303.

131. ——— and Statham.—"The Development of the Leishman Body in Cultivation," 'J. Army Med. Corps,' 3, 14 pp., 1 pl., March, 1905.

132. Manson, P.—"Note on Two Cases of Febrile Tropical Splenomegaly (Kala-Azar) and a Suggestion," 'Brit. Med. J.,' 1905, ii, p. 1261.

133. ——— and Low, G. C.—"The Leishman-Donovan Body and Tropical Splenomegaly," 'Brit. Med. J.,' 1904, i, p. 183, 12 figs.

134. ——— ——— "The Leishman-Donovan Body in Ulcerated Surfaces; a Possible Route of its Escape from the Human Body," 'J. Trop. Med.,' 7, p. 239, 1904.

135. Marchand, F., and Ledingham, J.—"On the Question of Trypanosoma Infection in Man," 'Lancet,' 1904, i, p. 149, 1 fig.

136. ——— ——— "Über Infektion mit 'Leishman'schen Körperchen' (Kala-Azar?) und ihr Verhältniss zur Trypanosomen-Krankheit.," 'Zeitschr. Hyg.,' 47, p. 1, 2 pls, 1904.

137. Mesnil, F., Nicolle, F., and Remlinger, P.—"Sur le Protozoaire du bouton d'Alep," 'C. R. Soc. Biol.,' 57, p. 167, 1904.

138. Rogers, L.—"On the Development of Flagellated Organisms (Trypanosomes) from the Spleen Protozoic Parasites of Cachexial Fevers and Kala-Azar," 'Q. J. Micr. Sci.,' 48, p. 367, 1 pl., 1904.

139. Rogers, L.—"The Conditions Affecting the Development of Flagellate Organisms from Leishman Bodies and their Bearing on the Probable Mode of Infection," 'Lancet,' 1905, i, p. 1484.

140. Ross, R.—"Notes on the Bodies recently described by Leishman and Donovan," 'Brit. Med. J.,' 1903, i, pp. 1261 and 1401, 17 figs.

141. ——— "A New Parasite of Man," 'Thompson-Yates Lab. Rep.,' (5), 2, p. 79, 1 pl., 1904.

142. Wright, J. H.—"Protozoa in a Case of Tropical Ulcer (Delhi Sore)," 'J. Med. Research,' Boston, 10, p. 472, 4 pls., 1903.

c. Relating to the "Spirochætæ."

143. Certes, A.—"Note sur les parasites et les commensaux de l'huitre," 'Bull. Soc. Zool. France,' 7, p. 347, 1 pl., 1882.

144. ——— "Sur le Trypanosoma balbianii, op. cit., 16, pp. 95 and 130, 1 fig., 1891.

145. Ehrenberg.—"Die Infusionsthierchen als vollkommene Organismen," Leipzig, 1838. [Describes and figures Spirochæta plicatilis; this form was originally diagnosed in 'Abh. Ak. Berlin,' year 1833, p. 313 (publ. 1835)].

145 a. Laveran and Mesnil.—"Sur la nature bactérienne du prétendu Trypanosome des huitres, T. balbianii," 'C. R. Ac. Sci.,' p. 883, 1901.

146. Perrin, W. S.—"A Preliminary Communication on the Life-history of Trypanosoma balbianii," 'P. Roy. Soc.,' 76 B, p. 367, 4 figs., 1905.

147. Krzysztalowicz, F., and Siedlecki, M.—"Contribution a l'étude de la structure et du cycle évolutif de Spirochæte pallida Schaud," 'Bull. Ac. Cracovie,' 6th November, 1905, p. 713, 1 pl.

148. Schaudinn, F.—"Zur Kenntniss der Spirochæte pallida (Vorl. Mittheilung)," 'Deutsch. med. Wochenschr.,' No. 42, 19th October, 1905, p. 1665.

149. ——— and Hoffmann, E.—"Über Spirochætenbefunde im Lymphdrüsensaft Syphilitscher," op. cit., No. 18, 1905, p. 711.

150. ——— ——— "Vorläufiger Bericht über das Vorkommen von Spirochæten in syphilitschen Krankheitsproducten und bei Papillomen," 'Arb. Kaiserl. Gesundheitsamte,' 22, p. 527, 1905.

151. ——— ——— "Über Spirochæte pallida bei Syphilis und die Unterschiede dieser Form gegenüber anderen Arten dieser Gattung," 'Berl. klin. Wochenschr.,' No. 22, 1905.

152. Vuillemin, P,—"Sur la dénomination de l'agent présumé de la syphilis," 'C. R. Ac. Sci.,' 140, p. 1567, 1905.

Notes on the Development, Structure, and Origin of the Median and Paired Fins of Fish.

By

Edwin S. Goodrich, F.R.S.,

Fellow of Merton College, Oxford.

With Plates 10—14.

Contents.

Introduction.

A great deal has been written in the last few years about the structure, development, and origin of the paired fins of fish, yet two rival and incompatible theories are still prevalent. According to the theory put forth by Gegenbaur (**14, 15, 16**), the paired fins have been derived from gill structures, the gill-arch having been modified into the limb-girdle, and the fin itself, with its skeleton, having been derived from the gill-flap or septum, and its supporting gill-rays. This may shortly be called the "gill-arch theory." The second theory, that of Balfour (**1, 2**), Thacher (**35**), and Mivart (**23**), holds that the paired fins are of the same nature as the unpaired median fins. According to this view, the limbs have been derived from paired longitudinal fin-folds, in which skeletal supports, the radials, or somactidia (Lankester), became developed as in the median fins, and subsequently gave rise to the limb-girdles. This may be called the "lateral fin-fold theory." Each of these theories may claim to have among its numerous supporters the names of some of the most eminent exponents of the morphology of the vertebrates. Dohrn (**10**), Haswell (**20**), Rabl (**31**), Mollier (**24, 25, 26**), Harrison (**19**), Wiedersheim (**36**), A. Smith Woodward (**37**), and Dean (**9**) have written in favour of the lateral fold theory; Davidoff (**8**), Fürbringer (**12**), Braus (**3–7**), and others have supported its rival. It is unnecessary for me in these notes to give a history of the discussions to which the question has given rise; the literature has been recently reviewed by Mollier and Braus, and the whole subject is familiar to zoologists. But there are certain essential points which seem to be in danger of being obscured from view in a cloud of controversy, and it is in the hope of clearing up some of these points and of filling up certain gaps in the evidence that these notes have been published.

As I am anxious to keep this paper within reasonable limits and not to overburden the already very bulky literature on the subject of the origin of the paired limbs, only some aspects

of the problem will be dealt with in detail. A brief and somewhat dogmatic statement of the case is made at the beginning, followed by a description of my own observations, and ending with a short summary.

The Lateral Fold Theory.

Balfour's conception of an originally continuous fin-fold, reaching from the pectoral to the pelvic region (**1**) is discredited because it has been found only (as an epidermal fold) in those forms, like Torpedo, in which the pectoral fins reach the pelvic fins in the adult, a condition which is probably rightly considered as secondary. Moreover, the appearance of an epidermal longitudinal fold, as a first indication of the development of the paired fins, is considered to be of little importance, and its presence between the paired fins is denied in sharks (Mollier **24**, Braus **4**, etc.).

Now, the continuity of the pectoral with the pelvic fin-fold is not an essential point. The important thing is to recognise that the paired fins always arise as a longitudinal fold or ridge, similar to that which gives rise to the median fins. That this is really the case is now admitted by all (Braus **7**). Even in Ceratodus, where the paired fins in the adult are set at a pronounced angle to the long axis of the body, they make their first appearance as longitudinal ridges (Semon **33**).

Possibly from the very first, in phylogeny, the paired fins were discontinuous, and differentiated into pectoral and pelvic. For conclusive evidence on this point we must look to palæontology; and it has not yet been obtained. But there is some evidence to be gathered from comparative anatomy and embryology in favour of Balfour's view, as has frequently been pointed out (Dohrn **10**, Mollier **24**).

For instance, the musculature of the fins is developed in Elasmobranchs, from buds given off from the ventral ends of the myotomes, and these buds have been shown to be produced not only on the myotomes in the region of the fins, but

on all the trunk myotomes situated between the pectoral and the pelvic fins in such sharks as Pristiurus and Scyllium, in which these fins are widely separated in the adult (Dohrn **10**, Rabl **31**, Braus **4**, and p. 343 below). Many of the intermediate buds seem to disappear entirely during development. In those segments which are near the fins the buds become better developed and more persistent, and a large number pass into the fin-fold. Muscle buds are also found in front of the pectoral fin and behind the pelvic fin, dwindling in size as they are farther removed from the fin-base. Thus, in these sharks, the muscles of the paired fins are formed by the great development in two regions of a continuous series of muscle buds, vanishing posteriorly behind the cloaca. The manner in which these vestigial buds disappear by reduction at either end of the fin rudiment, and in which the persistent buds become concentrated at the relatively narrowing base of the fin, has been admirably described and figured by Mollier (**24**) and Braus.

The fin-base of the adult occupies much less space relatively than the fin-fold of the embryo.

Now, the radial fin-muscles being developed from buds of the myotomes, naturally receive their motor nerve-supply from the ventral roots of the spinal nerves, and these correspond in number to the myotomes which share in the formation of the musculature. Owing to concentration, the nerves are found to converge toward the base of the fin. In front and behind the nerves may be drawn together so as to form a "collector" nerve or compound stem.

It is part of the lateral fold theory to suppose that the endoskeleton of the paired fins has been derived from a series of cartilaginous rods, radials, or somactidia, similar to those of the median fins (Thacher **35**, Mivart **23**). The various types of fin-skeleton, with their cartilage rays and basal pieces, would have been developed from such originally segmental radials by concentration and fusion. To this contention it is objected that in development the radials of Elasmobranchs arise in a continuous rudiment—a plate of procartilaginous

mesenchyme (Balfour **2**, Mollier **24**, Ruge **32**, and p. 357 below). It may be answered (Dohrn **10**, Mollier **24**) that, the radials being closely approximated, their procartilaginous rudiments with indefinite borders necessarily merge together to a considerable extent. As a matter of fact, the cartilage pieces appear as islands in the vaguely-defined rudiment, which correspond closely in position and number with the separate elements of the adult fin-skeleton. Some slight indications of recapitulation, some fusion of neighbouring radials, may be detected, which bears out the views so convincingly advocated by Thacher and Mivart. But it cannot be claimed that recapitulation is complete in this respect in the development of the paired fins. It is obvious, however, that if its absence is considered as evidence against the lateral fold theory it tells with equal force against the gill-arch theory, since the skeleton is, according to this view, also derived from originally separate (branchial) rays.

But the whole argument against the lateral fold theory collapses when we find that, as Balfour long ago showed, the radials of the median fins likewise arise in a continuous prochondral plate, in the median fins of Elasmobranchs, even when they are separate in the adult (p. 355 below). These median fins are much concentrated, and nothing proves so clearly that the early continuity of the rudiments is due to their approximation, for here the original metameric nature of the radials will not be denied. The most enthusiastic supporter of the gill-arch theory would not suppose that the continuous plate represents an early stage in the phylogenetic history of the skeleton of median fins! Unfortunately, we know but little concerning the development of the skeleton in unconcentrated median fins. Doubtless, in such cases the radials arise separately; Harrison, indeed, has shown this in his valuable paper on the salmon (**19**).

Yet other objections have been brought forward by Braus, in the elaborate and beautiful memoirs which have of late contributed so much to our knowledge of the structure and development of fins (**3**, **4**, **6**, **7**). It has been shown that two

muscle-buds are given off by each myotome to the paired fins in Elasmobranchs; that these pass outwards into the fin-fold, dividing into upper and lower halves, which give rise to the dorsal and ventral radial muscles. Between each pair of corresponding upper and lower buds develops a cartilaginous radial. Thus, as Rabl showed, since two radial muscles and cartilages correspond to each segment, the relation between the number of radials in the fin-skeleton, and the number of trunk vertebræ belonging to those segments which contributed to the formation of the fin, may be expressed in the formula $\frac{\text{Radials}}{2} = \text{vertebræ}$. Braus has endeavoured to prove that this formula does not hold good (p. 444, **3**). But it is quite obvious that, although in the main correct, it can only be intended to give approximate results when applied to whole fins. In most paired fins of Elasmobranchs the anterior and posterior regions are much modified by excessive concentration and reduction, and here the correspondence between muscles and radials becomes much disturbed. The formula applies perfectly over the greater part of a fin which is normally developed, as is seen in Braus's own figures (**4, 6**). More important is the contention that the adult radial fin-muscles do not correspond to the muscle-buds in the embryo. It is urged that the muscle-buds become mixed and that the adult muscles are no longer unisegmental and haploneurous, but are compound and polyneurous, and, in fact, bear no definite relation to the segments from which they arose.

It is true that, as Mollier has shown (**24**), the muscle-buds in Elasmobranch fins may be connected together at their base, at all events temporarily, by strands of tissue. It is also true that the mixed motor and sensory nerves form a complicated plexus at the base of, and round about, the radial muscles. But it does not follow that these muscles are either compound or polyneurous. So far as I am aware, it has never been proved that muscle-forming substance actually passes from one bud to another (p. 359); nor has it ever been proved that one radial muscle is really innervated by more than one motor

root. In fact, it seems to be very probable indeed that, even in the adult, the radial muscles are strictly segmental and haploneurous (see below, pp. 364–371). Some fusions may take place, some disturbances of the metameric order may occur, especially at the extreme anterior and posterior ends of the fins; but it is quite firmly established that each adult radial muscle develops from, and corresponds in position to, a single muscle-bud. It may be asserted with confidence that a radial muscle is derived, at least mainly, from that bud whose position it later occupies; and that the radial muscles in the normally developed region of the paired fin of an Elasmobranch corresponds accurately in number and position to the group of primitive buds from which they have been formed.[1]

There is a last objection which Braus persistently reiterates in his papers, and of which he makes a great deal. He alleges that the "concordance" which exists in the adult between the radial muscles and the radial cartilages is not primitive, but secondary. He states that in the early stages of development there are "discrepancies" between these elements, that the muscle-buds do not correspond exactly with the rudiments of the radials, and that the perfect correspondence, or concordance, is gradually established in later stages. This subject will be dealt with later on in greater detail (p. 357); but it may here be said that the evidence on which Braus bases his argument seems to be of the slenderest and most unconvincing nature. Not even in the adult is the concordance perfect; marked disturbances occur at both the anterior and the posterior extremities of the fins. The peripheral ends of the adult muscles correspond exactly with the radials in the middle

[1] If it is objected that in Ceratodus, where the adult paired fin has about thirty radials and radial muscles, only about three segments have been shown to contribute muscle-buds in the embryo (Semon **33**), it must be answered that this result is not trustworthy. Davidoff (**8**) and Braus (**3**) have found twelve spinal nerves contributing to the limb-plexus. It is probable that Rabl's formula holds good in Ceratodus (Mollier **24**), and that a large and sufficient number of segments really contribute muscle-forming cells to the limb, but not in the form of distinct buds.

region; but as they pass inwards to become attached to the base, or the girdle, the muscles no longer preserve the "concordance." On the other hand, nothing is so striking on examining sections through the developing fins of Elasmobranchs, whether paired or unpaired, as the extraordinarily regular "concordance"; it is obvious on the very first appearance of the procartilaginous radial (p. 358, figs. 5, 8, 9, 18).

These attempts to undermine the lateral fold theory, by showing that the adult muscles are compound and polyneurous, and that the concordance is secondary, are not borne out by the evidence. Moreover, even if it could be proved that the metamerism of the fin elements has been lost, the lateral fold theory would scarcely be affected, since it only claims that the muscles and skeletal radials formed a longitudinal series of metameric origin in the beginning. No one doubts that the metamerism has been obscured, or lost, in the higher vertebrates; it matters little, theoretically, whether it still persists in modern fish.

The Gill-Arch Theory.

Let us now pass to the rival theory. It is claimed that the initial stages in the phylogenetic history of the paired fins are more easily accounted for on the gill-arch theory of their origin. Now, according to the lateral fold theory the paired fins appeared, as they do in ontogeny, as longitudinal ridges, which, from their very first appearance, may have been useful as balancing and directing organs. Even in modern fish the paired fins are used not so much for progression as for guidance and balancing.

On Gegenbaur's theory the direction of the paired fins must at first have been dorso-ventral across the long axis of the body; such folds would probably be a hindrance to progression, and both the pectoral and pelvic fins would have been placed close together behind the head in a most unfavourable situation.

The position of the pelvic fin is accounted for by supposing

that it has migrated backwards from the head region. Now, there is no evidence of a more anterior position of the pelvics in primitive fishes generally, either living or extinct. Indeed, the only known fish in which the pelvics are far forward (some Teleostei) are acknowledged to be specialised in this respect. The presence in ontogeny of rudimentary muscle-buds in front of the pelvic fins, is supposed to indicate backward migration. This is negatived by the fact that similar rudimentary buds are found behind the pelvic fin (Braus **4**, Pl. 22, and in this paper, figs. 1, 4, 25). The fins could not have migrated both ways at once, and there is no reason to believe that they first migrated backwards to a point behind the cloaca, and then forwards towards the head.

Davidoff (**8**), Gegenbaur (**15**), and others have held that the presence in front of the pelvic fin of a collector nerve, composed of branches of a number of spinal nerves, and the greater extent of this plexus in the young than in the adult (Punnett **29, 30**), indicates backward migration. But, again, both a similar plexus and extension are found on the posterior side of the fin.

The question of the nerve supply of the fins will be discussed in greater detail later (p. 363); but in describing the general nerve-plexus at the base of the fins one must be careful to distinguish between the collector nerve formed by the convergence and combination of branches of a series of spinal nerves and the plexus proper, due to intertwining secondary branches, made up chiefly, if not entirely, of sensory nerve-fibres. The formation of a collector nerve is simply and easily explained as the result of concentration. The mere presence of a connecting plexus (mainly longitudinal) is due neither to concentration nor to migration (p. 367).

Moreover, both these arguments in support of the theory of migration are sufficiently answered by the fact that rudimentary buds are found both in front of and behind the median fins (Mayer **22** and p. 353 below), and that a longitudinal nerve-plexus may extend along their base even when

the fin is continuous and there is no possibility of migration. Longitudinal connecting nerves have long been known to exist at the base of the unconcentrated fins of Teleostean fish; I find them also at the base of the dorsal fin of Chimæra, which is scarcely, if at all, concentrated.

Objections to the Gill-Arch Theory.

We may now deal with some very serious difficulties in the way of the gill-arch theory. Firstly, it offers no intelligible explanation of the participation of a large number of segments in the formation of the paired fins. Yet it is always the case that a considerable, and sometimes a very large, number of spinal nerves and myotomes contribute towards its development.

Secondly, if the skeleton of the paired fins were derived from gill-rays we should expect the muscle supply to be drawn, not from the myotomes at all, but from the unsegmented "lateral-plate," or visceral, musculature, which is innervated by the dorsal roots of the spinal nerves. It is true that the trapezius muscle attached to the scapula is of lateral-plate origin, and is supplied from the vagus nerve; yet it does not enter into the fin, does not, in fact, belong to the fin musculature. At all events, in the pelvic region there is no trace whatever of other than segmented muscles.

A third, and perhaps still more important, objection to Gegenbaur's theory is this: the position of the limb-girdles in relation to the nerves, blood-vessels, cœlom, etc., is exactly the reverse of what it should be if they were derived from visceral arches. The cœlom, the subintestinal vessel (heart, etc.), the myotomes and their nerves, all pass outside the visceral arches. The limb-girdles, on the contrary, lie morphologically outside these structures, so that the nerves frequently pass through the girdles to reach the fins. In fact, the girdles lie in the outer body-wall, while the visceral arches lie in the wall of the alimentary canal. No mere superficial resemblance in shape of the girdle to the

arch in a developing Elasmobranch, such as is insisted upon by Braus (**5**), no mere opinion, unsupported by evidence, that the relative position of the girdle has been altered, such as is expressed by Fürbringer (**12**), can outweigh these facts.

The fourth and last objection which we shall urge against the gill-arch theory is one which will probably seem to most zoologists to be the most fatal of all: the theory gives no explanation of the remarkable resemblance borne by the paired fins to the unpaired fins. The resemblance is not vague and indefinite, it is minute; it can be followed out in every detail both of their structure and of their development. In no respect is this more striking than in the development and differentiation of the dermal fin-rays in the various groups of fishes.

All these facts, which clearly support the lateral fold theory, are so many deadly blows aimed at the rival gill-arch theory. Far from being difficulties which have to be explained away, they become evidence actually in favour of the fundamental likeness of the paired and unpaired fins.

The Apparent Migration of Fins.

We have now to account for the apparent migration of limbs from one place to another on the body of vertebrates. Every trunk segment may be said to be capable of producing muscular, nervous, and skeletal "limb elements" of a paired character. This "potentiality" is actually called into force in the case of the Rajidæ throughout the trunk region, with the exception of a few anterior segments (see Rabl **31**, Mollier **24**, and especially Braus **3**). In Torpedo, for instance, the 4th to the 30th spinal nerves supply the pectoral fin, and the 31st to the 42nd the pelvic fin. In Trygon the 3rd to the 59th supply the pectoral, and the 60th to the 71st the pelvic fin (Braus). The same conclusion is indicated in the case of forms like Pristiurus and Scyllium, where the paired fins are widely separated, by the development of muscle-buds on all the trunk segments (see

figs. 1, 25). It is also borne out by a comparison of the range of extension of the fins in various genera; for instance, whilst the paired fins occupy segments 5–23 and 47–65 in Zygœna, they occupy segments 2–19 and 29–50 in Heptanchus and segments 2–15 and 19–37 in Chimæra (Braus).

The conclusion that every trunk segment is capable of producing muscular, nervous, and skeletal elements of the median dorsal fin is likewise reached on examining the structure and development of that organ. It is well known that a more or less perfectly continuous dorsal fin still exists in many modern Teleostei, and was present in many extinct forms (Dipnoi, Pleuracanthus). I shall be able to show below (p. 353) that the muscle-buds giving rise to the widely separated adult dorsal fins of Scyllium form a continuous series in the embryo.

Every trunk-segment, then, is potentially able to produce paired and unpaired "fin-elements." But, even if the ancestral Gnathostome was provided with continuous-paired finfolds, the position of the paired limbs of vertebrates can not be accounted for merely on the supposition that these folds have survived in this or that region. The paired limbs have certainly altered in position since they were first established with regard to the numerical order of the segments they occupy. In fact, it is clear that a perpetual shifting of the position of the limbs has taken place in all classes of Gnathostome vertebrates.

It seems to be often held that these changes of position are brought about either by the actual shifting or migration of the limb from one place to another, or by the excalation and intercalation of segments. We cannot, in this paper, enter into a discussion as to the origin and significance of metameric segmentation in vertebrates; but something must be said about the theory of excalation and intercalation, strongly supported many years ago by v. Jhering (**18**). Already it has been so severely and successfully attacked by Fürbringer (**11**) that it can be very shortly dismissed.

In the case of the pelvic fins of Teleosts, for instance, there

are fifteen trunk segments between the pectoral and the pelvic nerve-plexus in Esox lucius, three in Cyprinus tinca, and none at all in Gadus. To account for this by v. Jhering's theory, we must suppose that a new trunk, presumably also new viscera, have developed behind the pelvic fins, while the old trunk and viscera have disappeared in front! Moreover, in Lepidoleprus and Uranoscopus the 3rd spinal nerve shares in both the pectoral and the pelvic plexus.

Still more difficult to explain by excalation and intercalation is the case of the Elasmobranchs. There are twenty-three segments between the pectoral and the pelvic plexus in Zygæna, only three in Pristis, and none at all in many Rajidæ; yet, of course, the other parts remain unaffected.

The evidence of embryology is also thoroughly opposed to such a theory. Comparing various forms, such as Rana with Necturus, Lacerta with a snake, etc., we find large, sometimes vast, differences in the number of segments; we might, therefore, expect to discover in the embryo zones where segments are either being formed or absorbed. Not a trace occurs of such zones of growth or absorption.

The nerve-plexus of the pectoral fin of Spinax occupies ten segments, that of Torpedo twenty-seven, that of Trygon fifty-seven; no sign whatever of zones of excalation or intercalation has been found in their development. It is unnecessary to multiply instances (Fürbringer **11**, Braus **3**).

But if it is difficult to account for the varying position of the paired limbs on the theory of excalation and intercalation, the task becomes impossible if we attempt thus to explain the varying position of both the paired fins and the unpaired fins; for we find that the various fins alter in position and extent independently of each other. No scheme of excalation and intercalation, however ingeniously devised, can ever account for the position of the first dorsal fin opposite the pectoral in Lamna, between the pectoral and the pelvic fins in Alopecias, opposite the pelvic in Scyllium, and well behind it in Raja.

Returning, now, to the other explanation of the change of

position of paired fins, we find that Gegenbaur seems to have held that the whole girdle and fin-skeleton could move from its place of origin, dragging to some extent the muscles and nerves with it. He pointed to the collector nerves and rudimentary buds as evidence of this actual migration of the ready-formed pelvic fin. This argument has already been dealt with above (p. 340), and will be further answered below. Braus believes that he has proved that actual migration of the paired fins takes place during the development of Acanthias. His excellent figures, however, afford convincing evidence to the contrary. It is obvious that if a fin, in ontogeny, moves as a whole, no one part of it can remain in its original position. If now we compare his figure of the earlier with that of the later stage in the development of the pelvic fin (figs. 1, 2, 3 and 4, Pl. 22), we find that the muscle-buds and nerve belonging to segment 36 remain throughout in approximately the same position. The neighbourhood of segment 36, therefore, represents a fixed point. It is true that the fin-fold extends further forward in the earlier stages than it does in the later, and further back in the later than it does in the earlier; but this is due to the fact that the fin develops, on the whole, from before backwards, and undergoes more reduction in front than behind. The apparent migration of the fin from segments 21–30 to segments 30–39, during development, is brought about, not by the actual motion backwards of the whole fin structure, but by the concentration of the fin towards a central region, and by the great reduction of its anterior border.[1]

A fin-fold will appear to move, during development, backwards or forwards, according as there is concentration and reduction, more in the one direction than in the other.

In agreement with this, it is found that a fin-fold, and its contained muscular, nervous, and skeletal elements, are derived from that region of the trunk which is occupied by the adult fin (see further, p. 360).

[1] I am inclined to doubt the correctness of the enumeration of the segments in Braus' figures. No such extensive apparent migration occurs in Scyllium.

The exact origin of the muscles of the paired fins is rarely as easily traceable as in the Elasmobranch; but, as far as is known, the above rule holds good for all Gnathostomes. Unfortunately, in many forms distinct muscle-buds are not produced, and the muscle-producing cells are budded off separately from the Myotomes. Nevertheless, the derivation of the limb muscles has been distinctly traced in the case of various Elasmobranchs, of Salmo (Harrison **19**), of Acipenser (Mollier **26**), Cyclopterus (Guitel **17**), and of Lacerta (Mollier **26**). In all cases where the development has been followed it has been shown that the nerve-supply ("limb-plexus") in the adult is a sure guide to the identification of the segments from which the muscles have been derived. Segments before, and behind, those of the limb-plexus may have ceased to contribute, owing to reduction during development, but adult nerve-supply shows which segments have contributed most.

Unfortunately, with regard to the skeletal element the facts are not so well established. From the very nature of the case, it is much more difficult to deal with. The cartilaginous radials are merely local differentiations in continuous connective tissue, or mesenchyme. And although probably this tissue has itself been derived from segmental sclerotomes, yet the limits of the segments have long ceased to exist when the radials develop. There is, however, no valid reason for believing that radials are less constant than the muscles with which they are related. Nor is there any evidence that the skeleton of the pelvic limb, for instance, is formed of tissue derived from any other segments but those belonging to its muscles.

Of course, limb elements may undergo relative displacement in the course of ontogeny. In the development of fins the anterior muscle-buds are relatively displaced backwards, and the posterior buds are relatively displaced forwards—this is the process of concentration. It may also happen, in the higher vertebrates, that a limb may be shifted a segment or two up, or down, the vertebral column with which

it becomes connected. In the case of the Gadidæ, with jugular pelvic fins, it is clear that these have moved to their position in front of the pectorals. But—and this is the important thing to remember—these limbs do not really lose their original connections, the displacement can be traced in ontogeny, and the nerve supply in the adult infallibly betrays its course.

The Faithfulness of Muscle and Nerve.

That in a series of metameric myotomes and nerves each motor nerve remains faithful to its myotome, throughout the vicissitudes of phylogenetic and ontogenetic modification, may surely be considered as established. That a motor nerve is unable to forsake the muscle in connection with which it was originally developed to become attached to some other seems to be in the highest degree probable, both on physiological and on anatomical grounds. As a matter of fact, this appears always to be the case in the development of limbs.

Now, the paired limbs, and also the median fins, are supplied by branches from a number of segmental nerves forming a "limb plexus." In such a plexus the branches may fuse to common stems, or become joined together by connecting twigs, so that the nerve-fibres appear to become inextricably mixed; at all events, they form a network of mixed fibres (motor and sensory). The motor "plexus" of a limb, so far as it can be said to exist (see p. 366), is brought about, not by the nerve deserting one muscle for the sake of another, but by the combination of muscles derived from neighbouring segments. (I venture to make this dogmatic statement in spite of the fact that the embryological evidence is still, unfortunately, very incomplete because it seems to me to result inevitably from what has been ascertained concerning the anatomy and development of muscles and nerves generally.)

We may thus get compound muscles formed which receive motor branches from more than one spinal nerve. Strictly

speaking, even in this case the nerves in all probability remain faithful to the muscle substance of their own segment, for it has been proved that each motor root supplies its own special muscle-fibres, which are merely bound together in the same muscle (Sherrington **34**).

It seems to me very doubtful whether such compound muscles are ever produced in the fins of fish, and I shall show later (pp. 359 and 369) that there is good reason for believing that the adult radial muscles are both unisegmental and haploneurous. However, compound polyneurous muscles may perhaps be found in fish, as they are in higher vertebrates. Thus segmental nerves, involved in a limb-plexus, may apparently, but only apparently, become connected with muscles belonging to other segments than their own.

The Shifting of Limbs Explained.

Briefly we may repeat, the muscle and nerve-supply is drawn in the embryo from the segments of the region occupied by the limbs in the adult; in cases where the development is unknown, the nerve-supply indicates to which segments the limbs belong. The size of the nerves composing the plexus may be considered as proportional to the importance of the share the several segments take in the formation of the muscles. The muscle-buds and adult muscles in fins are usually better developed in the central regions of the fins than at their two ends. So the nerve components of a limb-plexus are usually stouter in the middle than in front and behind. Just as the muscular elements dwindle or increase in size, owing to the backward or forward extension of the base of a limb, just so far may the nerves increase or diminish in thickness.

The position of a limb-plexus may shift backwards or forwards in all Gnathostomes; no one would suppose that the nerves actually pass up or down through the vertebræ, etc. Fürbringer has clearly shown how the shifting may take place in his important and beautiful works on the anatomy of birds

and reptiles (11, etc.). As may be seen in the diagram (fig. 27), the alteration in position of a limb is due to the contribution made to the limb-muscles, etc., of certain segments at one end becoming less and finally ceasing altogether, while the contribution made by certain segments at the other end becomes correspondingly large. Thus new segments may be taken in at one end and old segments may drop out at the other, or the number of segments contributing may be merely increased or diminished.

A limb may in this way undergo change of position without necessarily undergoing any change of form or structure. The only change involved in the process is that the limb, instead of being derived from a certain set of segments in one region, is derived from a similar set of segments farther up or down the trunk. This is Fürbringer's principle of imitative homodynamy, or parhomology, accompanying the progressive metameric modification of a plexus. To borrow Professor Lankester's illustration, it may be compared to the transposition of a tune from one key to another on the piano. The tune remains the same, but it is played on different notes.

We conclude, then, that the change of position of limbs is not due to the actual migration of the limb-rudiment, or limb-substance, but to reduction on one side and growth on the other. The migration is apparent, not real. It is, if one may be allowed the expression, the calling forth of the potentiality of the segments, which shifts, passing up or down like a wave. This view might be called "the theory of the transposition of the limbs."

The same argument applies to the girdles. In some Elasmobranchs (Braus 3), for instance, the pectoral girdle is pierced by thirty-six nerves belonging to the limb plexus (Trygon), in others by twenty (Torpedo), or by three (Læmargus), in Ceratodus by none at all. These diazonal nerves may each pass through separate foramina, or several may pass through the same foramen. It seems probable, therefore, that the material (scleromere) of a varying number

of segments may share in the formation of the girdle, and that when no diazonal nerves are present only one cartilaginous segmental element is fully developed, at all events at the point where the nerves pass outwards to the limb. When several nerves pass through the same foramen we may suppose that the cartilaginous elements between them have been suppressed. It is interesting to note that in the case of the Chondrostei (Thacher **35**, Wiedersheim **36**, Mollier **26**) the pelvic girdle still shows distinct traces of segmentation. Since, however, the girdles are structures which grow inwards, enveloping the nerve-plexus, with which they only come into secondary connection, it is quite possible that all strict metameric concordance between the two has been modified or lost in most cases. But a limb-girdle may be transposed, like a plexus, by the addition of new elements at one end and their disappearance at the opposite end. And thus is brought about the apparent backward or forward motion of a girdle through a number of segmental nerves, or, in other words, the passage of nerves through a girdle.

To this theory of transposition it may be objected that, if true, the limbs and girdles of the Gnathostomata are not strictly homologous. Now, if by the homology of two structures we mean that they are produced by the same number of segments, occupying in both cases the same place in the metameric series, the limbs and girdles are certainly not always homologous. In this strict and narrow sense they are often not homologous amongst closely allied species, nor in individuals of the same species, nor even on the two sides of the same individual. Fürbringer, Braus, and Punnett have clearly demonstrated the great variability of the nerve-plexus supplying the paired limbs. So long as a distinct individuality and persistence are attributed to each segment, so long as segment x of one animal is considered to be represented only by the same segment x in another animal, the term "homology" can only be applied in a general sense to the limb and its nerve-plexus, etc., as a whole. And let it not be imagined that we can escape from this conclusion by

calling in the aid of the theory of excalation and intercalation (see above, p. 344). The pectoral fin of Spinax, with its ten segments, and that of Trygon, with its fifty-seven segments, cannot be strictly homologous on any theory, whether the extra forty-seven segments have been added in the latter genus, or withdrawn in the former.

Observations on the Structure and Development of the Fins of Elasmobranchs.

The material used was obtained chiefly from the Plymouth Laboratory of the Marine Biological Association; but I also have to thank Prof. Dohrn and Mr. Adam Sedgwick for the generous gift of valuable embryos.

The lateral fold theory is founded on the similarity between the median and the paired fins, yet comparatively little has been published on the development and structure of the median fins of Elasmobranchs since the pioneer work of Thacher (**35**) and Mivart (**23**).

Balfour (**2**) studied their development, and described the origin of the cartilaginous radials from a continuous prochondral plate. An epoch in our knowledge of the median fins dates from the appearance of an important paper by Mayer (**22**). He there describes the development of the skeleton in Pristiurus, and of the radial muscles from muscle-buds, which had already been noticed by Doran (**10**). Attention is drawn to the presence of abortive buds behind the dorsal fins, and the collector nerves and general nerve-plexus is described in many adult forms. But Mayer was unable to trace accurately the relation borne by the buds to the myotomes, nor did he follow out the process of concentration in detail.

Harrison (**19**) has published an excellent account of the development of the median fins in Salmo; in this fish, however, the conditions are somewhat different, and the concentration much less pronounced.

Finally, Braus has lately described some stages in the

ontogeny of the dorsal fin of Acanthias (**5**). But this fin is too much modified to yield much for our purpose.

Development of the Median Fins of Scyllium canicula.

Fig. 1 is a careful reconstruction from longitudinal serial sections of a portion of an embryo about 18 mm. long. Unfortunately, this specimen was cut short at the forty-ninth segment, so that only the first dorsal fin is included.

At this stage is very well shown the origin of the muscle-buds from the myotomes. One bud only is given off by each myotome, not two as was surmised by Mayer. Already the first steps in concentration are discernible in the convergence of the buds towards a central region (about the forty-third segment). The buds dwindle in size on both sides from this region. They can be traced with certainty to the thirty-second segment, and, more doubtfully, even beyond to the twenty-eighth. There appear to be some eighteen buds in all. The rudiment of the fin-fold itself, with its ridge of mesenchymatous tissue indicated by shading in the figure, extends over at least a dozen segments, passing off gradually in front.

Fig. 25 is drawn with a camera from a specimen, 19 mm. long, mounted whole in Canada balsam. The median fins are here slightly more advanced, but only the largest muscle-buds can be made out clearly on this preparation owing to the smaller ones being hidden below the edge of the myotomes. The hinder edge of the first dorsal fin is about at the level of the forty-third ganglion, and that of the second dorsal at the fifty-seventh ganglion. The buds are rather more concentrated.

In fig. 4 are reconstructed the buds of two dorsal fins of an embryo 19 mm. in length. It is important to notice that there is, at this stage, no gap between the two fins. The first bud passing towards the second dorsal lies immediately behind the last given off towards the first dorsal fin.

The two dorsal fins of an embryo 24 mm. long are reconstructed in fig. 2. In the second dorsal the origin of each

bud from its myotome can still be traced, for the most part, with ease, but in the first dorsal, which is a little more advanced in development, concentration is more pronounced. Here some of the posterior and anterior buds are seen to be breaking up into irregular masses of cells, and are rapidly losing their connection with, and becoming separated from, the myotomes from which they arose.

Fig. 3, a reconstructed first dorsal of an embryo 26 mm. long, shows a slightly different case of the same process of concentration. The anterior buds have become separated off in irregular masses, leaving slender stalks, probably nerve rudiments, attached to the myotomes.

An embryo 28 mm. long (fig. 6) shows the muscle-buds beginning to acquire their definitive structure. At their peripheral ends they are still buds of embryonic epithelial tissue; but towards the base of the fin they are becoming changed into muscular tissue (indicated by a paler tint in the reconstruction). A more detailed view of these growing radial muscles is given in fig. 19.

At this stage we can already distinguish twelve well-marked developing radial muscles, corresponding to twelve original buds. That these become gradually converted into twelve adult radial muscles, and were derived from the buds of twelve myotomes, there can be no possible doubt. An examination of numerous intermediate stages proves it.

A mass of tissue derived from muscle-buds is becoming converted into radial muscle at each end of the series. Whether each of these masses is derived from a single bud or from several it is extremely difficult to determine. As already noticed, the buds at the extreme anterior and posterior ends of the fin become irregular in shape and heaped up close together, so that it is impossible to make certain how many persist to form the adult muscles at these two points. That some few of the buds disappear altogether in the course of development seems to be almost certain; but possibly two, or even three, persist at this stage.

The above description applies also to the development of

the second dorsal, which differs only from the first in being rather smaller, and in developing a little later.

Passing now to later stages (figs. 7, 11, 16, and 13), the radial muscles are seen to become thoroughly differentiated, retaining all the while their individuality. The little mass described above at each end develops into a bundle of radial muscle-fibres, which in some cases appears to represent only a single segment. For instance, in the dorsal fins of a Scyllium canicula, about a foot long, dissected in Naples, and shown in fig. 23, there are only twelve muscles altogether. Possibly, however, even here the anterior muscle is compounded of two at least. As a rule the anterior and posterior bundles of muscle-fibres show traces of subdivision in the adult.

The adult dorsal fins vary in their extent and in the number of their constituent parts. Neopolitan specimens generally have twelve radial cartilages, with ten or eleven clearly-defined radial muscles (figs. 22 and 23). Dog-fish from Plymouth usually have thirteen radial cartilages, with eleven or twelve distinct muscles, not counting the complex muscle bundle at each end. Sometimes there are fifteen radials and sixteen muscles (fig. 26).

Development of the Skeleton of the Median Fins.

The first indication of the endoskeleton of the median fins is seen in embryos about 30 mm. long. By this time the muscle-buds are concentrated, but are only just beginning to become converted into muscle-fibres. A slightly darker zone appears near the base of the fin (fig. 17 *b*). Here the nuclei of the mesenchymatous layer filling the fin-fold are rather more closely crowded together. This denser zone spreads a little, and soon between each pair of right and left muscle-buds is seen a dark streak of crowded nuclei, the first rudiment of the radials (fig. 5); this embryo, 28 mm. long, is, however, more advanced than the previous one. In an embryo 33 mm. long the procartilaginous rudiments of the radials are clearly shown (fig. 20). The whole future skeleton is

now faintly indicated; the radials have no definite outlines, and merge together above and below. At this stage the radial muscles are well differentiated (fig. 7).

Cartilage begins to appear near the middle of each radial when the embryo is about 36 mm. in length (fig. 10), thence it spreads upwards and downwards to the joints, where it stops. The proximal and the distal elements are separately chondrified. The dorsal, or distal, edge is the last to become cartilage (fig. 15).

The skeleton of a first dorsal fin drawn in figs. 15 and 16 is of some interest, for unusual concrescence has taken place. Not only are the first two and the last two radials fused ventrally, as is usually the case, but also the 10th and the 11th, and the 10th has fused dorsally with the 9th.

With regard to the very origin of the skeleton—whether the radials appear as separate rods or not—the evidence is somewhat obscure. It is true that a patch of denser mesenchyme is first seen (fig. 17 *b*), but it can hardly be called a plate of procartilage. It is merely a cloud of more densely packed nuclei, and the radials, as such, make their appearance as a series of denser zones separate from each other, and extend outwards into a region not previously occupied by the "plate." This stage may, perhaps, more justly be considered as representing their first appearance. Their apparent continuity I believe to be due to their close approximation, as Mollier has suggested (**24**).

The Development of the Paired Fins.

Little need be said about the paired fins, which have been so thoroughly studied by others.

In Scyllium each muscle-bud, having divided into an upper and a lower secondary bud, develops into a pair of radial muscles. These are regularly formed from their corresponding buds throughout the greater part of the fin (figs. 1, 25, 6, and 9). But at each end, especially the anterior end, is an indistinctly segmented mass of muscle-fibres, probably derived from

several buds. As a rule twenty to twenty-two pairs of radial muscles can be made out in the adult pectoral and pelvic fins.

A denser region of closely-packed nuclei at the base of the fin-folds is the first sign of a skeleton, as in the case of the median fins. Then the girdle, basals, and radials make their appearance as procartilaginous tracts, all in continuity with each other (fig. 9). The radials, however, arise as streaks of denser mesenchyme, which are separate along the greater part of their course. They are continuous only at their base, where they join the girdle or the basipterygium (metapterygium). Even this basal shows faint indications of segmentation as if it had been formed by the concentration and fusion of the radials.

Cartilage is first formed in the girdle and basipterygium (fig. 10), then in the radials. The joints remain unchondrified.

The Concordance between the Muscles and the Radials.

In the adult fins there is almost perfect "concordance" of these elements in the peripheral regions, one cartilage being lodged between two corresponding muscles. But, especially at the anterior edge of the paired fins, and both at the front and hind ends of the dorsals, the agreement is imperfect. Here excessive concentration has taken place; the cartilages are possibly compound, and the muscles are indistinctly subdivided into bundles, which do not agree exactly with them either in number or in shape (figs. 11, 12, 16, 13, and 26). The muscles also generally extend beyond the cartilages. There can be little doubt that most fins contain more muscular than skeletal metameres. Not every segment which contributes a muscle-bud necessarily contributes a skeletal radial.

Now, on tracing the development both of the paired and of the median fins, we find that, so far as the two elements co-exist, they are in exact correspondence. This concordance of the muscles with the radials, which is, indeed, more perfect in the young than in the adult, can be clearly demonstrated

by sections, reconstruction, and whole preparations. Horizontal sections through the dorsals, at a stage when the procartilaginous radials are just beginning to appear, show the concordance quite plainly (fig. 8). In figures of longitudinal sections, passing obliquely through two pelvic fins (fig. 18) the correspondence is obvious. A reconstruction of one of these pelvic fins, in which the ventral muscle buds are drawn in the anterior half and the dorsal buds in the posterior half, is no less conclusive (fig. 9). The same may be said of a reconstruction of the first dorsal of an embryo 33 mm. long, in which the radials are beginning to appear (fig. 7), and also of reconstructions of both a pelvic and a dorsal fin, in which the cartilage is developing (figs. 11 and 12).

The contention of Braus (**4, 7**, see p. 339 above), that the concordance is secondarily established late in development, is utterly at variance with all my observations.

Concentration.

The process of concentration in the median fins can be followed on comparing the whole series of stages from the earliest appearance of the buds to the adult condition (figs. 1–4, 6, 7, and 11).

At first the fin rudiment extends over as many segments as produce buds—about sixteen to eighteen. This ideal first stage is, however, never perfectly recapitulated, since the central buds develop first and become slightly concentrated before the other buds have appeared (figs. 1 and 25).

In later stages the body segments lengthen much faster than the fin rudiment, so that the buds have the appearance of actively growing towards the base of the fin from both sides. As a matter of fact, they probably remain passive during the process. It is possible, however, that they may grow to a slight extent towards the fin, but such a growth would be very hard to prove. On the other hand, they undoubtedly grow outwards into the developing fin-fold.

Both the fin-fold, with its contained muscle-buds, and the

body are growing rapidly; but they lengthen at a very different rate, and it is to this fact that "concentration" is due. An embryo 19 mm. long has the first dorsal fin muscles extending over some fourteen segments (fig. 1), an embryo 26 mm. long over about ten segments, an embryo 28 mm. long over about four and a half segments. Finally, in the adult dog-fish, the base of the muscles occupies about the length of three segments. A minimum of fourteen fin segments has, then, been relatively concentrated into the length of three trunk segments.

During concentration the hinder limit of the dorsal fins remains approximately at the same place—about the level of the forty-second and fifty-sixth ganglion respectively. The two dorsal fins retain their relative distance during the whole of development; the second dorsal is always fourteen segments behind the first. Concentration takes place very much more at the front than at the hind end of the fins. Their anterior edge, then, moves backwards relatively to the body. Each fin, as a whole, remains throughout in the same position.

In the case of the paired fins concentration takes place in exactly the same way. But here it is not so pronounced, and the apparent motion of the fins is less.

The Fusion of the Muscle-Buds.

It has been stated above that in the normally developed region of the fins each muscle-bud gives rise to one radial muscle. Mollier (**24**) describes and figures certain strands of cells which at a certain stage unite the bases of the developing radial muscles, suggesting that the adult muscle may contain cells derived from several buds (p. 338, above). I find similar strands joining the bases of the muscles in both the paired and the median fins of Scyllium canicula (figs. 6 and 19). They are most conspicuous in embryos of about 28 mm. in length. In the early stages of development, when the buds are still of embryonic tissue, no such connecting bands are present. On the other hand, when the radial muscles are

histologically differentiated, the connecting strands can no longer be clearly seen. In late embryos the radial muscles appear to be quite as distinctly separated from each other as in the adult. But in these later stages one can find in sections the network of nerve-fibres which in the adult fin runs along the base of the muscles, and passes from one to the other in a complicated intermuscular plexus.

It seems, therefore, highly probable that the connecting strands of embryonic tissue found by Mollier, Braus (**4**), and myself are really the rudiments of the nerve-plexus. I am, unfortunately, unable actually to prove this; but there is no doubt that the strands occur before the nerve-plexus can be found, and at about the stage when we should expect it to develop. At all events, there is no evidence that any muscle-forming cells pass from one muscle-bud to another.

The Position of the Fins.

In estimating the exact position of the fins at various stages in development only approximate results can be obtained. It is not possible to compare different stages in the growth of one individual, and there is considerable variation amongst several. Moreover, it is probable that, in the course of growth, segments may become incorporated into the occipital region of the head, where myotomes and their nerves may be reduced or obliterated. We can, therefore, never make quite certain that a given segment, say the twentieth, in one adult dogfish corresponds to the twentieth segment in another adult, or to the twentieth in an embryo.

The position of the fins in an adult Scyllium canicula is shown in text fig. 1, and in an embryo, about 19 mm. long, in text fig. 3. In the first the myotomes are not represented; in the second the nerves are omitted, the ganglia only being indicated.

In enumerating the nerves of the adult the spinal nerve issuing immediately behind the skull was counted as the first.

The thirteenth nerve is generally the last to contribute to the pectoral fin plexus. Occasionally the fourteenth also sends a twig, while sometimes the twelfth is the last of the plexus. The second and third nerves generally send branches which, together with the fourth nerve, pass through a foramen in the girdle to reach the fin muscles. The nerves 4–13 pass behind the girdle.

About eleven nerves supply the pelvic fin. Of these the last usually belongs to the thirty-fifth segment and the first to the twenty-fifth segment. The first three nerves may form a collector passing through the girdle. The twenty-fourth and twenty-third may also contribute some fibres in front, and the thirty-sixth and thirty-seventh behind.

The plexus of the first dorsal fin is made of branches from about the twenty-seventh to the forty-third nerves. Very small twigs possibly enter into it from the twenty-sixth and twenty-fifth nerves, but it is probable that these, and perhaps also those of the twenty-seventh and twenty-eighth, are merely sensory. The plexus of the second dorsal spreads from about the forty-fourth to the fifty-seventh nerves.

In the embryo the first nerve was taken to correspond to the first ganglion. Several small myotomes, some four or five, occur in front of the first ganglion. They appear to be represented in the adult by those small myotomes which lie in front of the first spinal nerve, and are supplied by the spino-occipital nerves. Text figure 2 represents the condition of the fins in the adult if concentration had not taken place. The fins have here been deconcentrated.

Now, it is only necessary to compare these three diagrams to see that the position of the fins has remained approximately the same throughout development. Concentration, however, has brought about considerable apparent shifting of the pelvic fins, but there is a fixed point in the neighbourhood of nerves 28–30. In the case of the pectoral fin the drawing back of the anterior margin of the fin has been almost entirely compensated by the drawing forward of the posterior margin, so that in spite of great concentration the position of the fin

3
n.1
n.13
n.25
n.35
1d
n.44
2d
n.57
a
pl
pt
2
n.1
n.13
n.25
rm
n.35
n.44
cr
n.57
1
n.1
n.13
n.25
ac
n.35
n.44
pc
n.57

is unchanged. Much more pronounced is the apparent shifting backwards of the dorsal fins. While their hinder margin is only slightly carried forwards, the anterior margin retreats over some eight or nine segments. Here, again, there are fixed points about the fortieth and fifty-fourth nerves which do not move at all (pp. 343–352).

On the Nerve-Supply of the Fins.

We must now more closely examine the structure of the plexus of nerves which supply the fins, dealing more particularly with the median fins.

Mayer (**22**), to whom I am much indebted for many useful hints on the best methods for this purpose, has described and figured the nerve-plexus of the median fins of many Elasmobranch fish. But he did not follow out in detail the relation of the nerves to the fin muscles and to the body segments. With the object of continuing and extending his researches, I have dissected the nerve-plexus in a large number of specimens. For this purpose material has been used after treatment with hot water, or after maceration in weak nitric acid. Osmic acid added to these preparations brings out the nerves most distinctly. Owing to the delicate and complex nature of the plexus of the median fins and to the very brittle state of the nerves, it is very difficult indeed to obtain a perfectly complete dissection of the plexus in a single specimen. The

---

EXPLANATION OF TEXT-FIGURES.

Text-figure 1.—Diagram of an adult Scyllium canicula, showing the nerve-supply of the fins.

Text-figure 2.—Diagram of an adult Scyllium canicula. The fins are expanded, and their nervous, muscular, and skeletal segmental elements are distributed as if concentration had not taken place. The nerve foramina in the girdles are indicated by shaded oval areas; the girdles themselves are not shown.

Text-figure 3.—Diagram of an embryo Scyllium canicula about 19 mm. long, in which are shown the ganglia, the myotomes, and the muscle-buds. *a*, Anal fin; *ac*, anterior collector of first dorsal fin; *cr*, cartilaginous radial projecting beyond the radial muscles; *n* 1–57, spinal nerves and ganglia; *pc*, collector nerve of second dorsal fin; *pl*, pelvic fin; *pt*, pectoral fin; *rm*, radial muscle; 1*d* and 2*d*, first and second dorsal fins.

plexus supplying the paired fins is stronger and much less difficult to expose. The pectoral and pelvic plexus have been admirably described and figured by Braus (**3, 6**) in a large number of Elasmobranchs, while Punnitt (**29, 30**) has studied the pelvic plexus in Mustelus and Acanthias.

The nerve-plexus of the paired fins is very variable, both as regards the number of nerves which contribute towards it and the exact course of its secondary branches. Such is also the character of the nerve-plexus of the dorsal fins; but here it is less easy to decide as to the exact number of nerves which enter into its composition. In minor details no two specimens correspond, and even the two sides of the same individual may differ considerably. However, on the whole, there is great constancy in the character and metameric value of the plexus of the median fins, as is shown by comparing a large number of specimens. Unfortunately, it is so complex, and the nerve branches are so fine, that I have not found it possible to trace out its formation in ontogeny.

The plexus of the dorsal fins.—We have seen above that a dorsal fin contains some fourteen muscle segments. We should, therefore, expect at least fourteen spinal nerves to join in its formation. Moreover, since concentration takes place to a much greater extent in front than behind, we should expect the longitudinal collector, formed by the gathering together of various nerve components, to be situated chiefly in front of the fin-base. Now this is just what dissection reveals.

Figs. 21 and 22 show the general nerve-supply of the two dorsal fins. A comparatively stout collector is seen to run forwards from the base of each fin (*alc.*). It is composed of a number of twigs derived from the rami dorsales of some dozen segments. The collector increases in bulk as it passes backwards, and more nerves enter into it. Where the collector begins is often very difficult to determine, in the case of the first dorsal especially; for its first components are so extremely slender that they are very hard to discriminate from the intercrossing plexus of nerve-twigs which are present all along the median dorsal septum.

The collector gives off branches to the fin as soon as it reaches its base, and often ceases about half way down the fin. Then come one or two nerves which give off branches independently to the fin. In many fins all the nerves passing to the fin are joined together by communicating branches, continuations of the collector (fig. 26).

At the hind end of the fin are one or two nerves with a short, and often ill-defined, posterior collector (*plc.*).

When pterygial nerves, passing out from the collectors, reach the base of the radial muscles, they run in amongst them and branch repeatedly. A plexus of extraordinary complexity is thus formed round and through the muscles and along the cartilages outwards to the web of the fin.

We find, then, that some fourteen to sixteen spinal nerves undoubtedly contribute to the innervation of the first dorsal fin, and that the rami pterygiales of those situated in front of the middle of the fin-base always combine to a longitudinal collector. The collector clearly shows that the radial muscles derived from these segments have been displaced backward. The one or two rami pterygiales gathered into a posterior collector indicate a similar but very much less extensive concentration forwards.

An examination of the nerve-supply of the second dorsal fin yields the same results. The anterior collector of this fin begins immediately behind the posterior collector of the first dorsal.

The anatomy of the adult fully bears out the conclusion arrived at from a study of the development; the dorsal fins are made up of a large number of greatly concentrated segmental elements—muscular, nervous, and skeletal. The lateral fold theory is, then, strongly supported by our knowledge of the structure and development of the median and paired fins, since the paired fins have long been known to be constructed and developed on exactly the same principle.

But there remains one important, though not essential, question to discuss: How far is the original metameric structure preserved in the adult?

With regard to the musculature, we have already decided that there is no definite evidence that the metamerism is lost (p. 359). The skeleton is still obviously segmentally divided in the dorsal fins of Scyllium, in spite of the slight concrescence of some of the radials at their base. The radials of the paired fins have undergone much greater fusion and modification. But there is nothing in their structure or development which precludes the idea that even the basals were once metamerically segmented. In modern sharks, however, this segmentation of the skeleton of the paired fins is to a great extent lost. It is to the nerves that appeal is generally made for evidence against metamerism (pp. 338–340); let us, therefore, examine further the nerve-supply of the fins.

On the real nature of the nerve-plexus.—Many anatomists seem to consider that the nerve-plexus is formed by a combination of several nerves, which lose their individuality, and are then redistributed to the limb, somewhat as a number of blood-vessels may anastomose and supply a gland. In such a case the nerves would be so mixed in the plexus that even their motor fibres might lose all trace of metamerism.

But such is not really the case, even in the highest vertebrates, as Herringham (**21**), Patterson (**27**, **28**), and others have shown.

Now, we may well ask whether in the Elasmobranch there is really any motor plexus at all, if by plexus is meant a mixing of nerve-fibres bringing about a disturbance or destruction of the original metamerism. If the nerves could be traced to each radial muscle of a fin, it would be easy enough to prove whether or not it is the case. Unfortunately, dissection can help us but little in settling this point. Most of the nerves to the paired fins pass directly to the fin-base; but as soon as they reach it they become joined together by a complicated system of connecting nerves, even before they enter the muscles. When they reach the latter they become involved in such a complex network that it becomes impossible to determine for certain whither the nerve-fibres lead. That on the whole each nerve supplies two radial muscles in

regular order can be fairly well established; but it cannot be asserted that they do not also supply others. In fact, I have found it impossible to prove by mere dissection that these muscles are haploneurous. Nevertheless, it can be shown that the muscles of the paired fins are innervated in regular order from before backwards by the spinal nerves, each of which supplies a pair above and below.

Turning to the dorsal fins, we find that not only do the rami pterygiales form a longitudinal collector, in which it is impossible to follow out for certain the nerve-fibres from individual segments, but also that the branches running to the fin from the collector form a plexus of even more complicated structure. Over and over again have I tried in vain to follow the nerve-fibres from a spinal nerve to a radial muscle. It must be remembered that the rami pterygiales are nerves of mixed character, containing motor and sensory fibres. The real difficulty is, not to trace a branch to a muscle, but to make sure that no motor fibres from that nerve pass on elsewhere to other muscles along the ramifying twigs of the plexus.

Having failed to analyse the plexus of the dorsal fin by dissection, it remained to be seen whether any fin could be found in which the motor fibres are distinguishable from the sensory. Such a condition I discovered in the ventral lobe of the caudal fin of Scyllium.

The small radial muscles with which this lobe is provided, unfortunately, do not develop from regular muscle-buds, so they cannot be traced in ontogeny to the myotomes. They are subdivided into a large number of small bundles, much more numerons than the segments, and are developed from cells which come off from the proliferating lower edge of the myotomes. The same thing occurs in the anal fin.

Fortunately, in the tail of Scyllium the nerves from the ventral motor roots do not combine into mixed trunks with those from the dorsal sensory roots. Both the motor and the sensory branches pass obliquely downwards to the base of the fin. Here they form an elaborate plexus (fig. 24), in

which can be distinguished a large, longitudinal "collector" and twigs running outwards to the radial muscles and skin of the fin. Now, by careful dissection, under the high powers of the binocular microscope of Zeiss, one can follow out the motor and the sensory fibres to their destination. It soon becomes evident that, while the latter combine to form the longitudinal trunk and the plexus of anastomosing nerves, which send branches at intervals to the skin (fig. 24), the motor fibres pass through the plexus without really becoming involved in it. Each spinal nerve sends down motor branches supplying a considerable number of radial muscles. It is, of course, by no means easy to follow out every twig to its ending; but from a careful and minute study of several tails I have satisfied myself that the motor branches of one segment do not anastomose or mix with those of another segment —the area supplied by one motor root begins where that of another ends. In the specimen figured there is one twig (marked with an *), in two segments, which seems to join one segment to the next behind; but I am inclined to believe that the fibres do not mix peripherally. In other tails investigated since, I have found no such junction. At all events, the facts are quite compatible with the view that no mixture takes place.

There appears, then, to be no such thing as a real motor plexus in the caudal fin. Whether there is a true sensory plexus, or whether it is more apparent than real, I am unable to determine for certain, as the fibres cannot be disentangled.

Seeing that the so-called motor "plexus" in the caudal is probably only apparent, we may well ask whether a true motor plexus exists in any of the dorsal or paired fins. May it not be that here also the motor fibres pass through a sensory network and do not lose their original metameric order?

I am strongly of opinion that this is the case, and that the radial muscles are haploneurous, the original metamerism of the fin being preserved in the adult. Since this question cannot be answered by anatomy, we must appeal to experiments on the living tissues.

Experiments on the nerve-supply of the fins.—While occupying the British Association table at the Naples Aquarium last winter, I had the opportunity of conducting some experiments with a view to tracing out the nerve-supply of the radial muscles. I have to thank Prof. Gotch for advice as to the best way of stimulating the nerves, and Mr. G. W. Smith for helping me to carry out the experiments.

If in the limb-plexus the motor fibres of various segments were crossed or mixed, and if, as some authors contend, the radial muscles were polyneurous, we should expect to produce a general, or at least an extensive, contraction of the fin-muscles on the stimulation of one nerve. To test these views experiments were made on the pectoral fin of Raja. Owing to its great size, the muscles and nerves of this fin are peculiarly well adapted for the purpose.

The first series of experiments were made by directly stimulating individual spinal nerves, and watching the contraction of the radial muscles. To insure definite and correct results, the nerves were first severed near the spinal cord, and were then stimulated in various ways at their proximal end, the necessary precautions having been taken to keep the tissues in good condition. The electric stimulus is the easiest to use; but a mechanical stimulus, applied either by snipping the nerves with scissors, by ligaturing with a thread, or by pinching with ivory forceps, gives, perhaps, the surest result.

It was found that the series of radial muscles of one side could be made to contract regularly, in pairs, from before backwards, by stimulating the successive nerves of the plexus, beginning at the anterior end. Similarly the muscles contract regularly in pairs from behind forwards on stimulating the nerves in the reverse direction. It was determined with absolute certainty that the stimulation of one nerve does not produce a general contraction of the muscles of the fin, but only of a limited portion of the musculature corresponding in position to the nerve.

Owing to the fact that the radial muscles lie very near

together, closely bound to each other and to the skeleton by the connective tissue which surrounds them, it is difficult to establish, without the possibility of doubt, that the contraction is restricted to two radial muscles only. Nevertheless, after repeated trials, I am quite convinced that such is really the case. There can be no doubt whatever that the contraction does not spread to several neighbouring muscles.

If all the nerves, excepting one or two, are severed from the spinal cord, and if then a general stimulation be induced through the cord, only those pairs of muscles contract which correspond to the one or two nerves left intact.

The following experiment was also repeatedly made to determine whether the nerve-supply of neighbouring segments overlaps. Three consecutive nerves of the plexus, A, B, and C, were severed from the spinal cord. The two outer ones, A and C, were then excited by constant application of the electric stimulus until the corresponding pairs of radial muscles scarcely, if at all, responded. Then the middle nerve B was stimulated, and its muscles were found to respond in perfectly normal fashion. They contracted equally well whether the outer nerves were still being stimulated or not. This seems to me to prove, without the possibility of doubt, if not that there is no overlap whatever, at all events that it can only be very slight.

So clear and definite was the evidence derived from these and other experiments of a like nature, that I have no hesitation in stating my opinion that each pair of radial muscles (containing two dorsal and two ventral elements) derived from a single segment, is supplied exclusively with motor fibres from the ventral root of the nerve belonging to that same segment. In fact, no plexus exists in the pectoral fin of Raja in the sense of a mixture or overlapping of the areas supplied by the segmental motor nerves.

So far as experiments were conducted on the pelvic fins they gave the same results.

Unfortunately, the dorsal fins of Scyllium do not lend them-

selves so readily to experiment. I have not yet been able to apply so decisive a set of tests to the delicate "plexus" supplying these fins. But it can be easily shown that the successive stimulation of the rami dorsales sharing in the "plexus" induces the successive contraction of the series of corresponding radial muscles.

It results from these experiments that the metamerism of the fin elements may remain undisturbed in the paired, and probably also in the unpaired, fins of Elasmobranchs. In this they agree with the evidence of embryology.

Summary and Conclusion.

The chief observations described above may be summarised as follows: The development of the median dorsal fins is essentially similar to that of the paired fins. They arise as longitudinal folds, into which grow buds from the myotomes. Some fourteen or sixteen myotomes contribute to the fin each one muscle-bud. Concentration sets in almost from the first appearance of the buds; it is chiefly, if not entirely, due to the body growing faster than the fin. Along the greater part of the dorsal fin each muscle-bud becomes converted into one radial muscle. At the extreme ends of the fins the exact metameric origin of the muscles is difficult to trace and is somewhat obscured. Only here fusion of neighbouring segmental buds perhaps takes place. At certain stages slender strands of embryonic tissue connect the bases of the radial muscles; these are probably rudiments of the nerve-plexus. Neither the study of development nor of the adult structure affords any definite evidence that the primitive metamerism of the musculature is lost. Experiments seem to establish that the radial muscles remain haploneurous, retaining their primitive connection with the nerve belonging to that myotome from which they have been developed. The nerve-"plexus" of the fins is composed of intertwining sensory fibres, along or through which the motor fibres proceed to their destination without mixing with those of other segments. There is

probably no real motor plexus, but the motor nerves may be gathered together into more or less longitudinal collectors, and become again sorted out on reaching the musculature. Such collectors are found at the base of the dorsal fins, compounded of some fourteen to sixteen segmental rami pterygiales. All the fins remain throughout development in approximately the same position. Apparent change of place may be brought about by concentration being greater in the one direction than in the other. This is especially the case with the dorsal fins, the anterior edge of which may undergo a relative shifting over some ten segments.

The general bearing of these results has been sufficiently discussed in the Introduction (p. 334), and need not again be dealt with here. But it may be pointed out how completely they support the lateral fold theory of the origin of the paired fins.

List of References.

1. Balfour, F. M.—"A Monograph on the Development of Elasmobranch Fishes," 'Journ. of Anat. and Phys.,' 1876–8.

2. Balfour, F. M.—"On the Development of the Skeleton of the Paired Fins of Elasmobranchii," 'Proc. Zool. Soc. London,' 1881.

3. Braus, H.—"Ueber die Innervation der Paarigen Extremitäten bei Selachiern," 'Jen. Zeitschr. für Naturw.,' Bd. xxix, 1895.

4. Braus, H.—"Beiträge zur Entwickl. d. Muskulatur u. d. periph. Nervensystem d. Selachier," 'Morph. Jahrb.,' Bd. xxvii, 1899.

5. Braus, H.—"Thatsächliches aus der Entwickl. d. Extremitätenskelettes," 'Haeckelfestschrift Denkschr. Med.-Nat. Ges.,' Jena, 1904.

6. Braus, H.—"Die Muskeln u. Nerven der Ceratodusflosse," 'Zool. Forsch. Denkschr. Med.-Nat. Ges.,' Jena, 1901.

7. Braus, H.—"Die Entwickl. d. Form. d. Extremitäten u. d. Extremitätenskeletts," 'Hertwig's Handbuch d. vergl. u. exp. Entwick. der Wirbeltiere,' 1904.

8. Davidoff, v. M.—"Beiträge z. vergl. Anatomie der hinteren Gliedmasse der Fische," 'Morph. Jahrb.,' Bd. v, 1879.

9. Dean, B.—"Historical Evidence as to the Origin of the Paired Limbs of Vertebrates," 'Am. Nat.,' v. xxxvi, 1902.

10. Dohrn, A.—"Studien z. Urgeschichte des Wirbelthierkörpers: VI. Die Paarigen u. Unpaaren Flossen der Selachier," 'Mitth. Zool. Sta. Neapel.,' Bd. v, 1884.

11. Fürbringer, M.—"Zur Lehre von den Umbildungen des Nervenplexus," 'Morph. Jahrb.,' Bd. v, 1879.

12. Fürbringer, M.—"Ueber die Spino-occipitalen Nerven," 'Festschrift für Carl Gegenbaur,' Bd. iii, 1897.

13. Fürbringer, K.—"Beiträge z. Morph. des Skeletes der Dipnoer," 'Zool. Forsch. Denkschr. d. Med.-Nat. Gesell.,' Jena, 1904.

14. Gegenbaur, C.—'Untersuchungen z. vergl. Anatomie der Wirbelthiere,' II, Leipzig, 1865.

15. Gegenbaur, C.—"Zur Gliedmassenfrage," 'Morph. Jahrb.,' Bd. v, 1879.

16. Gegenbaur, C.—"Das Flossenskelet der Crossapterygier u. das Archipterygium der Fische," 'Morph. Jahrb.,' Bd. xxii, 1895.

17. Guitel, F.—"Recherches sur le développement des nageoires paires du Cyclopterus lumpus, L.," 'Arch. u. Zool. exp. et genér.,' 3rd série, v, 1896.

18. Jhering, v.—'Das Periph. Nervensystem d. Wirbelthiere,' Leipzig, 1878.

19. Harrison, K. G.—"Die Entwickl. der Unpaaren u. Paarigen Flossen der Teleostier," 'Arch. f. Mikr. Anat.,' Bd. xlvi, 1895.

20. Haswell, W. A.—"On the Structure of the Paired Fins of Ceratodus," 'Proc. Linn. Soc. New South Wales,' vol. vii, 1883.

21. Herringham, W. P.—'The Minute Anatomy of the Brachial Plexus," 'Proc. Roy. Soc.,' vol. xli, 1886.

22. Mayer, P.—"Die Unpaaren Flossen der Selachier," 'Mitth. Zool. Sta. Neapel.,' Bd. vi, 1886.

23. Mivart, St. G.—"Notes on the Fins of Elasmobranchs," 'Trans. Zool. Soc. London," vol. x, 1879.

24. Mollier, S.—"Die Paarigen Extremitäten der Wirbelthiere: I. Das Ichthyopterygium," 'Anat. Hefte,' Bd. i, 1893.

25. Mollier, S.—"Die Paarigen Extremitäten," etc.: II. "Das Cheiropterygium," 'Anat. Hefte,' Bd. v, 1895.

26. Mollier, S.—"Die Paarigen Extremitäten," etc.: III. "Die Entw. d. Paar. Flossen des Störs," 'Anat. Hefte,' Bd. viii, 1897.

27. Patterson, A. M.—"The Position of the Mammalian Limb," 'Journ. of Anat. and Phys.,' vol. xxiii, 1889.

28. Patterson, A. M.—"The Origin and Distribution of the Nerves of the Lower Limb," 'Journ. of Anat. and Phys.,' vol. xxviii, 1893–4.

29. Punnett, R. C.—"On the Formation of the Pelvic Plexus, etc., in Mustelus," 'Phil. Trans. Roy. Soc. London,' vol. B cxcii, 1900.

30. Punnett, R. C.—"On the Composition and Variations of the Pelvic Plexus in Acanthias vulgaris," 'Proc. Roy. Soc. London,' vol. lxix, 1901.

31. Rabl, C.—"Theorie d. Mesoderms," 'Morph. Jahrb.,' Bd. xix, 1892.

32. Ruge, E.—"Die Entwickl. Skeletes der vorderen Extremitätem von Spinax niger," 'Morph. Jahrb.,' Bd. xxx, 1902.

33. Semon, R.—"Die Entwickl. d. Paarigen Flossen des Ceratodus forsteri, 'Zool. Forsch. and Jen. Denkschr.,' Bd. iv, 1898.

34. Sherrington, C. S.—"Notes on the Arrangement of some Motor Fibres in the Lumbo-sacral Plexus," 'Journ. of Phys.,' vol. xiii, 1892.

35. Thacher, J. K.—"Median and Paired Fins," 'Trans. Connecti. Acad.,' vol. iii, 1877.

36. Wiedersheim, R.—'Das Gliedmassenskelet der Wirbelthière,' Jena, 1892.

37. Woodward, A. S.—"The Evolution of Fins," 'Natural Science,' 1892.

EXPLANATION OF THE PLATES.

Illustrating Mr. E. S. Goodrich's paper, "Notes on the Development, Structure, and Origin of the Median and Paired Fins of Fish."

Lettering of the Figures.

a. f. Anal fin. *a. l. c.* Anterior longitudinal collector nerve. *an.* Connecting strand of tissue. *bp.* Basipterygium. *br. ar.* Branchial arch. *ca.* Nerve canal. *c. r.* Cartilaginous radial. *c. t.* Connective tissue. *d. f.* Dorsal fin. *d. r.* Dorsal root. *g.* Ganglion. *h. m.* Hypoglossal musculature. *l. s. c.* Longitudinal sensory collector nerve. *m.* Myotome. *m. b.* Muscle-bud. *m. f.* Motor fibres. *n.* Nerve. *n. a.* Neural arch. *n. cd.* Nerve-cord. *p. g.* Pelvic girdle. *p. l. c.* Posterior collector nerve. *p. r.* Procartilaginous radial. *pt. f.* Pectoral fin. *pv. f.* Pelvic fin. *r.* Radial or somactid. *r. m.* Radial muscle. *r. pt.* Ramus pterygialis. *s.* First indication of the skeleton. *s. f.* Sensory fibres. *v.* Vagus. *v. r.* Ventral root. *x.* is placed in front of a number which could not be accurately determined.

All the figures refer to Scyllium canicula, and the arrows point towards the head. In several of the figures the myotomes, their muscle-buds, and the radial muscles are drawn in red. Blue in fig. 24 represents sensory nerves.

The spinal nerves in the embryo are numbered from the first ganglion, in the adult from the first which issues behind the skull. About five myotomes are found in embryos in front of the first ganglion. When portions of the embryos were cut off before imbedding, the number of the ganglia was estimated, and an *x* inserted before it to denote its uncertainty, as in fig. 4.

PLATE 10.

Fig. 1.—Reconstruction from serial longitudinal sections of a portion of an embryo about 18 mm. long. It is cut off behind the first dorsal fin. The pectoral fin has been cut off near its base.

Fig. 2.—Reconstruction of the first and second dorsal fins of an embryo 24 mm. long.

Fig. 3.—Reconstruction of the first dorsal fin of an embryo 26 mm. long.

Fig. 4.—Reconstruction of the first and second dorsal fins and of a portion of the pelvic fin of an embryo 19 mm. long.

Fig. 5.—Longitudinal vertical section of the hinder region of the first dorsal fin of an embryo 28 mm. long. The radials are beginning to appear; the extremities of several muscle-buds are seen above. 'Cam. Ob. Z.,' *a a*, oc. 3.

PLATE 11.

Fig. 6.—Reconstruction of a portion of an embryo 28 mm. long (same as that in fig. 5), with the first dorsal and pelvic fins.

Fig. 7.—Reconstruction of the first dorsal fin of an embryo 33 mm. long. Both the muscles and the procartilage radials are represented.

Fig. 8.—Longitudinal horizontal section of the first dorsal fin of an embryo 32 mm. long. Z. A. oc 3, Cam.

Fig. 9.—Reconstruction of the pelvic fin of an embryo 28 mm. long. The procartilage skeleton is represented complete; the anterior twelve ventral muscle-buds and ten posterior dorsal muscle-buds are indicated. At this stage the base of the muscle-buds is being converted into contractile tissue, which is not represented in the figure.

Fig. 10.—Reconstruction of a portion of the vertebral column, the skeleton of the first dorsal, and of the pelvic fin of an embryo 37 mm. long. The cartilage is beginning to develop.

Figs. 11, 12.—Reconstructions of the first dorsal fin (fig. 11), and the pelvic fin (fig. 12), of the series represented in fig. 10. The muscles are outlined in red and the skeleton in black.

PLATE 12.

Fig. 13.—The skeleton and muscles of the first dorsal fin of an adult.

Fig. 14.—Oblique longitudinal section of the pelvic fin reconstructed in fig. 9. Traces of segmentation extend into the basipterygial region.

Fig. 15.—Reconstructed skeleton of the first dorsal fin of an embryo 55 mm. long. Procartilage extends along the dorsal edge.

Fig. 16.—Portion of the vertebral column, skeleton of the first dorsal fin (without the procartilage), and radial muscles of the embryo 55 mm. long represented in fig. 15.

Fig. 17A.—Reconstructed muscle-buds of the first dorsal fin of an embryo 30 mm. long.

Fig. 17B.—Longitudinal vertical section of the first dorsal fin, drawn to the same scale as fig. 17A and taken from the same series, showing the first indication of the skeleton.

Fig. 18.—Longitudinal section of the pelvic fin of an embryo, 33 mm. long.

Fig. 19.—The base of three radial muscles, showing connecting strands of tissue (*an.*). Embryo 28 mm. long. 'Cam. Ob. Z. D.,' oc. 2.

Fig. 20.—Longitudinal vertical section of the first dorsal of an embryo 33 mm. long, reconstructed in fig. 7.

PLATE 13.

Fig. 21.—Dorsal branches of the spinal nerves 24–57 of an adult, showing the longitudinal collectors near the dorsal fins.

Fig. 22.—Similar figure of an individual about 25 cm. long. The skeleton of the fins is indicated.

Fig. 23.—First dorsal fin of the same individual showing the radial muscles.

Fig. 24.—Nerve-supply of the anterior region of the ventral lobe of the caudal fin of an adult. The muscles are shown only in one part. The motor-nerves are in black and the sensory are drawn in blue. * indicates twigs which appear to mix with neighbouring nerves.

PLATE 14.

Fig. 25.—Embryo, 19 mm. long, mounted in Canada balsam. Cam.

Fig. 26.—Skeleton (indicated by a dotted line), and muscles of the first dorsal fin of an adult. The nerve-plexus at the base of the fin is shown and the beginning of the collector of the second dorsal fin.

Fig. 27.—Diagram to illustrate the transposition of limbs.

Preliminary Account of a New Organ in Periplaneta orientalis.

By

Ruth M. Harrison,
Lady Margaret Hall, Oxford.

With Plate 15.

While dissecting a cockroach in the Zoological Laboratory at Oxford I noticed a pair of small oval pouches lying below and to either side of the nerve-cord between the fifth and sixth abdominal ganglia. Being unable to find either description or figures of any such structure, I proceeded to examine it further by means of dissection and serial sections.

The position of the organ is shown in fig. 1. This is a dissection of the posterior segments of a male cockroach from which the tracheal system has been removed. In a freshly-killed specimen it has a yellowish, transparent appearance which renders it somewhat inconspicuous; but it was present in every full-grown cockroach that I examined, both male and female. In the male it measures about 2 mm. in length, but in the female it is very much smaller, never being more than about half the size of that of the male; sections, however, show no histological difference. If the nerve-cord is stretched to one side, the pouches, which before appeared to be separate, are seen to be two lobes projecting upwards and forwards from a median structure, which opens below to the exterior by a single aperture on the ventral surface of the animal between the sixth and seventh sternites. This is clearly seen by a comparison of figs. 4 and 5, which

are transverse sections through the same specimen. The section represented in fig. 4 is taken just behind the fifth abdominal ganglion, and shows that anteriorly the pouches are quite separate; fig. 5 represents a more posterior section, showing that the two lobes unite to form a single pouch. The median portion of the anterior border of the seventh sternite is hollowed out in the form of a crescent, the edge of which forms a distinctly thickened rim of chitin; this is seen in longitudinal section in fig. 3 and in transverse section in fig. 5. Fig. 3 also shows the opening to the exterior in its natural position; the sternites overlap to a considerable extent, so that only half of each plate is exposed to a surface view; this gives the appearance in section of a long duct leading to the exterior, but the limit of the organ is marked by the thickened chitinous rim on sternite 7.

A dorsal view of the structure has the appearance represented in fig. 2. In this figure the nerve-cord has been removed altogether, but the tracheal system has been preserved intact. The paired main ventral longitudinal vessels are seen to lie above the organ, and a complicated system of smaller vessels is distributed over its entire surface.

In preparing specimens for microscopic examination various methods of softening or dissolving the chitin were attempted, but the most satisfactory results were obtained by painting with collodion and cutting through the chitin, thus preserving the soft structures in their natural position. Corrosive-acetic and Perenyi's chromo-nitric proved the best fixatives, and borax carmine and hæmatoxylin were used as stains.

In section the structure of the wall of the organ at once suggests some kind of gland. Round the anterior lobes and on the dorsal surface is a layer of elongated cells with their long axes vertical, and having large rounded nuclei placed towards the periphery; the cytoplasm shows a very fine granular consistency, and in each cell is a stoutish tube, one end of which appears to open near the nucleus, and the other is directed towards the lumen of the gland. Round this tube the cytoplasm is denser, the granules being here more closely

accumulated. In a surface view of the wall these cells are seen to be hexagonal in shape and to fit together with great regularity. Towards the external opening these cells pass imperceptibly into the epithelial layer below the cuticle. Within this layer is a mass of tissue where all cell outline has become entirely obliterated ; the nuclei are quite irregular in shape, and the cytoplasmic portion of the cell is represented by scattered fragments bounding large vacuoles. Lining the whole cavity internally is a thin transparent membrane, continuous with the chitinous skeleton of the insect. This appears to be of the nature of chitin since it remains unaffected by the action of potash. It is very much folded and crumpled, and attached to it, projecting away from the lumen of the gland, are numerous bunches of extremely long and fine hair-like processes, which also seem to be chitinous, remaining unchanged by maceration in potash. These are not very obvious in sections except at the point where each hair is attached to the lining membrane, for here a distinct minute circular spot is visible. In a preparation in which the protoplasmic structures have been dissolved in potash these processes become clearer. They are attached irregularly in little groups of two or three to about a dozen or more, while some occur singly.

From the above description the glandular nature of the organ becomes apparent. It would seem that the secretion is stored in the form of granules in the cells of the outer layer, and that these cells migrate inwards—i. e. towards the lumen of the gland (figs. 6, 7), where they disintegrate.

In a young specimen the gland consists of a single layer of epithelial cells of a non-granular character with large oval nuclei, lined by a comparatively broad belt of chitin continuous with the cuticle of the animal. The non-granular condition of the epithelial layer, and the entire absence of any intermediate tissue like that present in the adult organ, is doubtless due to the fact that the gland has not yet become actively secretive. An inspection of the sections represented in figs. 4, 5 seems to afford evidence that the cells do actually

migrate inwards and disintegrate; the animal from which this gland was taken was a fine large male, and the scarcity of granular cells may possibly be explained by the fact that the organ is ceasing to be functional, and is in a degenerate condition. The nature of the secretion and the function of the whole organ I have not so far investigated; but this I hope to do, together with a further study of the individual elements and their relations to each other.

The curious tubes in the outer granular layer bear a strong resemblance to certain tubes described and figured by Claus in a stink-gland of the larva of the Coleopteron Chrysomela. But the hairs are quite unlike those described by Minchin in the dorsal glands of this same species of Periplaneta, or those described by Kraase in a dorsal stink-gland of the Blattid Aphlebia, in that they project inwards, away from the lumen of the gland. Whether there is any definite relation between these tubes and the bunches of hairs I have not been able to ascertain. The tubes seem to come to an abrupt end near the nucleus, and I cannot trace them beyond the limit of the granular cells; the hairs are comparatively stout and cylindrical at their bases, but appear gradually to taper away at their free extremities. But it seems doubtful that this should actually be the case; for the chitinous lining membrane is quite continuous, and it is difficult to see how the secretion reaches the exterior unless these hairs serve in some way as a means of communication between the lumen of the gland and the secreting cells.

In the above description it has been shown that Periplaneta orientalis possesses a glandular organ on its ventral side, lying in the sixth abdominal segment, between the fifth and sixth abdominal ganglia, and opening to the exterior between the sixth and seventh sternites. This opening is median, and from it the gland extends upwards and forwards as two distinct lobes. It is composed of a layer of modified epithelial cells lined by a chitinous membrane continuous with the external chitinous skeleton of the insect. The epithelial cells of the adult are finely granular,

and in each a denser patch of granules surrounds a stout tube which extends from the region of the nucleus to the inner edge of the cell. Attached to the lining membrane, sometimes singly, sometimes in groups, are immensely long hairs with free extremities, directed towards the epithelial layer. The cells of this outer layer appear to migrate inwards, and there degenerate, leaving only irregular nuclei very unlike the large round nuclei of the granular cells. In a young specimen the epithelial cells are not granular, and there is no intermediate tissue between them and the chitinous lining. This suggests that the granules represent the secretion of the gland, and their absence in the young indicates that the organ is immature.

This work has been carried on in the Department of Comparative Anatomy in the Oxford University Museum, and I wish to express my warm thanks to Mr. Goodrich for much kind help throughout the course of my research.

References.

Claus.—'Zeit. f. wiss. Zool.,' Bd. xi, 1861.

Kraase.—'Zool. Anz.,' Bd. xiii, 1890.

Minchin.—'Quart. Journ. Micr. Sci.,' vol. xxix, 1888.

EXPLANATION OF PLATE 15,

Illustrating Miss Ruth M. Harrison's paper, "Preliminary Account of a New Organ in Periplaneta orientalis."

List of Reference Letters.

G. The gland. *c.* Cerca. *epi.* Epithelium of body wall. *epi.g.* Epithelium of gland. *f.b.* Fat body. *gr.c.* Granular cells. *h.* Hairs. *l.* Lumen of gland. *l.m.* Lining membrane. *m.gl.* Mushroom-shaped gland. *n.* Nuclei of disintegrating cells. *r.* Rectum. *s.* Style. *th.r.c.* Thickened rim of chitin on sternite 7 round the external aperture. *tr.v.* Tracheal vessel. *tu.* Tubes in the granular cells. *5th abd.g.* and *6th abd.g.* Fifth and sixth abdo-

minal ganglia. st_5—st_7. Sternites five to seven. t_4, t_5—t_{10}. Tergites four, five to ten.

Figs. 3—12 have all been drawn with the aid of a camera lucida.

PLATE 15.

Fig. 1.—Dissection of the six posterior segments of a large male cockroach × 5.

Fig. 2.—The gland removed with the tracheal vessels associated with it. × 22.

Fig. 3.—Longitudinal sagittal section through a female cockroach in the region of the gland. The nerve cord has been cut just below the fifth abdominal ganglion. The external opening between the sixth and seventh sternites is shown, also the thickened chitinous rim at the anterior end of the seventh sternite. × 43.

Fig. 4.—Transverse section through a male cockroach at the anterior end of gland, showing that two lobes project forward. × 70.

Fig. 5.—Transverse section through the same cockroach a little further back, showing that posteriorly the lobes unite. The thickened chitinous rim of sternite 7 has been cut near the posterior limit of the crescent. × 70.

Fig. 6.—Portion of the wall of a gland from a male cockroach more highly magnified. The tubes in the cells of the epithelial layer have been cut in a good many cases. × 330.

Fig. 7.—Portion of the wall of the gland from another cockroach under a higher magnification. The tubes are not visible in this preparation, but the hairs attached to the lining membrane are shown. This section also shows some of the cells of the outer layer migrating inwards. × 400.

Fig. 8.—A small portion of the gland after maceration in potash so that only the chitinous hairs remain. The magnification in this figure is so high that it was impossible to get the whole of a single hair within the field of the camera. Only the attachments and beginnings of each hair were therefore traced with a camera, and the free extremities had to be drawn in separately. Oil imm. oc. 12.

Figs. 9 and 10.—Longitudinal sections through a young male cockroach about 1·5 cm. long. Fig. 9 is a median section showing the external openings; Fig. 10 is a section taken to one side of the middle line, showing the extent of one lobe of the gland. × 105.

Fig. 11.—An enlarged drawing of a portion of the same gland. The comparatively enormous breadth of the chitinous lining in the last three figures is probably artificial, due to a breaking apart in the section-cutting. × 400.

Fig. 12.—A small portion of the wall of the gland highly magnified, showing the tubes in the epithelial layer. × 960.

THE MARINE BIOLOGICAL ASSOCIATION

OF THE

UNITED KINGDOM.

——:o:——

Patron—HIS MAJESTY THE KING.

President—Prof. E. RAY LANKESTER, LL.D., F.R.S.

——:o:——

THE ASSOCIATION WAS FOUNDED "TO ESTABLISH AND MAINTAIN LABORATORIES ON THE COAST OF THE UNITED KINGDOM, WHERE ACCURATE RESEARCHES MAY BE CARRIED ON, LEADING TO THE IMPROVEMENT OF ZOOLOGICAL AND BOTANICAL SCIENCE, AND TO AN INCREASE OF OUR KNOWLEDGE AS REGARDS THE FOOD, LIFE CONDITIONS, AND HABITS OF BRITISH FOOD-FISHES AND MOLLUSCS."

The Laboratory at Plymouth

was opened in 1888. Since that time investigations, practical and scientific, have been constantly pursued by naturalists appointed by the Association, as well as by those from England and abroad who have carried on independent researches.

Naturalists desiring to work at the Laboratory

should communicate with the Director, who will supply all information as to terms, etc.

Works published by the Association

include the following:—'A Treatise on the Common Sole,' J. T. Cunningham, M.A., 4to, 25/-. 'The Natural History of the Marketable Marine Fishes of the British Islands,' J. T. Cunningham, M.A., 7/6 net (published for the Association by Messrs. Macmillan & Co.).

The Journal of the Marine Biological Association

is issued half-yearly, price 3/6 each number.

In addition to these publications, the results of work done in the Laboratory are recorded in the 'Quarterly Journal of Microscopical Science,' and in other scientific journals, British and foreign.

Specimens of Marine Animals and Plants,

both living and preserved, according to the best methods, are supplied to the principal British Laboratories and Museums. Detailed price lists will be forwarded on application.

TERMS OF MEMBERSHIP.

ANNUAL MEMBERS	£1	1	0	per annum.
LIFE MEMBERS	15	15	0	Composition Fee.
FOUNDERS	100	0	0	„ „
GOVERNORS (Life Members of Council)	500	0	0	

Members have the following rights and privileges:—They elect annually the Officers and Council; they receive the *Journal* free by post; they are admitted to view the Laboratory at any time, and may introduce friends with them; they have the first claim to rent a table in the Laboratory for research, with use of tanks, boats, etc.; and have access to the Library at Plymouth. Special privileges are granted to Governors, Founders, and Life Members.

Persons desirous of becoming members, or of obtaining any information with regard to the Association, should communicate with—

The DIRECTOR,
The Laboratory,
Plymouth.

New Series, No. 199 (Vol. 50, Part 3). Price 10s. net.

AUGUST, 1906.

THE QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE.

EDITED BY

E. RAY LANKESTER, M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND OF THE ACADEMY OF THE LINCEI OF ROME, AND OF THE AMERICAN ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE ROYAL ACADEMY OF BELGIUM; HONORARY MEMBER OF THE NEW YORK ACADEMY OF SCIENCES, AND OF THE CAMBRIDGE PHILOSOPHICAL SOCIETY, AND OF THE ROYAL PHYSICAL SOCIETY OF EDINBURGH, AND OF THE BIOLOGICAL SOCIETY OF PARIS, AND OF THE CALIFORNIA ACADEMY OF SCIENCES OF SAN FRANCISCO; FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE AMERICAN PHILOSOPHICAL SOCIETY; DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE FULLERIAN PROFESSOR OF PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN; LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

FELLOW AND TUTOR OF TRINITY COLLEGE, CAMBRIDGE;

AND

SYDNEY J. HICKSON, M.A., F.R.S.,

BEYER PROFESSOR OF ZOOLOGY IN THE OWENS COLLEGE, MANCHESTER.

WITH LITHOGRAPHIC PLATES AND TEXT-FIGURES.

LONDON:
J. & A. CHURCHILL, 7 GREAT MARLBOROUGH STREET.
1906.

Adlard and Son, Impr.,] [London and Dorking.

CONTENTS OF No. 199.—New Series.

MEMOIRS:

On the Development of Nebalia.

By

Margaret Robinson,
Zoological Research Laboratory, University College, London.

With Plates 16—21.

Introduction.

It was at the suggestion of Professor W. F. R. Weldon that I began this investigation, and he kindly gave me the material which he had taken from the brood pouches of specimens obtained by dredging at Naples. Unfortunately it had been preserved some time when it came to me, and part of it (including some of the later stages) had been rendered of little use by the action of tannin from the corks of the bottles. I have, however, been able to work at six stages which are fairly consecutive, and seem important.

I may mention that I have tried to supplement my material by means of visits to places where Nebalia is abundant, namely, Jersey and Roscoff. At the latter place I received much kindness and assistance from Professeur Yves Delage and Monsieur A. Robert. Numbers of adult Nebalia were obtained from decaying crabs and lobsters, which had previously been placed under stones on the shore at low tide. None of these had eggs in the brood pouch, and though I spent a good deal of time and trouble in trying to make the animals breed in

dishes, I could never obtain a fertilised egg. I had previously tried to do this in Jersey, where Mr. James Hornell kindly provided me with specimens. This absence of females with eggs in the brood pouch from the shore leads me to believe that the animals go into deeper water to breed. All the people who have worked at the development of Nebalia—Metschnikoff, Claus, Butschinsky—have obtained their material from tideless seas.

From many practical hints and much other help I am indebted to the kindness of Professor E. A. Minchin. I wish here also to thank my many friends and fellow-workers for their assistance, particularly Dr. E. J. Allen, who has read through this manuscript and made many valuable suggestions and criticisms.

Historical.

The earliest notice of Nebalia is that by Otho Fabricius (1780) in his Fauna Grœnlandica. This is a not very exact description of the external features, and is accompanied by a little figure. The next account is that of Herbst (1796). It is a translation of the description given by Fabricius with a copy of his figure. Both of these authors call the animal Cancer bipes.

Montagu (1815) found Nebalia on the coast of Devon, and described it under the name of Monoculus rostratus. The first person to call it Nebalia was Leach, who described it in the 'Zoologist's Miscellany' (1813), and noted it as a very distinct genus belonging to the Crustacea Malacostraca.

Latreille, in his text-book (1831), places Nebalia with Cuma in an order which leads from the sessile-eyed crustaceans and those with stalked eyes, especially from Mysis, to the Cyclops. He calls the order Diclapoda "Ce nouvel ordre comprendra les genres Nébalie, Pontie, Condylure, et Cume, qui se lient d'une part avec les Mysis et de l'autre avec les Cyclopes." He lays stress on the fact that Nebalia carries its eggs in a brood pouch, as does Mysis.

Milne-Edwards (1828 and 1835) who found Nebalia on the coast of Brittany, and described it under the name of Nebalia Geoffroyi, after Geoffroi St. Hilaire, gave as his reason for not placing it among the Malacostraca the fact that the thoracic legs with their lamellate appendages do resemble those of Branchipus, and these gills are in no way like the gills of Decapods. In 1840 he writes of the Nebalidæ "Elles semblent à plusieurs égards établir le passage entre les Mysis et les Apus," but places them in the family "Les Apusiens" among the Phyllopods.

Kröyer (1847) (of whom I know only through Claus and Metschnikoff) stated that Nebalia could not be a Phyllopod because it carried its embryos in the brood pouch till they were practically adult.

Metschnikoff, in 1868, published a long account of the development of Nebalia. As this is an important paper of which unfortunately there is no published translation from the Russian, I give here a somewhat lengthy abstract of it. I must first thank Miss Zelda Kahan for translating the paper for me.

Metschnikoff begins his account of the development by describing the formation of the blastoderm. In this part I will give his words as nearly as possible: "Before total segmentation there is another division in the formative yolk, and there appears a polar vesicle. Both of these appear on the lower portion of the egg. Here there is formed a small accumulation of colourless protoplasm containing a large number of granules. This protoplasm, which is nothing more than generative yolk, separates itself from the egg envelope, and thus there comes into existence a small space between the shell and the yolk. In this space there appears a small globule of protoplasm which plays no part in development and soon disappears.

"The further development depends on the increase in quantity of the generative yolk. When it has increased so as to take up about one fifth of the volume of the entire egg it divides longitudinally into two parts oval in shape. These divide

meridionally, resulting in four cells. These again divide longitudinally. At first the cells only cover the lower pole of the egg forming two rows of cylindrical cells. Later they spread over the whole egg."

Then follows a description of seven stages in development. In the last of these only does the embryo become a true larva —for here alone it is free swimming; the other stages being passed through in the brood pouch of the mother.

First Stage.—In the first of these stages he notes a thickening on the ventral surface and the first appearance of the papilla.

Second Stage.—He describes an embryo with nauplius appendages, and an abdominal papilla which is bent over the ventral surface. In this stage he also notes the appearance of the endoderm and the way in which it begins to grow round the yolk.

Third Stage.—He notes two pairs of maxillæ and two thoracic legs, as well as the nauplius appendages. Here, too, he observes mouth and stomodæum, as well as anus and proctodæum.

Fourth Stage.—He describes the change in position of the appendages (they are now directed backwards instead of outwards), and the appearance of the third thoracic limb. He also notes the possession of dorsally directed palps by the maxillæ and thoracic appendages. In this stage, too, there is an increase in the size of the optic lobes and brain, and here he first describes the labrum. In each of these two last stages he describes the further circumcrescence of the yolk by the endoderm. He remarks, too, on the different appearances of the yolk in different places; that which is still not enclosed by endoderm being dense and granular, while the enclosed portion seems to be liquid and contains but few granules.

Fifth Stage.—The embryo now bursts the vitelline membrane, but is still enveloped by a cuticle, and the abdomen springs back so that now instead of lying on the ventral surface it is slightly curved in a dorsal direction. The cuticle

invests all the first five appendages (nauplius appendages and maxillæ) closely following all their curves and outgrowths. The rest of the appendages are covered by an unbroken skin which forms a general sac over them and the back. This skin separates itself gradually from the parts it covers, and is finally thrown off. In this stage the shell first appears as a dorsal crease. Some embryos, which closely resemble that last described, show the head flap, further lateral developments of the shell, and pigment in the eyes; but in these, as in the last described stage, the front part of the yolk is still unenclosed by endoderm.

Sixth Stage.—In this stage he notes:

(*a*) The shell valves covering the body of the embryo as far back as the fifth thoracic appendage.

(*b*) The head flap with its front end more rounded than in the adult.

(*c*) The body now segmented and covered with chitin, the latter forming rows of teeth on the two segments before the last, whilst in the adult these teeth surround all the six segments before the last in the form of belts.

(*d*) Segmentation of several of the appendages.

(*e*) All the yolk now enclosed by endoderm. Liver outgrowths which diverge in the shape of blind conical sacs lying at the sides of the intestine and containing grey matter (derived from the yolk) and some yolk. Also two shorter outgrowths directed forwards.

Seventh and Last Stage, which differs only from the adult in having three instead of four abdominal legs, in having fewer segments in the antennæ, and in having no vertical split in the middle laminæ of the gills of the thoracic legs.

In one place he regrets that he saw so little in the embryos. To us the wonder must be that he saw so much without the help of all our modern appliances and contrivances. He refers to the work of a previous observer, Kröyer, who found twenty-four appendages in the adult. Kröyer was of the opinion that Nebalia could not be a Phyllopod, as it did not leave the brood pouch of the mother until practically an

adult. He suggested further investigations to find out if it resembled the Decapods.

Metschnikoff himself is strongly in favour of the inclusion of Nebalia among the Malacostraca. He says that its most phyllopod-like feature, viz. the thoracic legs, differ really from those of the Phyllopods in number and development. Further, that the mouth-appendages resemble those of the decapods, that the digestive organs are not like those of the Phyllopods, and that the openings of the oviducts are in the wrong place for a Phyllopod. He concludes by saying that it has only a general similarity to the Branchiopoda.

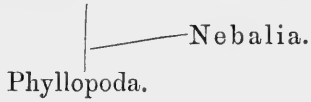
He then points out that Nebalia greatly resembles the Schizopods in the number and arrangement of the appendages, many of these having five to eight thoracic limbs with gills, though in their case the change of leg into gill does not go so far as it does in Nebalia. Then he remarks on the great likeness between the gill of Nebalia and the swimming leg of Euphausia. In conclusion Metschnikoff suggests that Nebalia be removed from the Phyllopods, where Milne-Edwards had placed it, to the Decapods, and that a special group be made for it side by side with the Schizopods. (One must suppose that by Decapods he means Malacostraca.)

Claus (1872) wrote concerning the anatomy and systematic position of Nebalia. He could not agree with Metschnikoff in placing it among the Decapods, as he thought it had no true Zoæa stage. However, he expressed the opinion that Nebalia must be very nearly allied to the Malacostraca.

v. Willemoes-Suhm (1875), in describing a new species of Nebalia found by the Challenger Expedition, places the Nebaliadæ among the Schizopods. In 1876 Claus, in his 'Crustacean System,' gave an account, with some figures, of Nebalia. He there drew attention to the likeness between Nebalia and the Mysidæ as regards heart, digestive canal, nervous system, antennary gland, and genital organs.

One might infer, from the words of Milne-Edwards and Latreille, that they believed that the Malacostraca were

descended from the Phyllopoda through Nebalia. Boas (1883), while considering Nebalia as transitional, is careful to point out that, in his opinion, this is not a case of direct descent. "Sie steht nicht auf dem geraden Weg von den Phyllopoden zu den Malacostraken, sondern etwas seitlich." He considers Thysanopus (an Euphausid) to be the most primitive Malacostracan, and the nearest to Nebalia, but it, like Nebalia, does not lie on the direct route from Phyllopoda to Malacostraca. His views are founded mainly on differences and likenesses in appendages and other external features. He expresses the result as regards Nebalia thus:



Sars (1885), on the other hand, in his Report on the Schizopoda of the Challenger Expedition (1885), expressly states that he cannot agree with Boas as to placing Nebalia either among or near the Schizopoda. He then regarded it as a Phyllopod.

In 1885 Claus again wrote on Nebalia. After alluding to Metschnikoff's paper he proceeds to give his own reasons for the assertion that Nebalia is "no Phyllopod." As to external features he says that there are only the following in which Nebalia resembles the Phyllopods—the shell, the thoracic legs, and the tail.

The form of the shell, he justly remarks, is one which is by no means confined to the Phyllopods. He thinks that, as in the case of the Euphausia larva, it is the original malacostracan shell which has been retained. Further, he says, it shows that the carapace of the Malacostraca and the shell of the Entomostraca had the same starting point. The thoracic limbs of Nebalia are, he continues, intermediate in character between those of the Phyllopoda and those of the Schizopoda. In internal structure Nebalia differs still more from the Phyllopoda, and is especially like the Mysidæ. With regard to the abnormal number of abdominal segments,

he considers that, as the two hindmost have not special ganglia, they are not true segments, but, rather, joints of a segment; and here he alludes to the jointing of the sixth abdominal segment in Gnathophausia, which he says is not accompanied by any corresponding division of the ganglion.

He regards the extra joint in Gnathophausia and the two extra joints in Nebalia as being representatives of the telson. In this paper he gives a schematic tree of the Crustacea. In the tree the Leptostraca, the Protoschizopoda and the Stomatopoda are made to come off together from the Protomalacostraca.

In 1887 Sars, in his Report on the Phyllocaridæ of the Challenger, following Dr. Packard (in 'Phyllopod Crustacea of North America'), is inclined to derive the Nebaliadæ from copepod-like ancestors. He says that the Podophthalmia are in no way related to them, but that the Branchiopoda probably came from the same stem, and have become altered to suit conditions of life, whereas the Nebaliadæ have preserved many primitive features.

In 1889 Claus published his last paper on Nebalia. In this he gives a very full and detailed account of the anatomy, and puts forward at some length his views as to the systematic position of the animal. He mentions several fresh pieces of evidence in favour of its being a Malacostracan. The chief of these are:

(*a*) That the openings of the genital ducts in both sexes are in the Malacostracan position. He had found the male ducts previously.

(*b*) The great complexity of the brain.

(*c*) The structure of the eyes and the optic ganglion, which resemble those in Mysis.

(*d*) The rudimentary shell gland and the eight pairs of ectodermal excretory glands on the thoracic legs. These function as well as the antennary gland.

(*e*) The two last abdominal segments, as representing the Malacostracan telson. The last but one of these he says has

a ganglion in the late larval stage which disappears in the adult.

Claus places the Leptostraca as one of the three orders into which the Malacostraca are divided by him, namely, Leptostraca, Arthrostraca, and Thoracostraca.

In 1892 Grobben, in a paper on the classification of the Crustacea, pointed out the several points of resemblance between Branchipus and Nebalia, but finally came to the conclusion that it would be harder to join Nebalia to the Phyllopoda than to the Malacostraca. He divided the Malacostraca into two main divisions, the Leptostraca and the Eumalacostraca, the latter division including the three orders Stomatopoda, Thoracostraca, and Arthrostraca. His paper contains a tree of the Crustacea, similar to that given by Claus (1885), in which the Leptostraca are made to come off from the Crustacean stem with the Protoschizopoda.

In 1893, Hansen suggested a new classification of the Malacostraca. Like Grobben he divided them into two groups, Leptostraca and Eumalacostraca, which latter consists of three orders (unnamed). The first of these orders contains the Mysidæ, the Cumacea, Isopoda and Amphipoda; the second, the Euphausids and the Decapods, while the third exists for the Stomatopoda only. He considers that the Leptostraca are decidedly the most primitive Malacostraca, and that of the Eumalacostraca the most nearly related to them are the Mysidæ. He found in the shaft of the second antenna of Nebalia five joints of which the last showed a tendency to consist of two pieces. This makes the shaft resemble that of the second antenna of Mysis. Other points of likeness which he mentions are (1) the development of the larvæ; (2) the form of the heart; (3) the fact that young embryos of Mysis have at the hindmost end of the body two small hard processes fairly well chitinised which must be homologous with the furcæ in Nebalia; (4) the presence of conical outgrowths at the openings of the male ducts.

In 1897, Butschinsky published in the 'Zool. Anzeiger' a

short account of the formation of the blastoderm, and 1900, a longer paper on the development in the same journal. His account of the formation of the blastoderm differs slightly from Metschnikoff's. He says that the cleavage which he observed was really intermediate between a discoidal cleavage and a superficial one. In his second paper he again alludes to this difference between his account of the formation of the blastoderm and Metschnikoff's, and gives a short summary of the development.

Methods.

The eggs were taken from the pouches and fixed in a hot concentrated solution of corrosive sublimate to which a little acetic acid was added. They were then washed and taken very gradually through alcohols of increasing strengths up to 80 per cent. For their most excellent fixation and preservation I am very grateful to Professor Weldon.

The shells of the early stages were removed by teasing with very fine sewing needles.

As yolk preserved in sublimate is extremely brittle, in order to cut it without its breaking I had recourse to the two following methods:—(1) Embedding in celloidin. (2) Painting each section with a mixture composed of equal parts of gum mastic and celloidin.

The orientation was done in the first case by cutting the celloidin into the required shape, and in the second by fastening the embryo in position on a piece of lardaceous liver before embedding in paraffin. The sections were cut with Jung's microtome, and are 4μ in thickness.

The embryos from which the surface views are taken were stained with Delafield's hæmatoxylin; the sections with Kleinenberg's hæmatoxylin, and orange.

The sections were all drawn under Zeiss's objective D D. and ocular No. 3 with Abbé's camera lucida, the details being filled in with the help of a $\frac{1}{12}''$ immersion objective.

The Development of the Embryo.

The Formation of the Blastoderm.

Metschnikoff describes a discoidal segmentation; but a series of sections made through a stage which is earlier than that shown in Plate 16, fig. A, leads one to suppose that we have in Nebalia not an instance of discoidal segmentation proper, but rather a case of Korschelt and Heider's (1893), type III b, in which the increase of the blastoderm is aided by the accession of new elements from the inside of the egg. It seems that the blastoderm, certainly at first, increases both by division of the cells already on the ventral surface and also by the reception of additional cells which come from within the egg itself (Plate 16, fig. 1). According to Butschinsky (1897) the first two divisions take place in the protoplasm while it is still lying in the centre of the egg within the yolk. The four cells resulting from these divisions travel to the ventral pole of the egg and there divide, thus forming a cap of eight cells. This cap, by subsequent divisions of its cells, gradually surrounds the yolk. He also describes a few cells which are smaller than the other cells of the blastoderm. These smaller cells have escaped my observation.

Stage A. (Plate 16, fig. A.)

Figure A shows an egg in which the yolk is still incompletely covered by the blastoderm. Sections through it contain none of the large cells within the yolk shown in fig. 1. Therefore it may be supposed that only in a very early stage is the blastoderm increased by the reception of additional cells coming from within the yolk.

The blastoderm cells are more or less oval in shape and their protoplasm is granular. The yolk is broken up into

small angular lumps some of which stain more deeply than the others. All through the development, until the yolk is completely liquefied, this irregular staining can be noticed. Even at this very early stage some cells, which, as will subsequently appear, may be regarded as vitellophags, are budded off from the blastoderm into the yolk (fig. 2, *vp.*).

Stage B.

External View.—The external appearance of the embryo closely resembles that of the next stage (Stage B′). On the ventral surface the blastoderm shows three areas of thickening which are arranged so as to form the apex and two adjoining sides of a triangle. This triangle, however, is much shorter than that outlined by the ventral thickenings in Stage B′. It is, in fact, an area which barely covers the posterior two thirds of the ventral surface of the blastoderm. It is outlined by two thickened strands which converge, with a thickened patch uniting their convergent ends.

These lateral thickened strands consist of large, rounded, granular cells which are thicker and rounder than the cells of the rest of the blastoderm. In some places these cells appear to give rise to others by a kind of tangential division (figs. 3 and 4). That some of the new cells formed in this way are vitellophags there can be little doubt, others are probably some of the first cells of the ordinary mesoderm.

The median posterior thickening (the thickened apex of the triangle) has in its centre a very narrow groove running in a longitudinal direction. This groove is short, extending through eight sections (each only 4 μ in thickness).

Of these sections I have drawn two, one (fig. 6) through the middle region of the groove, and the other (fig. 5) near its anterior limit.

Behind the region shown in fig. 6 the groove widens out a little. A section taken immediately behind the groove is shown in fig. 7.

The section through the middle region shows an inpushing

of five cells of the blastoderm, and on each side of this inpushing there is a cell (fig. 6, *mes.*).

There can be little doubt that the entrance to the groove represents a blastopore, and that there is here an invagination of blastoderm cells to form the endoderm. The cells on either side of the invagination I take to be mesoderm, and to have been budded off from the blastoderm in situ.

In the section close to the anterior limit of the groove the lumen is narrower. This makes one think the closure begins at the front end of the groove. Just dorsal to the narrow depression there are a few large rounded cells (fig. 5). These I take to be endoderm cells which have been invaginated or have arisen by proliferation from invaginated cells.

The section behind the blastopore also shows two layers of cells in the ventral region (fig. 7). I find it hard to say whether the inner layer consists entirely of mesoderm or not. The large almost central cell may be endodermal, the others are certainly mesoderm.

All the cells in this and in the next stage contain a very granular protoplasm.

Stage B′ (fig. B′.).

External View.—The embryo lying on what will be the ventral surface of the yolk shows three distinct regions of thickening, namely, the two optic thickenings at the anterior end, and the caudal thickening at the posterior end (*c.t.*). These three thickenings are connected by strands of cells in which strands there are again thickenings foreshadowing the antennæ.

One can also see in this external view an indication of the caudal groove between the caudal thickening and the blastoderm in front of it. Many dividing nuclei can be seen in the central region of the ventral surface of the blastoderm.

Internal Structure.—Transverse sections through the optic thickenings show them to consist of cylindrical cells (fig. 8), and of only one layer of these, though here and there

the beginnings of a second layer can be noticed. As one follows the sections farther back one notices that the cells gradually lose their cylindrical shape becoming flattened (figs. 9 and 10). Sections through the strands show that all along them mesoderm cells are apparently being budded off from the blastoderm (figs. 9 and 10, *mes.*). In the anterior region these cells are few and far between, but the farther back we trace them the more numerous do they become, so that on reaching the caudal thickening we find the strands to consist of two definite layers of cells—ectoderm and mesoderm (fig. 11).

Besides the above-mentioned mesoderm cells there are budded off from the blastoderm more cells of the type mentioned as occurring in the earlier stages (figs. 9 and 10). These cells have large much-vacuolated nuclei (about twice as large as the nuclei of the other cells), and a small amount of cytoplasm which is spread out in processes resembling pseudopodia. In fact each cell has the appearance of an amœba with an immense nucleus. They are to be found throughout the whole length of the embryo, but are not numerous. In the embryo from which the figured sections were taken there were about ten of these cells. Though they come to lie in the yolk which is ultimately surrounded by endoderm, these cells are distinctly mesodermal in origin, i. e. they are budded off from the blastoderm in the same manner, and in the same regions as the rest of the mesoderm. It has been suggested by Kowalewsky (1886), and Nusbaum (1886) that these cells help in some way to soften the yolk, and so render it easy of absorption by the protoplasm. That the yolk is of a different consistency in different stages can be seen even in preserved specimens, and in the later stages the vitellophags are first diminished in numbers and then gradually disappear. These facts taken together certainly support the above-mentioned view as to the function of these cells.

Sections through the posterior end of the embryo pass through the hind ends of the lateral thickenings as well as

the caudal thickening. They show, in the region occupied by the groove in Stage B, a band of large rounded cells, the endoderm, resulting from the invagination. On either side of this band in its anterior region the body wall consists of two layers of cells, ectoderm and mesoderm, since there are here the posterior convergent ends of the thickened lateral strands (fig. 11).

Unfortunately the sections of the series here drawn are a

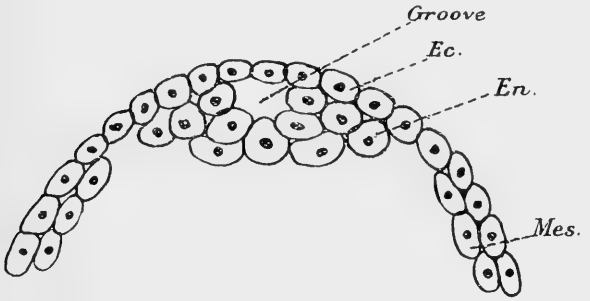


DIAGRAM 1.—Transverse section through the caudal thickening in Stage B′, showing the remains of the groove "roofed in" by the closure of the blastopore. *Ec.* Ectoderm. *En.* Endoderm. *Mes.* Mesoderm.

little oblique, or they would show more distinctly than they do the remains of the groove which is now roofed in, as it were, by the closing of the blastopore, as shown in the above diagram.

It can be seen from figs. 12 and 13 that the endoderm cells have already begun to proliferate; and from figs. 13 and 14 it would appear that their numbers were also increased by cells being pushed in from the ectoderm. Further back it becomes rather difficult to distinguish between the endoderm and the mesoderm; but there is a difference in the shape of the cells, those of the mesoderm being oval or almost almond-shaped, whilst the endoderm cells are much more nearly spherical.

All the protoplasm in this stage is very granular, and the

cells in the caudal thickening are many of them much vacuolated.

Putting together the facts concerning these two stages B and B′ one is forced to the conclusion that the cells which are invaginated at the blastopore give rise to the endoderm. I cannot help thinking that they give rise to that only, and that the mesoderm arises quite independently of this invagination. I think that fig. 6 points strongly to this. That the vitellophags are budded off from the blastoderm figs. 3 and 4 show, but unfortunately I have not been able to find in any of the embryos at this stage a blastoderm cell in process of division to form mesoderm, except in fig. 3, *div.*, and the results of these two divisions may be vitellophags after all.

Bergh (1893) in his account of the formation of the germ layers in Mysis describes two stages which almost correspond as to age with Stage B described above. They seem both to be a little younger than it, and also a third stage which is perhaps a little younger than Stage B′. He is careful to state that in none of these does he find any vitellophags. In the proliferation in the region of the blastopore he finds two kinds of cells, large and small, the large being mesoderm and the small endoderm.

In a stage a little older than the oldest of these three Bergh finds some vitellophags for the first time; and in summing up the results of this part of his work he states that the cell mass resulting from the invagination at the blastopore differentiates itself further into three regions of germination (Anlagen).

(1) The vitellophags,

(2) The entoderm disc,

(3) The eight original cells of the mesoderm.

The likeness between these stages in Mysis and those in Nebalia described above is very great; but there are differences.

(1) I find vitellophags in both Stage B and Stage B′, and indeed in an earlier stage than B, i. e. in Stage A, where the

blastoderm has not as yet completely surrounded the yolk. Therefore they must arise quite independently of the invagination.

(2) From fig. 6 it would seem that the mesoderm in this region arose also from the blastoderm independently of the invagination. This mesoderm Bergh calls metanauplial. The mesoderm of the nauplius appendages he calls nauplial, and says it is probably budded off from the ectoderm in situ. This obliges him to admit of two origins for the mesoderm.

(*a*) The invagination at the blastopore for the metanauplial mesoderm.

(*b*) Budding off from the blastoderm in situ for the nauplial mesoderm.

Now in Nebalia it seems to me that there are two possibilities, either

(1) All the mesoderm cells are budded off in situ from the blastoderm, i. e. the nauplial mesoderm in the places where the appendages are about to be folded off, and the metanauplial mesoderm from the blastoderm in the region of the blastopore at about the same time as the invagination takes place but quite independently of it—or

(2) All the mesoderm is budded off from the blastoderm in the region of the blastopore at about the same time as the invagination takes place (but quite independently of the invagination), and the cells which will form the nauplial mesoderm travel forwards.

Either of these possibilities seems a little more probable than the possibility that in the same animal part of the mesoderm should arise as the result of an invagination and part by division in situ.

Butschinsky (1900) is of opinion that both endoderm and mesoderm originate from a mass of cells to which in this stage the blastoderm gives rise at the caudal thickening; and this is the only point of any importance in which our accounts of the development differ.

Putting aside the difference with regard to the origin of the metanauplial mesoderm, there is a striking similarity

between the development of Mysis and that of Nebalia in these early stages which show the origin of the endoderm from an invagination which results in the formation of a solid band of cells.

Stage C (fig. c). Embryo with Nauplius Appendages.

External view.—In an external view of the ventral surface of the embryo at this stage one can distinguish :

(1) The eye thickenings.
(2) The first and second antennæ.
(3) The mandibles.
(4) The abdominal papilla which is bent forward.
(5) The mouth which is here a crescent-shaped depression lying between the first and second antennæ.

Internal structure.—As is usual among the Crustacea, embryos of approximately the same external appearance vary not only in size but also in internal development. I have therefore made drawings from three series of transverse sections taken from three embryos, each of which has the nauplius appendages. It is chiefly in the ectoderm of this stage that differences occur.

All three series show the optic thickenings in the anterior region, though in the very front these thickenings are not much pronounced, and indeed show little or no advance in size on those of the preceding stage (fig. 16). All three too show at a little distance behind the most anterior part of the optic thickenings on either side an invagination of ectoderm cells (*op. in.* fig. 17). This very closely resembles the optic invagination described by Reichenbach (1886) in the crayfish embryo with nauplius appendages.

In each of the three series, almost immediately behind the optic invagination on either side, there are a few ectoderm cells which are much larger than the rest, almost double the size, with large nuclei (fig. 18 *g.* 1). These I take to be the

first rudiment of the optic ganglion. They are continuous with similar cells lying near the base of the first antenna, and forming the rudiment of the antennulary ganglion (fig. 20, *g.* II). At the point of junction of these two rudiments there is a distinct groove or fold in the cells composing them (seen in transverse section, fig. 19, and in longitudinal section, fig. 23, between *g.* I and *g.* II). This fold makes it appear as though the optic ganglion cells were being nipped off anteriorly and laterally from those of the antennulary ganglion, these last lying nearer the middle line. Now, it might be argued that there is here a case of the optic ganglion being budded off from the antennulary ganglion; but one must bear in mind that these rudimentary ganglion cells are at present merely slightly differentiated ectoderm cells which still form part of the outer wall of the body of the embryo, and also that all the evidence we have seems to show that the differentiation has taken place simultaneously in front of, and just behind the groove, and in the cells of the groove itself. This groove or fold marks the division between the optic region and that of the first antenna. It is in fact almost the earliest trace of segmentation.

There are similar groups of specialised ectoderm cells near the bases of the second pair of antennæ and of the mandibles figs. 21 and 22, *g.* III, *g.* IV).

There are then in this embryo with nauplius appendages four pairs of rudimentary ganglia—(1) optic, (2) antennulary, (3) antennary, (4) mandibular (fig. 23).

Since the optic ganglia appear simultaneously with the other pairs of ganglia but independently of them; since too experiments made by Herbst (1896) and by others before him have shown that eye-stalks in Decapods may be replaced by antenna-like appendages, the optic region possesses two of the main essentials of a segment, and there seems to be little reason for not considering it the first segment of the body.

It was on account of the replacement of the eye-stalk of Palinurus by an antenniform palp that Milne-Edwards,

who was followed by Huxley, considered the eyes as the first pair of appendages. (Lankester, 1904.)

Reichenbach (1886) and Nusbaum (1887) found first traces of a nervous system in the crayfish and in Mysis similar to those above described, and they too looked upon the optic tract as the first body segment.

If this be so, then the eye region forms the first segment of the body not only in all the Malacostraca, but also in all the Crustacea, and in the rest of the Arthropoda.

Evidence against this opinion has been given by Claus in his papers on the development of Branchipus (1873 and 1886). Since he referred to the facts there recorded at great length in his last paper on Nebalia (1889) one feels bound to mention them, but it seems that his observations were not made on sufficiently early stages.

My observations on the development of the nervous system in Nebalia are merely additional evidence in favour of the opinion that in the Crustacea the optic region forms the first segment of the body. This is the view taken by Heymons (1901), and the one to which, lately, Prof. Lankester has given his support. (Lankester, 1904.)

In this paper I am using the terminology of Viallanes and Heymons (1892) for the different parts of the brain. Protocerebrum for that in the first or optic segment, deutocerebrum for that belonging to the second segment, and tritocerebrum for the pair of antennary ganglia in the third segment.

To continue the description of the nervous system at this stage. The cells of the four pairs of rudimentary ganglia, although they form part of the general ectoderm of the body-wall, differ from the other ectoderm cells in size, and also in the size and colour of their nuclei. They are larger than the cells of the rest of the ectoderm, and have larger and paler nuclei, which stain very slightly.

The ganglia of each pair are separated from each other by a median narrow band (one cell deep) of the cells of the ordinary ectoderm. The ganglia of the first pair are in

continuity with those of the second on either side, and these two pairs of ganglia lie in front of the mouth, while those of the antennary and mandibular segments lie behind it.

In one specimen a section through the first antenna shows the ganglion near its base to consist of two kinds of cells, as it does in Stage D. This is only worthy of notice as an instance of a difference in internal development between embryos which have the same external features.

Other Ectodermal Structures.—I find in this stage the rudiment of a labrum (fig. 24), and also a well-marked stomodæum, which runs a little forward from the mouth (fig. 26), but, though I have looked through several series of both transverse and longitudinal sections, I can find here no beginnings of either anus or proctodæum.

Endoderm.—The solid band of cells resulting from the invagination at the blastopore now spreads out laterally, i. e. its cells multiply laterally, so as to gradually enclose the yolk. The enclosure seems to take place in this way. Starting from the anterior end of the caudal thickening, just behind the depression, between it and the thorax, the cells grow backwards and dorsalwards, so that the first part of the yolk to be enclosed is that lying in the papilla (fig. 26). When this is done, i. e. when the yolk within the papilla is enclosed, the cells seem to increase more rapidly on the dorsal than on the ventral surface. A transverse section in front of the papilla through the farthest advanced of the embryos with nauplius appendages shows endoderm lying on the dorsal surface of the yolk, but none on the ventral surface. Similarly, a longitudinal section through an embryo belonging to the next stage shows endoderm stretching much farther forward on the dorsal side than on the ventral (see Diagram 2, p. 408). The endoderm cells in this stage begin to assume a columnar shape.

Mesoderm.—The mesoderm can be traced lying between the ectoderm and the yolk in chains of three or four cells on the ventral side, from the abdominal papilla to the optic invaginations. It is most abundant in the region just in

front of the papilla. The mesoderm cells can be seen being carried along with the ectoderm as it is being folded off to form the nauplius appendages (figs. 20—22).

Vitellophags.—These cells are now more numerous than they were in the preceding stages. Wherever one of them is seen the yolk round it has a granulated appearance. This granulation may be a step onwards towards liquefaction. It seems that the vitellophags, while transforming the yolk, undergo disintegration themselves. They certainly appear to lose their own protoplasm, and their huge swollen nuclei look as though they were ready to disintegrate (fig. 26, *v.p.*). Fig. 26, *v.p.*, shows a number of vitellophags, some with a little protoplasm, and others consisting of a nucleus only. This disintegration certainly militates against any idea that the vitellophags become blood corpuscles or take any part in the development of the embryo other than that of rendering the yolk more easy of absorption by the protoplasm.

Stage D (fig. D).

External Features.—The chief advances noticeable in an external view are the increase in the number of the appendages, and the growth farther forward of the abdominal papilla.

The embryo now has, in addition to the nauplius appendages, two pairs of maxillæ and rudiments of the first three pairs of the thoracic appendages. The mouth has moved farther back, so that now it is in the segment which bears the second pair of antennæ. Behind the mouth there is a distinct median ventral depression. In optical section the endoderm surrounding the hinder two-thirds of the yolk can be seen distinctly. This stage, Claus notwithstanding, bears a likeness to a Zoæa, and might certainly be called the stage with Zoæa appendages.

Internal Structure. Ectoderm; the Nervous System.—There is here a great advance in the complexity of the nervous system. In each segment there is a well-

marked pair of ganglionic swellings, and the first three of these pairs are now (since the second antenna and its ganglion have moved forward) joined to help in forming the syncerebrum. Of these three pairs of swellings those in the optic and antennulary segments are much larger than those in the segment bearing the second antennæ. Each ganglion now consists of two kinds of cells which may be distinguished by their nuclei.

(*a*) Cells with large clear nuclei, each nucleus being surrounded by an appreciable quantity of protoplasm, though cell outlines can now no longer be made out. These cells are seen to divide, hence it may be reasonably inferred that they give rise to—

(*b*) Cells with small darkly-staining nuclei. These lie for the most part on the inner side of the large cells. The small nuclei are closely packed together, and the protoplasm surrounding each of them is very small in amount, almost inappreciable. Some of these small nuclei are to be seen in each of the three pairs of appendages behind the eyes (first and second antennæ and mandibles) as well as in the ganglia.

The rudimentary brain here consists mainly of three pairs of ganglia, and may be spoken of as consisting of three parts —the proto- deutero- and trito-cerebrum.

In their most anterior region the two optic ganglia are separated, as they were in the last stage, by a narrow band of ectoderm cells only one cell deep (fig. 29), but posteriorly there lies between the optic ganglia a central mass of nervous cells which, from the appearance of the section shown in fig. 31 and others, seems to have arisen by an invagination from the cells of the band of ectoderm which, in the preceding stage, separated the ganglia of the first pair in this region.

In his work on the Crayfish, while describing the state of the nervous system in an embryo which is a little more advanced than this, Reichenbach (1886) figures a central mass lying between the two optic ganglia. This central mass consists of three parts, two large outer ones which he considers to be parts of the supra-œsophageal ganglion of either side

and a small median strand of cells which is clearly an invagination.

Nusbaum (1887) describes a median double cord of nervous tissue in the brain of a Mysis embryo, and in his account of the development of Oniscus (1886) he states that the brain in the optic region consists of four lobes, the two outer ones only being the optic lobes. This, indeed, is the state of things in this stage. The median cord here more closely resembles that in Mysis than it does the tiny invagination figured by Reichenbach (1886).

In neither of his papers does Nusbaum say how this median cord originates. That it does arise from the band of cells which connects the optic ganglia at their first appearance is, I think, certain, and from its appearance in figs. 31 and 32 it seems probable that it arises by invagination.

The central mass is itself double, and the optic ganglia are slightly bilobed, so that we may say that each ganglion here consists of two parts—a small inner one which is single, and a large outer one which is bilobed (fig. 31). But it seems to me that the real state of things is better expressed by saying that the protocerebrum in this region consists of a pair of large lateral bilobed optic ganglia, and a central mass of nervous tissue which is double.

Tracing the nervous system farther back we find that the central mass increases in size, and the two lobes of each optic ganglion gradually fuse into one (figs. 32—34). In a section just anterior to the first antenna (fig. 35) one can see a still further separation of the central mass from what is here the rest of the deuterocerebrum, while in the region of the first pair of antennæ the median mass is partly separated from the ganglia by a layer of ectoderm cells (fig. 36, *f.*). These cells have fusiform nuclei which look as though they might belong to connective-tissue cells, but the mesoderm at this stage is not sufficiently advanced for that to be the case.

On tracing the median mass backwards one finds that it first becomes single (fig. 37), and finally, in the region of the

second antenna and the mouth, disappears altogether (figs. 38, 39).

Summing up our knowledge of the brain at this stage one may say that it consists of three pairs of lateral ganglia and a median mass of nervous tissue which extends from the posterior region of the first pair of ganglia, where it begins as a double cord, to just in front of the third pair of ganglia, where it ends as a single cord.

The ganglia of the mandibles and first maxillæ at this stage are much spread out and flattened (figs. 40, 41), while the second maxilla has the merest rudiment of a ganglion.

The Eyes.—Sections through the anterior regions of the optic lobes show a slight depression in the ectoderm cells forming the outer wall (figs. 28, 29, *d.*). These cells are probably the forerunners of the crystalline cone cells or of the cells of the corneal hypodermis, or, as I believe, of both these sets of cells. Very slightly behind this depression lies the anterior limit of the optic ganglion, and on a level with this the optic invagination (fig. 30, *op. in.*). This has now the form of a solid cup. On the inner side of this now solid invagination there can be seen a few of the smaller nerve nuclei. Reichenbach (1886), in his account of the development of the eye in the Crayfish, says that the invagination first becomes solid and then divides into two layers, the inner of which furnishes cells to the optic ganglion, while the cells derived from the outer layer become retinulæ. The state of things shown in fig. 30 is very like that described and figured by him in a Crayfish embryo at about this stage.

Other Ectodermal Structures.—The stomodæum still runs forward from the mouth, and in this stage there is the first appearance of the anus and proctodæum (fig. 42, *proc.*). In a transverse section through the caudal papilla of an embryo with nauplius appendages I noticed two exceptionally large ectoderm cells. In this stage also I find two very large cells in similar sections just behind the anus (fig. 43, *l.c.*), but I have not succeeded in tracing these cells in later stages.

Nusbaum (1887) found similar cells in a very early stage in

Mysis. He believed them to be genital cells, and found them later in the abdomen, later again in the thorax in a ventral position, and later still in a dorsal position between the digestive canal and the heart. However, he is not very convincing in this part of his paper, and I am inclined to

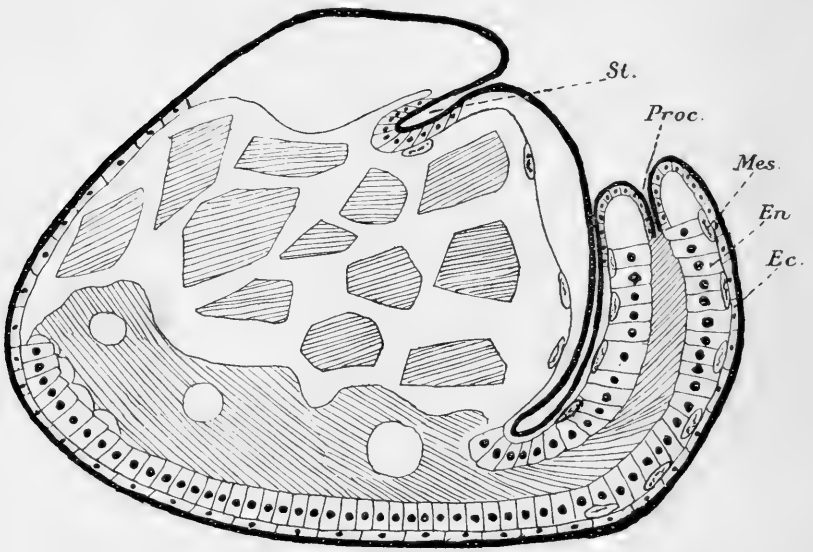


Diagram 2.—Median longitudinal section through embryo at Stage D. *Ec.* Ectoderm. *En.* Endoderm. *Mes.* Mesoderm. *Proc.* Proctodæum. *St.* Stomodæum.

think that these large cells, in Nebalia at least, are simply part of a zone of growth similar to the Knospungs-zone described by Reichenbach. This zone of growth is mentioned by Nusbaum, but has been much more fully described in Mysis by Bergh (1893). As I unfortunately did not notice it while making surface views of the embryos I have been unable owing to want of more material to describe it.

The Dorsal Organ.—I have not found a trace of a dorsal organ in any of my stages, and in this my account of the development differs from that of Butschinsky (1900).

In this stage in the region of the first antenna there is a decided thickening of the ectoderm on either side of the body-wall dorsal to the antennæ. These thickenings are lateral, but more ventral than dorsal in position. I take them to be the earliest traces of the shell valves (fig. 37, *s.v.*).

Mesoderm.—There is no great advance in this layer upon its condition in the embryo with nauplius appendages. We find mesoderm as far forward as the optic lobes, though not in the lobes. It is to be found in the appendages (i. e. in the first and second antennæ and mandibles (figs. 35—39). As yet there is no sign of a split in the mesoderm except in the second antenna (fig. 39) where there is the appearance of a split which may, however, be accidental and due to reagents. Vitellophags are still fairly abundant.

Endoderm.—This consists of large columnar cells with large nuclei. It is still confined to the hinder part of the body, extending on the dorsal side from the proctodæum to the region of the optic lobes. Ventrally it does not extend so far forward. Only in the abdominal papilla does it completely surround the yolk as is shown in the above diagram (2).

Stage E (fig. E).

External View.—The chief new features to be noticed in an external view of the embryo at this stage are the increase in the length of the appendages, the appearance of the median head flap, and the great increase in the size of the labrum.

There is also a change in the position of the appendages. They now more or less follow the outline of the body and are directed backwards instead of standing out at an angle to the body as they did in the last stage. The shape of the body itself too has altered a little. It is now longer and more oval than in Stage D. The posterior half is, however, much narrower than the front end, and the papilla stretches farther forward than it did in the last stage.

Internal Structure. Ectoderm; the Nervous

System.—The arrangement of the nervous elements which form the brain is very similar to that described in the last stage. The large ganglion cells still form part of the general ectoderm of the body. The protocerebrum still consists of two large lateral and two small median lobes (fig. 44), but the central mass reaches farther forward than it did, only the most anterior region of the protocerebrum being now without this central mass between the optic ganglia.

The most anterior parts of the optic ganglia resemble those in Stage D, i. e. they consist merely of the two kinds of nerve-cells there described.

The deuterocerebrum has grown considerably, and now consists of two parts:

(*a*) A median mass of nervous cells lying in front of the first antennæ.

(*b*) A pair of ganglia lying on a level with, and innervating the first pair of antennæ.

The anterior of these parts is, in reality, formed by that part of the central mass of nerve-tissue which lies immediately behind the optic region.

Reichenbach (1886) in describing the brain of a similar stage in the Crayfish says that the anterior parts of the supra-œsophageal ganglia are separated from their posterior parts on the one hand, and from the optic ganglion on the other by strands of connective tissue. Now, in a transverse section through the first antenna in Stage D (fig. 36, *f.*) there are to be seen cells which partly separate the median mass from the ganglion on either side, but these cells have the appearance of ectoderm cells. They do not resemble the mesoderm cells of Stage D, and, as yet, there is no mesoderm in the ventral part of the brain. In Stage E it is only just beginning to enter the brain from the dorsal, i. e. the yolk side.

Further, the cells lining the slight groove which separates the two lateral halves of the deuterocerebrum ventrally are certainly ectodermal. They can be seen forming a slight inpushing in fig. 47, and also as wedge-shaped cells in figs. 50, 46, and 34 (*wed.*).

In sections through developing Mysis Nusbaum (1887) showed ectoderm cells separating the different parts of the brain from each other. In my longitudinal sections through the brain at this stage I have not been able to demonstrate such cells, though fig. 48 shows the different parts of the brain fairly well.

In the optic lobes, and in both parts of the deutero-cerebrum, there are now to be seen fibres, as well as the two kinds of nerve-cells.

In the optic lobes the fibres are present in the posterior half only. Fig. 44 shows a transverse section taken about midway through the optic lobes. In it one can see the two lateral optic ganglia, each slightly bilobed, and the central mass, which is also bilobed, the lobing in both cases being much more distinctly marked on the dorsal than on the ventral surface. The median mass is, as I have said above, in direct continuity with the anterior part of the deutero-cerebrum. In this latter region it is widely bilobed on its dorsal side, and transverse fibres can be seen passing between its lateral parts (fig. 47). Fibres can also be traced from the antennulary ganglion into the antennule (fig. 50, *n.f.*).

The smaller nerve-cells are grouped very definitely and symmetrically all through the brain, forming more or less geometrical designs (figs. 44—46), but the main arrangement of the nervous elements is that large ganglion cells lie outside, within these smaller ganglion cells, and within these again, fibres (figs. 44—51).

Behind the region shown in fig. 47 the anterior part of the stomodæum pushes up, as it were, between the two latero-dorsal lobes of the central nervous mass, so that at the level of the first antenna there are no more transverse fibres to be seen, and the ganglia of the antennules appear to be in direct continuity with the ventral and dorso-lateral portions of the central mass, which here comes to an end (fig. 49 and 50). As in the preceding stage, the tritocerebrum, which consists merely of the pair of ganglia which innervate the second antennæ, is not nearly so large as either of the two

parts of the brain which lie in front of it (fig. 48, *t.c.*). These ganglia of the third pair are separated from each other by the labrum (fig. 51). In some specimens here, as in the first antennæ, one can see fibres running from the ganglion to the antenna. These fibres seem to run from the smaller dark-staining nuclei. This fact, taken with the arrangement of these small nuclei in the patterns alluded to above, leads one to think that the fibres originate from these small nuclei.

To sum up. The brain in this stage, as in the last, consists of three pairs of ganglia and a central mass of nervous tissue. This central mass extends farther forward than it did in the last stage, and in it, and in each of the ganglia, nerve-fibres have made their appearance. The deuterocerebrum consists of two parts lying one behind the other, but the anterior of these parts is not the anterior portion of the second pair of ganglia, but the posterior portion of the central mass of nervous tissue mentioned above. As has been stated above, this central mass does not originate from the primitive ganglia first seen in the embryo with nauplius appendages, but from the median band of cells lying between the ganglia of the first and of the second pairs.

In the stage at present under consideration it extends from almost the most anterior region of the space between the optic ganglia to the place where its two lateral lobes join the ganglia of the antennules. It consists now of two parts —(*a*) lying between the optic ganglia, (*b*) lying behind this and in front of the ganglia of the first pair of antennæ.

Between the antennary ganglion and that of the mandible on either side there is a narrow chain of nerve cells, representing a future commissure.

There is, as in the last stage, a deep groove between the two ventral halves of the mandibular ganglion. In the anterior part of this ganglion fibres can be seen in the middle of each half (fig. 52 *n.f.*). It is difficult to say whether the ganglion is formed by the fusion of merely the ganglia of either side, or by the fusion of three elements, the ganglia

of either side, and a median strand. I incline to think that it is formed from the fusion of two ganglia only, though the central part looks very like the central mass described above, and Reichenbach describes a median strand as forming part of the ventral chain in the Crayfish.

Posteriorly the mandibular ganglion is flattened and spread out as it was in the last stage, but the ganglion of the first maxilla has now assumed a more compact shape. It has no fibres as yet.

The Eyes.—Fig. 46 shows the optic invagination fairly well. Other sections in the same series show a closer contiguity between the cells with large colourless nuclei and the small dark-staining nuclei of the nerve-cells which here abut on them. It seems not improbable that these small nerve-cells have originated from the optic invagination, though I can find no more definite indication of it than that shown in fig. 30.

In contra-distinction to the other cells at this stage the cells of the outer wall of the invagination show distinct cell outlines. Their nuclei, too, are larger and paler than those of the other cells.

Other Ectodermal Structures.—The labrum (fig. 52 and fig. E) is much larger than it was in Stage D, and in some specimens there is a great advance in the growth of the stomodæum, the future stomach being distinctly foreshadowed (figs. 53, 54 *st.*).

The lateral ectodermal thickenings (future shell valves) have increased in size (figs. 51 and 53). In one or two specimens I have found a distinct median dorsal thickening of the ectoderm. This I take to be the first stage in growth of the dorsal part of the bi-valve shell (figs. 53, 54 *s.*).

Mesoderm.—The mesoderm cells have increased in number, and are now pretty evenly distributed along the whole length of the embryo, i. e. they are now no longer more abundant in the papilla than elsewhere. Fig. 54 shows several mesoderm cells lying round the stomodæum, some of them very closely attached to it.

In fig. 54 I have shown seven mesoderm cells (*h.c.*) lying just under the dorsal thickening of the ectoderm. These I think are probably the first traces of the heart. It is in just this way, according to Nusbaum (1887), that the heart first appears in Mysis.

At the base of the second antenna the rudiment of the antennary gland can be distinctly seen in some specimens

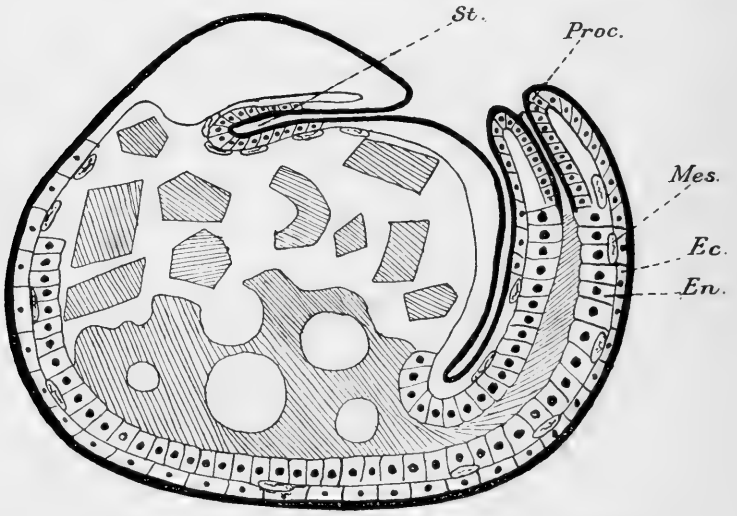


Diagram 3.—Median longitudinal section through embryo at Stage E. Lettering as in Diagram 2.

(fig. 55). It is, roughly speaking, triangular in shape with a triangular lumen. The cells, of which there are not more than six or seven, have a finely granular protoplasm with here and there a lump or rather globule of something which, since it stains in the same way as the yolk, I take to be the excretion. There are still some vitellophags to be seen.

Endoderm.—On the dorsal side the endoderm now extends along almost the entire length of the embryo, but ventrally still only a little way in front of the curve formed by the folding over of the papilla (Diagram 3).

Stage F (fig. F).

External View.—The embryo now has burst its shell, but is still enclosed in a cuticle. Metschnikoff says of this cuticle that "it invests all the first five appendages (nauplius appendages and maxillæ) closely following all their curves and outgrowths. The rest of the appendages are covered by an unbroken skin which forms a general sac over them and the back." In looking at the embryo under a dissecting microscope I have not succeeded in making out this cuticle, much less its disposition, but in many of my sections I have noticed pieces of it loosely enveloping the embryo.

In breaking the shell the embryo has become uncurled. It seems, in fact, to have sprung back with a rebound, so that now there is a slight dorsal curvature of both head and tail. It has now, besides the eyes, antennæ, mandibles, and maxillæ, seven, sometimes eight, thoracic appendages, sometimes also the rudiments of three abdominal legs. The optic lobes are now almost completely nipped off from the yolk. All the thoracic appendages are bilobed so that if the embryo were free-swimming one might call this the Mysis stage.

Internal Structure. Nervous System.—The nervous cells are now leaving the surface, i. e. they now no longer form part of the ectoderm of the body wall. This can be seen in fig. 56 *e.c.*, where there is shown a thin layer of ectoderm lying outside the large dividing cells of the ganglion of the antennule.

Protocerebrum.—Concomitant with an increase in size of the optic ganglia there is an increase in the number of fibres which they contain. The nerve cells in these ganglia have a very regular arrangement resembling that shown in Nusbaum's figures of the brain of the Mysis embryo (Nusbaum, 1887, fig. 80).

The central mass of nervous tissue between the optic ganglia has grown considerably. It is now very distinctly bilobed, and, in section, can be seen to be clearly marked off

from the central mass which forms the anterior part of the deuterocerebrum (fig. 57).

The Deuterocerebrum now consists of three distinct regions:

(*a*) An anterior, median, bilobed mass lying directly behind the central mass of the protocerebrum (fig. 57, *c.m.*).

(*b*) Two large outer lobes which lie outside of and slightly behind the hinder part of (*a*), and which are in direct continuity with—

(*c*) The ganglia of the antennules. These are slightly larger than they were in Stage E.

Tritocerebrum.—This has grown considerably and each ganglion now consists of two parts:

(1) A ventral mass of nerve cells lying close to the stomodæum, and—

(2) A larger dorso-lateral mass lying farther from the middle line (fig. 60).

These masses are in continuity with each other except at the point on either side where fibres pass from the ganglion to the antenna. The bay between these masses of cells is filled with fibres, and, posteriorly, each ventral mass is continued as a thin cord or chain of cells which connects the antennary ganglion with that of the mandible on the same side.

There is more mesoderm in the brain than was present there in the last stage. This mesoderm consists of chains of large cells which run more or less obliquely from the stomodæum to the dorsal body wall (figs. 59 and 60). These chains are the forerunners of the bands of connective tissue and muscle which connect the stomodæum with the dorsal body wall in the later stages.

The brain here closely resembles that of Mysis at a like stage of development.

In the mandibular ganglion the two dorso-lateral clumps of ganglion cells present in the adult are beginning to make their appearance, and, as in the adult, there are ganglion cells in the centre showing the double origin of this ganglion. The

space between the central mass of ganglion cells and the dorso-lateral mass on each side is spanned by fibres (fig. 61, *g.* IV, also Claus 1889, fig. 3, Taf. ix).

The ganglia of the first and second maxillæ both consist, at their widest parts, of a ventral mass of ganglion cells which is continued dorso-laterally into two rounded masses of cells, one on each side, the space between these masses being spanned by fibres. In shape these maxillary ganglia are very much like those of the adult, and, as in the adult, the fibres from them to the maxillæ go off between the dorso-lateral humps on each side and the ventral mass.

The first, second, and third thoracic ganglia are similar to those of the maxillæ. Between the ganglia there runs a double cord of nerve cells. The centre of each half of this cord is filled with fibres (fig. 64, *nf.*).

The posterior ganglia of the ventral chain are as yet triangular masses of nerve cells, each triangle having a slightly double appearance, and lying with its apex directed ventrally.

One cannot at this stage speak with certainty as to the future part to be played by the central mass of nervous tissue. It seems not improbable that it furnishes fibres in the later stages of brain development.

As will be seen from the above I have not been able to find a central mass in any of the ganglia of the ventral chain.

The Eyes.—The separation of the optic lobes from the yolk, which, indeed, began in the more advanced specimens of Stage E, is here almost complete.

On the outer and lower edge of each optic lobe the outer ectodermal layer can be seen in some places to be more than one cell deep (figs. 59 and 60), and this leads me to believe that this layer gives rise not only to the corneal hypodermis, but also to the cone cells.

The large cells with pale nuclei can be easily recognised again here (figs. 58, 59, and 60, *p.n.*), though cell outlines can now no longer be distinguished. Nor can one at this stage see any connection between these large cells and those of the optic ganglion.

Unfortunately this stage is not sufficiently advanced to enable one to write definitely about the future of the different parts of the eye mentioned here; still it seems almost certain that the outer cells of the optic thickening first seen in Stage B, seen also in Stages C and D, to lie in front and outside of (i. e. more lateral than) the optic invagination and the optic ganglion, and to be traced in the later stages, do really furnish the cone cells and the corneal hypodermis, while the optic invagination furnishes the retinulæ, and in the early stages certainly gives off some cells for the increase of the optic ganglion.

I have not been able so far to find any mesoderm cells between the optic ganglion and the future retina.

Since Reichenbach (1886) a great deal has been written about the development of the crustacean eye as a whole, and the optic invagination in particular. Kingsley (1887) seems to derive the whole eye, corneal hypodermis excepted, from the invagination which he says never becomes solid. Parker (1891) was of opinion that when an invagination occurs it is concerned with the optic ganglion only. In a later paper (1895), however, he seems to have veered round to Reichenbach's view as to the Crayfish eye, viz. that the outer wall caused by a division of the primary invagination forms the retina, and the inner furnishes cells to the ganglion. There can be little doubt as to the homology of the proliferation in Branchipus, the Lobster and Mysis, with the invagination in the Crayfish and Nebalia. Parker (1895) says that eventually in the Lobster, and probably in the Crayfish the ganglion loses its connection with this centre of growth, which continues as a growing area for the retina only. But Claus (1889), on the other hand, considered that the proliferation in the Branchipus larva was continued as the zone of growth in the adult, and that this zone furnished ganglion cells in the direction of the ganglion, and retina cells in the direction of the retina. He believed the zone of growth in the eye of the adult Nebalia to be homologous with that in the eye of Branchipus, and that in the Crayfish. In the

main he was in agreement with Reichenbach for he considered the proliferation in Branchipus to be virtually the same thing as the invagination in the Crayfish. Also, it is quite clear that he looked upon the zone of growth in the adult Nebalia as having originated in either a proliferation or an invagination.

Other Ectodermal Structures.—The stomodæum has grown considerably, and the shape of the future stomach is foreshadowed more definitely than it was in the last stage. Its two lateral walls are now composed of deep columnar cells arranged so as to form two curved pads which nearly meet in the middle (fig. 61, *st.*).

The labrum has grown larger, and in sections one can see that jointing has begun in the antennæ (figs. 57—59).

In the region of the mandibles the thickening of the dorsal ectoderm of the body wall mentioned in Stage E can again be seen (fig. 61). This, I think, is precedent to the formation of the dorsal part of the shell.

The two lateral shell thickenings have greatly increased in size, and fig. 64*f.* shows a fold beginning to form between one thickening and the rest of the ectoderm in its region.

Endoderm.—The yolk is now surrounded by endoderm except in the most anterior part of the embryo. In the thoracic region two latero-ventral outgrowths of the endoderm (which here completely surrounds the yolk) can be seen (figs. 63 and 64). These I take to be the beginnings of the liver lobes. It is in this way that the hepatic lobes in Mysis first make their appearance (Nusbaum, 1887).

Metschnikoff (1868) in describing this stage states that the yolk which is surrounded by endoderm is a coherent fluid mass, while that in the anterior region which is unsurrounded is broken up into cone-shaped lumps. My sections show a similar difference between the surrounded and unsurrounded yolk. Also, in the surrounded yolk I find no vitellophags, while in the unsurrounded portion some of these cells are still to be seen. This, I think, goes to prove that the vitellophags do in some way soften the yolk, and that when

it is ready for absorption by the endoderm they (having done their work) disappear. The appearance of yolk in preserved embryos is, of course, to a certain extent influenced by the fixative used.

Mesoderm.—There is an increase in the number of mesoderm cells, but as yet they show no very definite arrangement except in the region of the stomodæum where they are beginning to form a layer one cell deep round the stomach (fig. 61, *mes.*).

Figs. 59 and 60 show mesoderm cells running in oblique lines dorsalwards from the dorsal wall of the stomodæum. These lines doubtless represent the bands of connective tissue and muscle which in later stages run from the stomach and œsophagus to the dorsal body-wall.

In the region of the second maxilla, beginning at the posterior end of its ganglion, there are on either side stretching outwards from the ganglion towards the shell-thickening, three mesoderm cells which form a rudimentary muscle (fig. 62). The figure represents a transverse section taken slightly behind the ganglion. I have not been able to trace fibres from the ganglion to the muscle, but have little doubt that it is innervated from this ganglion (that of the second maxilla). Therefore it does not foreshadow the great transverse shell muscle of the adult which is innervated from ganglion of the first maxilla (Claus, 1889).

The Heart.—Of the seven mesoderm cells which were present under the dorsal ectodermal thickening in the last stage, I can find but one here (fig. 61). However I have little doubt that those cells do represent the heart in its earliest stage, for in a stage which is but very little older than this (Stage F) one sees in the same region in which this band of cells was present in Stage E (i. e. the region near the posterior end of the stomodæum) a few mesoderm cells arranged round a definite lumen just under the dorsal shell-thickening (figs. 55 *a*, 55 *b*, 55 *c*). This must be the anterior end of the heart or dorsal aorta. I imagine this to have been formed by a bending inwards of the mesoderm cells so as to

form a space between themselves and the dorsal ectoderm. It is much in this way that the heart in the Crayfish arises (Reichenbach, 1886).

The Antennary Gland.—In the stage with nauplius appendages mesoderm cells can be seen being carried into the appendages as they are folded off from the rest of the blastoderm (figs. 20—22, *mes.*). In Stage D mesoderm cells can be seen lying in the antennæ between the two layers of ectoderm, though there is there very little histological difference between ectoderm and mesoderm (figs. 37—39, *mes.*). Still, having traced mesoderm into the antennæ and finding no sign of an ectodermal invagination of any kind I have little doubt that the glands which appear at the bases of the second antennæ in Stage E are mesodermal in origin. These glands in this stage (F) are in the same condition as in Stage E (figs. 55, 59). There is no duct as yet, nor is there any sign of a gland in the second maxilla. Claus (1889) showed that in the adult Nebalia both glands function, and his observations have lately been confirmed by Bruntz (1904). In the adult the antennary gland is very rudimentary, and that in the second maxilla still less developed. Claus (1889) uses these facts as evidence in favour of Nebalia's belonging to the Malacostraca; for, he says, that while in Phyllopods the antennary glands appear first, and function, while the shell glands, if present, are insignificant and functionless, in the adult the antennary glands have dwindled, and it is the shell glands which function. In the Malacostraca, he continues, the relative importance of these two glands at the different ages is reversed, and in the adult it is the antennary gland which functions while the maxillary gland, if present, is comparatively insignificant. These statements have received further confirmation in Dr. Allen's work on the Nephridia of Decapods (1893). But it seems that logically speaking this piece of evidence, though it certainly helps to remove Nebalia from the Entomostraca, does not help to place it among the Malacostraca; for though in the adult Nebalia the antennary gland is the larger and the more

important it is also the first to appear in the embryo, and apparently functions before there is any sign of a gland in the second maxilla. I must add that I have found antennary glands in two stages which are more advanced than Stage F, and maxillary glands in one of those stages. There is no sign of degeneration in the glands of the adult as compared with the glands in those late embryonic stages.

It seems to me that these facts about the excretory glands, if they show anything at all as to the systematic position of Nebalia, point rather to its having come off from the original Crustacean stem (from a form in which both glands were equally developed) before either the Entomostraca or the Malacostraca, than to its being descended from either a primitive Phyllopod (Entomostracan) or a primitive Malacostracan.

Genital Cells.—In the thoracic region close to the hepatic outgrowths there is on each side, lying almost between the outgrowth and the mesenteron from which it has arisen, a small group of three or four mesoderm cells (figs. 63, 64). Nusbaum (1887) found what he considered to be genital cells in this place in a similar stage in Mysis, though he somewhat unsatisfactorily traced them from the abdomen to this position, and considered them to be ectodermal in origin.

Wagner (1894) states that in Mysis the genital cells appear very early as lateral outgrowths of Bergh's (1893) "entoderm disc," and that they travel from a ventral to a dorsal position, which last they reach at a very late stage, having on the journey become surrounded by a layer of flat mesoderm cells. It can be seen that neither of these authors is very convincing as to the origin of the genital cells in Mysis, and therefore it is with much hesitation that I suggest the possibility that these cells are genital cells. In my figure they certainly appear to be mesodermal, though they are darker, and more granular, as well as slightly smaller than the other mesoderm cells. If they are not genital cells they may possibly be future connective-tissue cells.

The vitellophags are much reduced in number. They are, in fact, only to be found in the unsurrounded yolk at the front end of the embryo.

Conclusion.

Claus's last paper is, among other things, a great summing up in favour of the Malacostracan position of Nebalia. The history of the development, as far as I have taken it, can do little but make his position still stronger. The thoracic limbs are perhaps the most Phyllopod-like feature that Nebalia possesses, and Claus has shown these to be intermediate in character, between those of a Phyllopod and those of the Schizopoda.

A Malacostracan feature in the development of Nebalia, to which, I believe, notice has not yet been drawn, is the sharpness of definition with which the embryonic stages are marked off from one another.

Among the Malacostraca the form which appears to be most nearly related to Nebalia is Mysis. The organs which are alike in the adults are alike also in their development. To begin with external points. The very definite early embryonic stages in Nebalia resemble very closely the early embryonic stages in Mysis, and in both animals the young stay in the brood pouch of the mother till they are practically adult. The brood pouches in the two animals are formed in the same way by spiny outgrowths on the coxopodites of the thoracic legs.

The peculiar form of gastrulation, the development of the endoderm in Stage B, the subsequent formation of the mid-gut by circumcrescence, and the development of the liver lobes in Nebalia all closely resemble the facts as recorded for Mysis (Nusbaum, 1887, and Bergh, 1893).

If the heart arises and develops as I have suggested above, then its development resembles that in Mysis more nearly than that in any Phyllopod. Another point of resemblance

lies in the development of the brain with its large central mass of nervous tissue (Nusbaum, 1889). There is also a likeness in the early stages of the development of the eyes, assuming that the proliferation in Mysis represents the invagination in Nebalia. If the cells mentioned above be indeed genital cells, there is still another point of resemblance in the development of the genital organs which in the adult Mysis and Nebalia are very much alike. The accounts of the development of these organs in Mysis given by Nusbaum (1887) and Wagner (1894) are certainly not very lucid, but they agree in one point. According to each of these accounts the cells travel from a ventral to a dorsal position, and this is what the cells shown in fig. 66 will have to do if they be really genital cells. They certainly resemble very closely those shown by Nusbaum in an embryo of Mysis.

It is very unfortunate that we have at present no account of the development of the excretory glands in Mysis. I have, in a somewhat circuitous way, found out that its antennary and maxillary glands develop in the order which obtains for these glands in Nebalia. Nusbaum (1887), though he carried his investigations on to late stages in development, says that he could find no trace of excretory glands in his specimens. I have cut sections through two Mysis embryos which, since their genital organs are slightly more dorsal in position than those shown in Nusbaum's figures, I take to be a little older than his latest stages. In each of these I found a well-developed antennary gland, but no true trace of a gland in the second maxilla.

In a paper on excretion Kowalewsky and Metschnikoff (1889) state that shell glands have been found by Claus in the larvæ of Mysidæ. Unfortunately I have been unable to verify this assertion, as they give no reference, and, though I have looked through numbers of papers by Claus, I can find no record of the observation. Assuming Claus's observation to have been correct, I am forced to the conclusion that in the embryos, in which I found antennary glands but

no maxillary glands, these latter had not yet appeared, and that, therefore, the antennary gland in Mysis, as in Nebalia, develops before that of the second maxilla. A maxillary gland has not yet been recorded for the adult Mysis. There can be no doubt that the glands on the thoracic legs in Nebalia, in spite of their alkaline reaction (Kowalewsky and Metschnikoff, 1899), are morphologically equivalent with the glands in the same position in Mysis, Squilla, and other Malacostraca.

As far back as 1868 Metschnikoff suggested that, in a classification of the Crustacea, Nebalia should be placed by the side of the Schizopods. Since then, as can be seen in the historical section of this paper, other observers have from time to time noted the likeness of Nebalia to the Schizopods or the Mysidæ. Claus (1886) and Grobben (1892), in their schematic trees (which differ only as regards the Stomatopoda) make the Lepostraca come off from the Protomalacostraca with the Protoschizopoda. In the present state of our knowledge this arrangement perhaps shows the relationship between Nebalia and the other Malacostraca better than any other, for the Mysidæ are undoubtedly the most primitive Schizopods.

The classification of the Malacostraca by Grobben (1892) and Hansen (1893) into Leptostraca and Eumalacostraca, and the division of the Schizopoda by Boas (1883) and Hansen (1893) also make apparent the nearness of the Mysidæ to the Leptostraca; but Calman (1904) in his amplification of Hansen's classification of the Malacostraca seems to have masked this nearness. He has done this by the introduction of Anaspides into the Eumalacostraca. We know very little about this animal, and nothing of its development. Judging from the account of its internal organs given by its discoverer (Thomson, 1894) it would seem to differ so much from the other Crustacea as to warrant its not being given a place at least until some well preserved specimens have been examined.

Since writing the above I have read a paper in the 'Quarterly Journal of Microscopical Science,' for December, 1905, in which the author, G. H. Carpenter, while suggesting that the Leptostraca are the most primitive Crustacea, and admitting their nearness to the Malacostraca, to which he says "they may be, to some extent, ancestral," states that he considers their nearest relations among the Entomostraca to be not the Phyllopods but the Copepods. This leads me to think that, since it has been my aim to emphasise the Malacostracan position of Nebalia, and its nearness to the Mysidæ, I have, perhaps, rather implied than explicitly stated (as I should have done), that though one cannot now think that Nebalia is descended from the Phyllopods, or, indeed, from any of the Entomostraca, yet I believe that its nearest allies among the Entomostraca are the Phyllopods. It seems to me that, leaving aside other points of resemblance between Branchipus and Nebalia, the likeness between the thoracic limbs of Nebalia and those of Branchipus and Apus cannot be accounted for by homoplasy. Thinking over this, I have been led again to Professor Lankester's illuminating papers (1881—1904), the reading of which has only strengthened my previous convictions.

It seems not improbable that Nebalia is the most ancient Crustacean of which we know at present. Perhaps the strongest piece of evidence for this view of its position lies in the fact that the adult animal has three pairs of cœlomoducts. If, however, I were to speculate as to the Ancestral Crustacean I should be inclined to imagine it as possessing the most primitive features not of Calanus, Nebalia, and Triarthrus, but of Apus and Nebalia.

March, 1906.

References to Literature.

Allen, E. J.—1893. "On the Nephridia and Body Cavity in some Decapod Crustacea," 'Quart. Journ. Micr. Sci.,' vol. 35.

Bergh, R. S.—1893. 'Zur Bildungsgeschichte des Keimstreifen von Mysis Zool. Jahrbücher' (Anatomie), Bd. vi, Jena.

Boas, J. E. V.—1883. "Studien ueber die Verwandtschafts beziehungen der Malakostraken," 'Morph. Jahrb.,' Bd. viii.

Bruntz, L.—1904. "Contribution à l'Étude de l'Excrétion chez les Arthropodes," 'Archives de Biologie,' tom. xx.

Butschinsky, P.—1897. "Die Furchung des Eies und die Blastodermbildung der Nebalia," 'Zool. Anzeiger,' Bd. xx.

——— 1900. "Zur Entwicklungsgeschichte der Nebalia Geoffroyi," 'Zool. Anzeiger,' Bd. xxiii.

Calman, W. T.—1904. "On the Classification of Crustacea Malacostraca," 'Annals and Mag. Nat. Hist.,' ser. 7, vol. xiii.

Carpenter, G. H.—1905. "Notes on the Segmentation and Phylogeny of the Anthropoda, with an account of the Maxillæ in Polyxenus lagurus," 'Quart. Journ. Micr. Sci.,' vol. 49, part 3, N.S., No. 195, Dec., 1905.

Claus, C.—1872. "Ueber den Bau und die System Stellung von Nebalia nebst Bemerkungen über das seither unbekannte Männchen dieser Gattung," 'Zeitschr. f. wiss. Zool.,' Bd. xxii.

——— 1873. "Zur Kenntniss des Baues und der Entwicklung von Branchipus und Apus," 'Abhand. Gesell. d. Wissensch.,' Göttingen, Bd. xviii.

——— 1876. "Untersuchungen zur Erforschung des Crustaceen Systems.'

——— 1886. "Neue Beiträge zur Morphologie der Crustaceen," 'Arbeit. Zool. Instit.,' Wien, Bd. vi.

——— 1886. "Untersuchungen über die Organisation und Entwicklung von Branchipus und Artemia," 'Arbeit. Zool. Inst.,' Wien, Bd. vi.

——— 1889. "Ueber den Organismus der Nebaliden und die Systematische stellung der Leptostraken," 'Arbeit. Zool. Instit.,' Wien, Bd. viii.

Fabricius, O.—1780. "Fauna Groenlandica," Hafniæ et Lipsiæ.

Grobben, C.—1892. "Zur Kenntniss des Stammbaues und des Systems der Crustaceen," 'Sitz. Kaiserl. Akad. d. Wissench.,' Wien, Bd. ci.

Hansen, H. J.—1893. "Zur Morphologie der Gliedmassen und Mundtheile bei Crustacen und Insecten," 'Zool. Anzeiger,' Bd. xvi.

Herbst, J.—1796. "Versuch. einer Naturgeschichte der Krabben und Krebse," Berlin.

Herbst, C.—1896. "Ueber die Regeneration von Antennenähnlichen Organen," 'Archiv f. Entwickelungs Mechanik.,' Bd. ii.

Heymons, R.—1901. 'Die Entwicklungsgeschichte der Scolopender Zoologica,' Bd. xiii, Heft xxxiii.

Kingsley, J. S.—1887. "The Development of the Compound Eye of Crangon," 'Journal of Morphology,' vol. i.

Korschelt, E., und Heider, K.—1893. 'Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere,' Bd. ii.

Kowalewsky, A.—1886. "Zur embryonalen Entwicklung der Musciden," 'Biol. Centralbl.,' Bd. vi.

——— und Metschnikoff, E.—1889. "Beitrag zur Kenntniss der Exkretionsorgane," 'Biol. Centralbl.,' Bd. ix.

Kröyer, H.—1847. 'Karcinologische Bidrag. Naturhist.,' Tidskrift ii, Raekke, Bd. iv.

Lankester, E. R.—1881. "Observations and Reflections on the Appendages and on the Nervous System of Apus cancriformis," 'Quart. Journ. Micr. Soc.,' N.S., vol. 21.

——— 1904. "The Structure and Classification of the Arthropoda," 'Quart. Journ. Micr. Soc.,' N.S., vol. 47.

——— 1904. "The Structure and Classification of the Arachnida," 'Quart. Journ. Micr. Soc.,' N.S., vol. 48.

Latreille, P. A.—1831. 'Cours d'Entomologie,' Paris.

Leach, W.—1814. 'Zoologist's Miscellany.'

Metschnikoff, E.—1868. "On the Development of Nebalia" (Russian), 'Acad. Imp. d. Sci.,' St. Petersburg, tom. xiii.

Milne-Edwards, H.—1828. "Mémoire sur quelques Crustacés nouveax," 'Ann. Sci. Nat.,' Paris, tom. xiii.

——— 1835. "Note sur le genre Nebalia," 'Ann. Sci. Nat.,' sér. 2, tom. iii.

——— 1840. "Histoire naturelle des Crustacés," tom. ii, Paris.

Montagu, G.—1813. "Descriptions of several New and Rare Animals discovered on the South Coast of Devonshire," 'Trans. Linn. Soc.,' Lond., vol. ix.

Nusbaum, J.—1886. "L'Embryologie d'Oniscus murarius," 'Zool. Anz.,' vol. ix.

——— 1887. "L'Embryologie de Mysischameleo," 'Archives de Zoologie, Expé. et générale, Deuxième sér., tom. v.

Parker, G. H.—1891. "On the History and Development of the Eye in the Lobster," 'Bull. Mus. Comp. Zool.,' Harvard, vol. xx and xxi.

——— 1895. "The Retina and Optic Ganglion in Decapoda," 'Mittheil. Zool. Stat. Neapel,' Bd. xii.

Reichenbach, H.—1886. 'Studien zur Entwicklungsgeschichte des Flusskrebses.'

Sars, G. O.—1885. 'Challenger Reports,' vol. xiii.

——— 1887. 'Challenger Reports,' vol. xix.

Thomson, G.—1894. "On a Freshwater Schizopod from Tasmania," 'Trans. Linn. Soc.,' London.

Viallanes, H.—1892. "Système nerveux des Articulés." 'Annales des Sciences naturelles,' 7e série, Zoologie, 13, 14.

v. Willemoes-Suhm, R.—1875. "On some Atlantic Crustacea from the 'Challenger' Expedition." 'Trans. Linn. Soc.,' London, ser. 2, Zool., vol. i.

Wagner, Jul.—1894. "Zur Entwicklungsgeschichte der Schizopodon," 'Zoologischer Anz.,' Bd. xvii, p. 437.

EXPLANATION OF PLATES 16—21.

Illustrating Miss M. Robinson's paper "On the Development of Nebalia."

Reference Letters.

ant. Antenna. *a.g.* Antennary gland. *a.p.* Abdominal papilla. *bd.* Blastoderm. *bp.* Blastopore. *cut.* Cuticle. *c.g.* Caudal groove. *c.c.* Crystalline cone cells. *c.t.* Caudal thickening. *c.m.* Central mass of nervous tissue. *d.* Depression in optic thickenings. *div.* Dividing cells. *dc.* Deuterocerebrum. *ec.* Ectoderm. *en.* Endoderm. *f.* Fold between shell thickening and body wall. *g.c.* Genital cells. *g.* Ganglion. *h.c.* Heart cells. *hep.* Hepatic lobes. *h.f.* Head flap. *l.c.* Large cells of the ectoderm. *lab.* Labrum. *m.* Mouth. *md.* Mandible. *mx.* Maxilla. *mes.* Mesoderm. *mus.* Muscle. *n.f.* Nerve fibres. *o.t.* Optic thickening. *op.in.* Optic invagination. *o.l.* Optic lobe. *pc.* Protocerebrum. *p.n.* "Pale nuclei" of the optic invagination. *proc.* Proctodæum. *s.* Shell. *st.* Stomodæum. *s.v.* Shell valve. *tc.* Tritocerebrum. *vp.* Vitellophag. *wed.* Wedge-shaped cells. *y.* Yolk. *y.s.* Yolk sac.

Exigencies of space have forbidden the drawing of more than the ventral half of each section in most cases.

The magnification of the sections is 475, except where otherwise stated.

PLATE 16.

FIG. A.—Stage A. Young embryo showing the blastoderm, which does not as yet completely surround the yolk.

FIG. B′.—Stage B′. Late gastrula stage in which the blastopore is closed, and the optic and antennal thickenings are definitely apparent.

FIG. C.—Stage C. Embryo with nauplius appendages.

FIG. D.—Stage D. Embryo with zoæa appendages.

FIG. E.—Stage E. Embryo in which the appendages more or less follow the outline of the body.

FIG. F. Stage F. Earliest stage in which the embryo is free from the shell. On account of its bifid appendages this might be called the Mysis stage.

FIG. 1.—Longitudinal section through the ovum, younger than Stage A, showing cells within the yolk as well as on the ventral surface. × 238.

FIG. 2.—Longitudinal section through Stage A, showing the yolk as yet incompletely surrounded by the blastoderm. × 238.

FIG. 3.—Transverse section through the lateral thickenings in Stage B. *div.* Dividing cells.

FIG. 4.—Transverse section through the lateral thickenings (behind Fig. 3) in Stage B.

FIG. 5.—Transverse section through the anterior region of the caudal thickening (front end of the groove) in Stage B. × 238.

FIG. 6.—Transverse section through the posterior thickening at the middle of the groove in Stage B. × 238.

FIG. 7.—Transverse section through hind end of the caudal thickening in Stage B (behind the groove). × 238.

PLATE 17.

FIG. 8.—Transverse section through the optic thickenings in Stage B′.

FIG. 9.—Transverse section through Stage B′, just behind the optic thickenings.

FIG. 10.—Transverse section through Stage B′, taken midway between the optic thickenings and the caudal thickening.

FIGS. 11—15.—Transverse sections through the caudal thickening in Stage B′.

FIG. 16.—Transverse section through the anterior part of the optic segment in Stage C. *ot.* Optic thickening.

Fig. 17.—Transverse section through the anterior part of the optic segment in Stage C, behind Fig. 16. *op.in.* Optic invagination.

Fig. 18.—Transverse section through the optic segment in the region of the ganglion in Stage C.

Fig. 19.—Transverse section through the posterior end of the optic ganglion in Stage C, at its junction with the ganglion of the first antenna.

Fig. 20.—Transverse section through the first antennæ in Stage C.

PLATE 18.

Fig. 21.—Transverse section through the second antenna in Stage C.

Fig. 22.—Transverse section through the mandibular segment in Stage C, showing ganglion.

Fig. 23.—Longitudinal section through Stage C, showing the first four appendages and the first three ganglia.

Fig. 24.—Transverse section through Stage C, just in front of the mouth, showing the labrum.

Fig. 25.—Transverse section through the mouth of Stage C.

Fig. 26.—Longitudinal (nearly median) section through Stage C.

Fig. 27.—Section through vitellophag in Stage C.

Fig. 28.—Transverse section through the anterior region of the optic thickenings in Stage D, in front of the optic ganglia. *d.* Depression.

Fig. 29.—Transverse section through the optic thickenings in Stage D, going through the most anterior region of the optic ganglia.

Fig. 30.—Transverse section through the optic thickenings in Stage D, behind Fig. 29, showing the optic invagination and the ganglion.

Fig. 31.—Transverse section through the posterior region of the optic thickenings in Stage D. *c.m.* Central mass.

Figs. 32—34.—Transverse sections through the hindmost part of the optic segment in Stage D.

Fig. 35.—Transverse section through the most anterior part of segment II in Stage D, showing the ganglia of the antennules and the central mass of nerve tissue lying between them.

PLATE 19.

Fig. 36.—Transverse section through the middle region of segment II in Stage D.

Fig. 37.—Transverse section through the hindmost region of segment II n Stage D.

Fig. 38.—Transverse section through the anterior region of segment III in Stage D.

Fig. 39.—Transverse section through the middle region of segment III in Stage D.

Fig. 40.—Transverse section through segment IV in Stage D.

Fig. 41.—Transverse section through the ventral part of segment V in Stage E.

Figs. 42, 43.—Transverse sections through hindmost end of the embryo in Stage D.

Figs. 44—46.—Transverse sections through the optic segment in Stage E. Fig. 44 is the most anterior.

Fig. 47.—Transverse section through the anterior part of the deutero-cerebrum in Stage E. The posterior ends of the optic lobes are cut through in this section.

PLATE 20.

Fig. 48.—Longitudinal section through the brain in Stage E.

Fig. 49.—Transverse section through the posterior region of the deutero-cerebrum in Stage E, showing the two ganglia of the antennules with the stomodæum between them.

Fig. 50.—Transverse section through the ventral part of the segment II in Stage E, showing the antennules, the antennulary ganglia (posterior part of the deuterocerebrum) and the stomodæum.

Fig. 51.—Transverse section through the ventral part of segment III in Stage E, showing the antennary ganglion, the shell thickenings, labrum, and mouth.

Fig. 52.—Transverse section through the ventral part of segment IV (mandibular segment) in Stage E, showing mandibular ganglion with its cleft, labrum, etc.

Figs. 53, 54.—Transverse sections through the antennary segment (behind that shown in Fig. 52) in Stage E, showing the thickening of the dorsal ectoderm and mesoderm cells (future cardiac cells) lying just below it. × 238.

Fig. 55.—Transverse section through the ventral part of the antennary segment in Stage E, showing the first trace of the antennary gland.

Fig. 55, *a*, *b*, *c*.—Three consecutive transverse sections through the dorsal side of a stage a little older than Stage F, showing mesoderm cells turning inwards to form the heart.

PLATE 21.

Fig. 56.—Transverse section through segments I and II in Stage F, showing shell flap, optic and antennulary ganglia, central mass of nervous tissue and labrum. × 238.

Fig. 57.—Transverse section just behind that shown in Fig. 56, showing divisions of the central mass of nervous tissue, the stomodæum, and the first appearance of jointing in the antennules. × 238.

Fig. 58.—Transverse section through Stage F, behind that shown in Fig. 57. × 238.

Fig. 59.—Transverse section through Stage F, behind that shown in Fig. 58. × 238.

Fig. 60.—Transverse section through Stage F, behind that shown in Fig. 59. This figure shows the cuticle. × 238.

Fig. 61.—Transverse section through stomodæal region in Stage F. × 238.

Fig. 62.—Transverse section through ventral side of Stage F, between segments VI and VII, showing rudimentary muscles. × 238.

Figs. 63, 64.—Transverse sections through the first thoracic segment in Stage F, showing the ventro-lateral out-pushings of the mesenteron, genital cells, and shell thickenings. Fig. 63 shows the first thoracic ganglion at its largest part. × 238.

On the Early Stages in the Development of Flustrella hispida (Fabricius), and on the Existence of a "Yolk Nucleus" in the Egg of this Form.

By

R. M. Pace (née Clark),

Late Scholar of Girton College, Cambridge.

With Plates 22—25.

Contents.

Introduction.

In the following paper an attempt has been made to trace out the earlier stages in the development of Flustrella hispida. The larva of this familiar Bryozoan has previously

been studied by Hincks (**13**), Redfern (**22**), Joliet (**14**), Barrois (**2**), and Prouho (**20**), but the first four authors studied only the living larva, of which Barrois has given an excellent account with numerous figures, while Prouho, who has described the later stages of the larval history in detail, has paid but little attention to its earlier stages.

The research was undertaken at the suggestion of Dr. S. F. Harmer, with the view to determine whether that structure overlying the internal sac in the mature larva of Flustrella is to be regarded as a stomach comparable to that which he had described (**11**) as present in Alcyonidium. As the work proceeded it has seemed expedient somewhat to extend its scope, and to follow out the history of the egg from its first appearance; and the presence of a "yolk-nucleus" being detected, this structure has also been studied in some detail.

The work, which has been conducted partly at Cambridge, partly at Plymouth, and partly at Brighton, has been greatly assisted by a grant from the Government Grant Committee of the Royal Society, to whom my best thanks are due. I would also take this opportunity to express my thanks to Dr. Harmer for his kindly interest and criticism, and for the loan of some of his own preparations of later larval stages, and for permission to reproduce one of his drawings (Pl. 25, fig. 65 *a*), to Dr. E. J. Allen for granting me the use of a table at the Plymouth Laboratory of the Marine Biological Association, to the authorities of Newnham College, Cambridge, for permission to work at the Balfour Laboratory, and to Prof. J. Graham Kerr, Dr. E. G. Gardiner, and Mr. W. Wallace, for advice on technical points.

Material and Methods.

Collecting Material.—The material on which this paper is based was collected at the following places and dates: Swanage, March, 1902; Totland Bay, Isle of Wight, April, 1903; Plymouth, February to April, 1903, February to July, 1904; Brighton, May to July, 1903.

At each of the above places Flustrella hispida grows abundantly between tide marks on Fucus, and occasionally also on other Algæ. The colonies form characteristic, dark, mossy-looking patches encrusting the algal fronds: the Fucus, when growing near low water mark, is often almost entirely covered with this Bryozoan, but nearer high water mark the Flustrella is not nearly so abundant, nor is it so well developed. Young colonies occur mainly on Fucus of the same season's growth.

For the study of the larval development, colonies of one or two seasons' growth taken from close to low water mark have proved the most suitable. Such colonies contain abundance of spermatozoa or of ova and larvæ, according to the season. Older colonies contain larger masses of dead zoœcia, larvæ being relatively less abundant, while these latter are wanting in such colonies as are presumably of the current year's growth. Again, in colonies taken near high water mark, larvæ and eggs are comparatively scarce, thus suggesting that the conditions of life are not so favourable as at a lower tide level, possibly because the colonies are uncovered by the water for a considerable part of the day. Flustrella colonies containing ripe reproductive elements or larvæ may be recognised by the presence of numerous dark-brown blotches.

The reproductive period commences early in February and continues until the beginning of August. Generally speaking, it appears that spermatozoa are abundant in February and March, and that they are not in evidence after the latter month. Young ova are scarce early in February and increase in number in March; during this latter month segmenting eggs and young larvæ are also abundant and some mature larvæ are present. Sections of the younger portion of a colony taken in March have shown the presence of segmenting eggs and larvæ in the older zoœcia, and of spermatozoa, together with a few young ova, in the younger zoœcia near the apex of the colony (Pl. 22, fig. 1). The maximum development of young embryos

is from April to June, while in July and August larvæ in advanced stages are still abundant, but at this time young stages and ova are rare. On the other hand, even in March and April, colonies may be found which contain only mature larvæ. It should be stated that in any one colony the majority of the larvæ are at approximately the same stage of development.

Flustrella hispida will readily live in standing water for from one to five days according to the time of year, while in running water it has been kept in good condition for over a week,[1] but it is best whenever practicable to work with quite fresh material, as the larvæ appear less healthy in colonies which have been kept even a few days.

Methods.—The work has been done partly by the study of entire larvæ and partly by means of sections. The larvæ were examined both in the living state and after fixation. For the latter purpose large numbers of eggs and larvæ were removed from the colonies and preserved and in addition portions of colonies were fixed entire in order to study the larvæ in situ.

Fixing Reagents.—The preservatives used were:—

(1) Cold saturated solution of corrosive sublimate, with the addition of 5 per cent. glacial acetic acid.

(2) 100 parts 5 per cent. chromic acid, with five drops of glacial acetic acid.

(3) Flemming's solution.

(4) Hermann's solution.

(5) Dr. Allen's chromo-nitro-osmic acid mixture.

(6) Acetic alcohol containing sublimate to saturation.

(7) Kleinenberg's solution.

Preserving Larvæ in situ.—For preserving larvæ in situ it has been found best to cut the colonies with the seaweed on which they are growing into small portions, and to immerse these in the fixing solution for some time to allow

[1] At Millport, owing to the exceptional purity of the water, there appears to be no difficulty in keeping Flustrella alive in the tanks for a quite indefinite period.—R. M. P.

complete penetration. As soon as possible after fixation the colonies were removed from the Fucus and after washing transferred to 70 per cent. alcohol. Chromo-acetic acid and corrosive acetic have given the best results when dealing with material fixed in bulk.

Isolated Larvæ.—The removal of larvæ from the colonies is best effected by slicing off the front wall of the colony with a sharp razor; the larvæ lie immediately below this wall, enclosed in the tentacle-sheath of the polypides, and they can then be readily removed by means of a scalpel. Before attempting to preserve the larvæ a considerable amount of washing is necessary in order to free them from a mucus-like substance in which they lie imbedded.

The best fixing reagents for the isolated larvæ appear to be corrosive acetic, and acetic alcohol saturated with sublimate; chromo-acetic acid sometimes gives good results; and the fixing reagents containing osmic acid have proved useful, especially in the study of entire eggs and larvæ before clearing. Material preserved in chromo-acetic requires very prolonged washing and frequently proves difficult to stain.

Entire Eggs and Larvæ.—The external characters of isolated eggs and larvæ of all stages have been studied during life. After fixation the larvæ were again examined unstained in 70 per cent. alcohol, and were then stained either in borax carmine followed by acid alcohol, or in safranin, and re-examined. After clearing with cedar-wood oil or clove oil—both of which reagents gave good results—the larvæ were either mounted entire in Canada balsam or imbedded in paraffin for sectioning. Staining the larva with borax carmine after acetic alcohol and corrosive sublimate or corrosive acetic brings out the nuclear spindles and also the yolk nucleus very clearly, and so greatly assists in the interpretation of the external appearance of a segmenting egg. In a few cases the embryo was removed from Canada balsam after having been examined and drawn, and was imbedded in paraffin for sectioning.

Preparation of Sections.—Sections were made both of the isolated larvæ and of portions of colonies containing larvæ. The watch-glass method of imbedding was found the most convenient, especially when dealing with isolated larvæ. Groups of from twenty to thirty isolated larvæ were imbedded en masse and sectioned, sections thus being obtained in a variety of planes. The larva at nearly all stages has a definite axis, which renders it possible to orientate it and so to obtain sections in any desired plane. To serve as a guide in determining in which direction unorientated larvæ had been sectioned, a set of standard sections was prepared by carefully orientating single larvæ which had been first studied entire.

Finally, portions of colonies were imbedded and cut with the larvæ in situ. To insure thorough impregnation, it was found best to soak the material in xylol for about a week, then to leave it in a mixture of xylol and paraffin for about six hours in a warm place, such as the tray of the water-bath, and finally to transfer to pure paraffin for about an hour. In cutting such material great difficulty has been experienced owing to the fact that the larvæ lie close under the front wall of the colony : this wall, being beset with chitinous spines, renders it difficult to imbed and to cut in such a manner that the razor encounters none of the spines when passing through the larvæ, since the chitin is sufficiently hard to notch the razor, thereby of course causing the section to tear. This difficulty is less marked in the case of transverse than of longitudinal sections.

Staining.—The most useful stain for sections appears to be Heidenhain's iron hæmatoxylin, followed by eosin dissolved in 90 per cent. alcohol. By this treatment the structure of the yolk nucleus and of all nuclear bodies is brought out very clearly. Borax carmine and safranin have given good results, and double staining with methyl blue and eosin has also been found useful. Mayer's alcoholic cochineal, picronigrosin, hæmatoxylin with a few drops of Kleinenberg's solution, hæmatoxylin and methyl orange have also been utilised; Mayer's mucicarmine was used for the detection of mucus.

Spermatogenesis and Oogenesis.

In Flustrella hispida the zoœcium is hermaphrodite, but the spermatozoa are chiefly developed earlier in the year than the ova. In February and early in March, however, ova and spermatozoa are found to occur simultaneously in the zoœcium; the spermatozoa are in such cases fully developed, while the ova are immature. Pl. 22, fig. 1, shows a section through a very young colony taken early in March; ova are seen to be present in one zoœcium, and in the anterior portion of the same zoœcium spermatozoa also occur.

Early in February the colonies assume a very puffed and spotted appearance, large dark-brown patches becoming visible. On cutting a section of such a colony these brown patches are found to be due to the presence of an immense number of spermatozoa, which can be removed in the same way as the ova by slicing off the front wall of the colony. As has already been described by Calvet (**8**), the spermatozoa are developed from the mesenchyme lining the lateral walls of the zoœcium, and the mother cells lie in masses close to the front wall in the region of the tentacle sheath (Pl. 22, fig. 1, *T.*). When ripe the spermatozoa have the typical flagellate form. Frequently masses of spermatozoa are seen to be lying with their heads imbedded in a central mass of protoplasm, and with their tails vibrating at the periphery. No attempt has been made at present to work out the details of spermatogenesis. The spermatozoa decrease in number towards the middle of March, and they are not in evidence after the end of that month.

The ovary lies at a point to the rear of and at a lower level than that at which the spermatozoa are developed. It is situated on a funicle passing from the mesenchymatous lining of the lateral zoœcial wall to the intestine (Pl. 22, fig. 1, *Ov.*). At first the young ovary shows no indication of cell-walls, but consists merely of a protoplasmic mass containing numerous large nuclei (Pl. 22, figs. 1 and 4, *N.*). Cell-walls subsequently arise in this protoplasmic mass, four or five of the ovarian

cells being differentiated in this manner (Pl. 22, fig. 5) and developing into ova, while the remainder give rise to the follicle cells. As maturation proceeds, the follicle cells increase in number and appear to grow in among the primitive ova, so that when these latter are ripe each ovum is surrounded by a follicular membrane (Pl. 22, figs. 2 and 3, *Fo.*).

Generally speaking, all the ova contained in the ovary are of about the same age (Pl. 22, figs. 2–5).

The Maturation of the Egg: the Yolk Nucleus.

The chief point of interest in the process of the maturation of the egg of Flustrella is the appearance of a "yolk nucleus," apparently homologous with that type described by van Bambeke (1) as occurring in the egg of Pholcus phalangioides. The existence of a yolk nucleus does not appear to have been hitherto recorded in any of the Ectoproctous Bryoza, although a similar structure has been figured by Braem (4 and 6) and by Kraepelin (17) as being present in the egg of Plumatella among the Entoprocta.

The Yolk Nucleus.—The history of this body in the egg of Flustrella hispida is briefly as follows:

In very young ovaries in which the ovarian cells are only just recognisable, there are, in addition to the germinal vesicle, certain darkly-staining granules surrounding the nucleus and lying in close contact with the latter (Pl. 22, figs. 4–5; Pl. 23, fig. 29, *Y.N.*). These granules at a later period coalesce to form the structure, which, following van Bambeke, may best be termed the "yolk nucleus." They originate, as has been said, quite close to the germinal vesicle, and their appearance is so very similar to that of certain intra-nuclear elements as to suggest that they have originated from the nucleus. In fact, in one case (Pl. 22, fig. 5; Pl. 23, fig. 29) these granules seemed to be in process of actually passing out from the germinal vesicle. The granules at this period are homogeneous in appearance, and their behaviour with any of

the staining reagents employed exactly resembles that of the chromatin granules of the nucleus.

Shortly after this stage these extra-nuclear granules coalesce, and eventually come to form a crescentic body—the yolk nucleus—which (Pl. 22, figs. 8, 11, 13) becomes surrounded by what appears to be a clear space; and at an earlier stage a similar clear zone frequently also occurs around individual granules, or groups of granules, prior to their complete coalescence (Pl. 22, figs. 6–7, *x*). This clear area may abut directly on to the germinal vesicle, or it may be separated from it by a thin layer of protoplasm (Pl. 22, figs. 11, 13, 14). At about this time also, vacuoles begin to appear in the body of the yolk nucleus (Pl. 22, figs. 11 and 13).

The appearance of the yolk nucleus as seen in sections depends, of course, upon the point through which the section is taken (Pl. 22, figs. 9–12). In figs. 9 and 10 the position of the yolk nucleus is marked only by the clear area which usually surrounds it; in fig. 11 the yolk nucleus shows a crescentic cross section, and it is seen to contain numerous vacuoles; while in Pl. 22, fig. 12, it has the appearance of a cap overlying the nucleus.

The yolk nucleus gradually passes from a crescentic to a hemispherical form (Pl. 22, fig. 14); and its growth being proportionately more rapid than that of the egg as a whole, this hemispherical form becomes still more marked in later stages, so that in certain sections the yolk nucleus may even appear as a complete ring encircling the nucleus (Pl. 22, fig. 15). The vacuoles increase in number, and frequently contain crystalloid bodies at this stage (Pl. 22, figs. 13, 14, *cr.*).

The yolk nucleus next loses its originally homogeneous appearance and shows signs of degeneration. This is evidenced by the appearance of a peculiar reticulate structure, the substance between the meshes staining less deeply than the network (Pl. 22, figs. 15, 16). The yolk nucleus then loses its regular outline (Pl. 22, figs. 16, 17), and it finally breaks up into more or less finely-divided, darkly-staining fragments, surrounded each by a clear zone. The process of fragmenta-

tion of the yolk nucleus appears to vary somewhat in its details in different eggs (Pl. 22, figs. 18–21). The fragments of the yolk nucleus retreat towards the periphery of the egg, and there for a time form a disconnected ring of darkly-staining substance, each segment of which is still surrounded by a clear zone (Pl. 22, figs. 22, 23). Soon after this the fragments of the yolk nucleus lose their identity, and at about the same time the first indications of the true yolk make their appearance as small granules, which are scattered about in the cytoplasm (Pl. 23, fig. 24, *Y*.). At the same time vacuolisation of the cytoplasm occurs; the yolk spherules come to lie within these vacuoles and rapidly increase in size until they appear to occupy the entire egg (Pl. 23, fig. 25).

The whole process of the growth and disintegration of the yolk nucleus and the formation of the yolk takes place some time before the egg is released from the ovary.

That the yolk nucleus is a true cell organ, and not merely an appearance due to the coagulation of proteid by the fixing reagent used, is proved by the fact that it is visible in the living egg as a dark mass surrounding or overlying the nucleus.

The nature and origin of the clear zone or space which has been described as surrounding the yolk nucleus are somewhat doubtful. It may possibly contain a fluid, but all attempts to prove by means of staining reagents that this is so have failed; and it would seem perhaps more probable that this apparent space is only an artificial one caused by the contraction of the substance of the yolk nucleus during fixation. This latter view is supported by the fact that the clear zone has not been detected in the living egg, nor in those eggs (Pl. 23, figs. 26, 27) which have been fixed by chromo-nitro-osmic acid, and also by the fact that the shape of the supposed space corresponds so exactly with that of the yolk nucleus which it surrounds.

With the view to determine whether the formation of oil globules, which has been described and figured by von Bambeke (1) as preceding yolk formation in the case of Pholcus

phalangioides, occurs also in the egg of Flustrella, a number of eggs were preserved with Dr. Allen's chromo-nitro-osmic mixture; these were sectioned without previous bleaching, so that any fatty matter might remain intact, but the results obtained have so far proved somewhat difficult of interpretation. Even in very young eggs in which the yolk nucleus is still in quite the initial stages of development, large drops of a fatty substance are found to be present in the region of the developing yolk nucleus; and as the egg enlarges, the number of the fatty drops also increases, but the latter always remain in the immediate neighbourhood of the yolk nucleus, while similar globules are also visible within that body itself (Pl. 23, figs. 26, 27). The amount of fatty material increases as degeneration proceeds. It collects especially towards the periphery of the egg, and at the same time the development of the yolk commences. The two substances increase in quantity side by side, so that the mature egg has the appearance of a mass of yolk spherules interspersed with fat globules (Pl. 23, fig. 28). This condition, the presence of fat and yolk side by side, continues so long as any yolk is discernible in the larva—that is until after the degeneration which precedes metamorphosis has commenced. At no period are the fat globules arranged in any definite relation to the nucleus. It is hoped that it may be possible to elucidate this question of the relation of the fat globules and the food yolk to one another and to the yolk nucleus, by investigating in greater detail the history of the yolk nucleus in the eggs of such other Bryozoa in which it may prove to be present.

Prolonged treatment with xylol will cause the fat globules to disappear from the yolk.

The Germinal Vesicle.—To determine whether there is any connection between the yolk nucleus and the germinal vesicle, the latter body has also been carefully studied. Pl. 23, fig. 29, shows a young germinal vesicle to which particles of the yolk nucleus are in close approximation, and it will be seen that one of these latter appears to be actually in process

of passing out through the nuclear membrane. The chromatin network is somewhat dense, and it has at its nodes deeply staining granules, which are similar in their appearance to the original elements of the yolk nucleus, while a faintly staining substance occupies its interstices.

The germinal vesicle at first grows relatively more rapidly than the egg as a whole, and the chromatin network becomes more attenuated (Pl. 23, figs. 30–32); but this latter fact is probably due rather to the increased size of the nucleus than to any emission of chromatin from it. No further changes take place in the germinal vesicle until after the formation of yolk has been completed, although the irregular contour of the nuclear membrane observed in certain sections (Pl. 23, figs. 24, 25) during the period of yolk formation may possibly denote amœboid movements in connection with the latter process: such amœboid movements have been described by Bambeke (1) in the case of the egg of Pholcus phalangioides.

After the completion of yolk formation the chromatin network begins to thicken (Pl. 23, fig. 33), and the substance between its meshes now stains more deeply (Pl. 23, fig. 34). The nucleolus also becomes relatively very large, and at the same time the nuclear membrane loses its regular outline (Pl. 23, figs. 34, 35). These processes continue until all trace of the chromatin network has disappeared, and the nucleus stains uniformly throughout (Pl. 23, fig. 36). At this stage, which is immediately prior to that of the formation of the polar bodies, the nucleus begins to decrease in size relatively to the rest of the egg and becomes amœboid: the nucleolus is still present.

The Nature and Function of the Yolk Nucleus.—The term "yolk nucleus" has been applied by various authors to bodies which appear to be totally different in their origin, development, and appearance, and which would seem to have only this much in common, that all have been regarded as being in some way connected with the phenomenon of yolk formation. In the present instance,

the body described approximates very closely to that type of yolk nucleus described by Bambeke (**1**), Crampton (**9**), Wallace (**26**), and Calkins (**7**) for the eggs of Pholcus phalangioides, Molgula manhattensis, Zoarces viviparus, and Lumbricus respectively.

It is not proposed in the present paper to enter into a detailed consideration of the former work bearing on this subject. A very complete bibliography of the yolk nucleus is furnished by the papers of Jordan (**15**), Mertens (**18**), Henneguy (**12**), Calkins (**7**), Wilson (**27**), Bambeke (**1**), and Crampton (**9**).

As has already been stated, the yolk nucleus of the egg of Flustrella hispida is closely comparable with that type described by Bambeke, Calkins, Crampton, and Wallace, but on comparison with these apparently closely related bodies certain points of difference may be noted.

Bambeke (**1**) describes in the egg of Pholcus phalangioides a type of yolk nucleus which corresponds closely in its appearance and in its mode of growth and degeneration with that occurring in Flustrella hispida. He recognises four stages in the history of the yolk nucleus and nutritive yolk of the egg of Pholcus: (*a*) the appearance of small, darkly staining granules, which he believes to be of nuclear origin, and which coalesce to form a crescentic structure containing vacuoles, with included crystalloid bodies, and surrounded by a clear zone; (*b*) the degeneration of the yolk nucleus; (*c*) the appearance of oil drops; (*d*) the formation of the true yolk. The most important difference between Bambeke's account of the yolk nucleus and the present one is that in the case of the egg of Pholcus the nucleus appears to take an active part in the process of yolk formation, and that the yolk nucleus itself gives rise to the vitellus by first undergoing a metamorphosis leading to the formation of oil globules, these latter becoming resorbed by the protoplasm from which the true yolk is then elaborated. Now, as has already been pointed out, the presence of oil globules in the case of the egg of Flustrella hispida bears but little

obvious relation to the process of yolk formation. In this form oil globules are present in very young eggs, both in the yolk nucleus itself and also scattered throughout the surrounding protoplasm; and, although they are certainly present in increasing numbers as the yolk develops, they do not disappear with the completion of yolk formation, but persist throughout larval life. So far, also, in Flustrella it has not been possible to confirm Bambeke's views as to the importance of the nucleus as a factor in yolk formation. The irregular shape of the nuclear membrane, which is sometimes observed in the early stages of yolk formation in the egg of Flustrella (Pl. 23, fig. 24), may possibly indicate amœboid movements; but these have no apparent reference to the distribution of the oil drops, which collect chiefly towards the periphery of the egg rather than in the immediate neighbourhood of the nucleus; and neither the oil globules nor the yolk spherules show the radial arrangement which is described by Bambeke.

Crampton (**9**), in his paper on the early history of the Ascidian egg, described a yolk nucleus of similar type in the egg of Molgula manhattensis; and he attempted to determine its chemical nature by differential staining. The general account given by Crampton of the history of the yolk nucleus in Molgula agrees with the above description of this structure in Flustrella. Crampton, among other stains, made use of Heidenhain's iron-hæmatoxylin, and states that this stain is taken up by the chromatin of the germinal vesicle, but that it has no effect on the yolk nucleus; he also mentions the difficulty experienced in washing out this stain from the yolk after yolk formation, without at the same time decolourising nuclear structures. In the case of the egg of Flustrella, iron-hæmatoxylin stains both the yolk nucleus and the chromatin elements of the nucleus with equal intensity, and a similar difficulty with regard to the washing of eggs containing yolk has been experienced. Pl. 23, fig. 25, shows a section of an egg in which, even after prolonged washing with iron-alum, the centres of many yolk spherules remained darkly stained. Crampton's researches led him to

the conclusion that the yolk nucleus, although of nuclear origin, does not consist of chromatin, as has been maintained by several writers, owing to its apparent origin from and similarity of appearance to the chromatin granules of the germinal vesicle; but that it is either purely albuminous or consists of nucleo-albumin containing a large percentage of nucleic acid constituents. According to this author, accounts of the origin of the yolk from the cytoplasm at a point distant from the nucleus, or from several centres, or of its formation all over the egg, refer only to its later history, and do not take into account an earlier stage, which is marked by the appearance of the yolk nucleus. If Crampton's view be the correct one, a study of younger eggs should in such cases lead to the discovery of this supposed early stage.

Wallace (**26**) describes in the egg of Zoarces a yolk nucleus of the type occurring in Flustrella; and by Mr. Wallace's courtesy, I have been enabled to examine many of his preparations and drawings, which show that the yolk nucleus agrees in almost all respects with that of Flustrella hispida. Wallace, however, found that fixing reagents containing nitric acid dissolved out the yolk nucleus, while, as has already been stated, this is not the case with the egg of Flustrella. Further, Wallace agrees with Bambeke that the formation of oil-drops precedes true yolk formation.

The Yolk Nucleus and Yolk Formation in Bryozoan Eggs.—As has been stated earlier in this paper, no yolk nucleus has hitherto been noted in the eggs of any of the Ectoprocta.[1] Among the Entoprocta, however, a structure, which appears to be similar to the yolk nucleus occurring in the egg of Flustrella hispida, has been figured by Kraepelin and by Braem as present in the ovum of Plumatella.

Kraepelin (**17**) points out that shortly before the egg

[1] It has lately been possible by the courtesy of Miss A. Heath to examine some preparations of a species of Alcyonidium which contained young ova. The material, which was collected at Millport in September, 1905, contained abundance of young ova, and these were found to contain "yolk nuclei" apparently similar to that which has been described in Flustrella.

ripens, the germinal vesicle becomes more or less surrounded by a differentiated mass of protoplasm, which persists until after fertilisation, but subsequently vanishes.

Braem (**4** and **6**) mentions the presence of a light zone of protoplasm surrounding the nucleus, which makes its appearance in the ovum at an early stage. This zone is at first not clearly defined from the outer layer of the protoplasm, but later on it becomes sharply demarcated. The outer zone takes no part in segmentation. Small, darkly-staining bodies next arise in the outer zone of the protoplasm. These bodies are of varying sizes, and are each surrounded by a clear area. Braem states that they are similar in appearance to the nucleolus, and suggests that they resemble the latter in chemical composition. He believes that they originate from the outer zone of the cytoplasm, but similar bodies may also occur in the inner zone. Braem suggests that these bodies in the ovum of Plumatella may be homologous with the yolk nucleus of certain other animal ova.

In the case of the eggs of Flustrella hispida, the facts so far obtained all point to the conclusion that the yolk nucleus is a true cell organ originating from the nucleus at an early stage in the history of the ovum; that after undergoing a series of changes it finally disintegrates; and that the process of disintegration is in some manner intimately connected with yolk formation. So far as can be seen, the germinal vesicle plays no direct part in the formation of the yolk. It has not yet been practicable sufficiently to investigate the presence or otherwise of a yolk nucleus in the eggs of other Bryozoa, and the results of this investigation must be reserved for a future paper.

The Centrosphere.—No trace of a centrosphere has yet been detected in the ovum of Flustrella hispida.

The Polar Bodies.—The formation of the polar bodies had only been observed in one case. The egg (Pl. 24, fig. 51) had already passed into the tentacle sheath, and the first polar body (*P.B.*) was lying on the surface of the ovum, while the second was in process of formation.

Fertilisation, and the Passage of the Egg into the Tentacle Sheath.

Fertilisation.—The act of fertilisation has not yet been observed, only such ova as were either preparing for, or had already undergone, fertilisation having been obtained. It has already been stated that the spermatozoa and ova ripen for the most part at different times, and no ripe spermatozoa have been observed in the zoœcial cavity after March.

Passage of the Eggs into the Tentacle Sheath.—The development of the ova takes place within the tentacle sheath, and the eggs pass from the zoœcial cavity into the tentacle sheath in the interval between the degeneration of a polypide and the formation of a new bud. This bud attains complete maturity, and not, as has been described by Joliet (**14**) in the case of Valkeria cuscuta, only partial development. As has been mentioned, from four to five eggs generally ripen at the same time, and these enter the tentacle sheath together and develop side by side. As development proceeds, the larvæ, while still enclosed in the tentacle sheath, increase in size, and gradually come to fill the entire cavity at first occupied by the polypide, and the latter now ceases to exist. Those zoœcia occupied by advanced larvæ contain large quantities of a slimy, mucus-like substance, which surrounds the developing embryos.

Segmentation, and the Formation of the Germinal Layers.

The earlier stages of segmentation have been studied in detail in the hope of elucidating the problem of the origin and subsequent history of the mesoderm and of the endoderm in this form. So far, however, the formation of mesoderm has not been definitely traced, but it is hoped that it may be possible to determine this point more satisfactorily at a later date.

The Primitive Cleavages.—After fertilisation the egg becomes separated from the vitelline membrane by a wide space (Pl. 23, fig. 37). The first cleavage (Pl. 23, figs. 37–42)

divides the egg into two symmetrical halves, each containing equal quantities of yolk: it is completed in about twenty minutes. This division is followed by another, in a plane at right angles to the first, dividing the egg into four spheres, which are to all appearance equal in all respects (Pl. 23, fig. 43; Pl. 24, fig. 52).

To simplify the following account of the cell-lineage of Flustrella, these four first-formed cells have been distinguished in the figures by the letters A, B, C, and D. Cells arising from these are denoted by the letter of the particular cell from which they have been derived, with the addition of a negative index to indicate the generation to which the cell belongs, and of a positive index to denote the number of cells in that generation at the moment of the formation of any given cell, and the order of their formation: thus A^4_5 denotes the fourth cell derived from A in the fifth generation.[1]

The 8-cell Stage.—The four cells A, B, C, and D, again dividing in a plane at right angles to each of the former divisions, give rise to eight cells: of these, the four lower cells are larger, and contain more yolk than the four upper ones (Pl. 23, fig. 44).

The polar bodies are seen in the living egg to lie on the surface of the smaller cells, and these smaller cells and their derivatives always lie on the upper surface of the egg, which later on becomes the dorsal or aboral surface of the larva.

The 12-cell Stage.—The four small upper cells next divide (Pl. 23, figs. 45 *a-b*) each into two unequal cells by a cleavage lying at an angle of 45° to the primitive cleavage plane. The ring of small cells which is thus formed becomes shortly afterwards rearranged into two rows of four cells each, so that the embryo now consists of two parallel series of small cells overlying the four large yolk-laden ones.

Owing to this new arrangement of the embryonic cells, it is

[1] In view of the use which has been made of a somewhat similar system of notation by other authors, it should be stated that the symbols here used have been adopted only for the sake of clearness, and that they have no reference to any of the theories of cell genesis which have been put forward.

now possible to distinguish between a longitudinal (long) and a transverse (short) axis of the larva. At a later period, however, cell division is found to occur more rapidly along the transverse than along the longitudinal axis, and the larva assumes a spherical shape; so that it is not therefore practicable to establish any direct correlation between the long and short axes of these early stages and the long and short axes of the mature larva.

The 16-cell Stage.—Each of the four large lower cells next divides into two cells of unequal size. The plane of cleavage is a vertical one lying more or less at right angles to the long axis of the embryo, and the four central cells formed are the larger; so that now the oral surface of the larva also consists of two rows, each of four cells, which immediately underlie those of the upper series. The larva (Pl. 23, figs. 47*a–c*) is now, therefore, built up of sixteen cells arranged in four parallel rows, which are disposed in two tiers; that is, it consists of an upper, aboral tier of two rows, each of four cells, and of a lower, oral tier of larger cells, also of two rows, each of four cells. All of these sixteen cells belong to the fifth generation (the unsegmented ovum being regarded as the first generation). The four central cells of the oral series are much larger than the lateral ones of the same series, and these again are larger than those of the upper tier.

At this stage, the segmentation cavity (*S.C.*) becomes visible, and it is noteworthy that it contains a substance which stains feebly with eosin, safranin, borax-carmine, etc.

It may be mentioned that the arrangement of the cells in two tiers of two parallel rows each of four cells is characteristic of many Polyzoan larvæ at the 16-cell stage.

The 20-cell Stage.—The next division (Pl. 23, fig. 48) also takes place in the four large central oral cells, which each again divide into two unequal cells—A $\frac{1}{6}$ and A $\frac{2}{6}$, B $\frac{1}{6}$ and B $\frac{2}{6}$, C $\frac{1}{6}$ and C $\frac{2}{6}$, and D $\frac{1}{6}$ and D $\frac{2}{6}$—cleavage taking place in a vertical plane at right angles to the previous division. The embryo (Pl. 23, figs. 49 *a–c*) now consists of

twenty cells; that is to say, there is an aboral series of eight small cells which are disposed in two parallel rows and belong to the fifth generation; below these a ring of eight larger cells, four of which—A $\frac{3}{5}$, B $\frac{3}{5}$, C $\frac{3}{5}$, and D $\frac{3}{5}$—are of the fifth generation, and four others—A $\frac{1}{6}$, B $\frac{1}{6}$, C $\frac{1}{6}$, and D $\frac{1}{6}$—of the sixth generation; while the oral surface is formed by four large central cells—A $\frac{2}{6}$, B $\frac{2}{6}$, C $\frac{2}{6}$, and D $\frac{2}{6}$—which are also of the sixth generation, and which project upwards into the segmentation cavity (Pl. 24, fig. 53). The aboral series of cells contains, generally speaking, less yolk than the lower series of cells, of which the before-mentioned four large oral cells are particularly rich in yolk (Pl. 24, figs. 53, etc.).

The 32-cell Stage.—The beginning of the next stage is shown in Pl. 24, figs. 54 *a–b*, and 55 *a–b*. The small cells composing the two rows of the upper tier first divide horizontally, giving rise to four rows each of four cells, and arranged in two tiers (Pl. 23, fig. 50 *b*, and Pl. 24, figs. 54 *a–b*, 55 *a–b*). This cleavage is followed almost immediately by the division of the four cells—A $\frac{3}{5}$, B $\frac{3}{5}$, C $\frac{3}{5}$, and D $\frac{3}{5}$—which were the first cells originally budded off by the large oral cells. These four cells divide (Pl. 24, figs. 54 *b*, and 55 *b*) by a vertical cleavage lying at an angle of 45° to the primitive cleavage of the segmenting ovum. The embryo (Pl. 23, figs. 50 *a–c*, and Pl. 24, fig. 56) now, therefore, consists of thirty-two cells, which are all of the sixth generation, and which are arranged in the following manner. The aboral surface of the larva is composed of sixteen small cells, disposed in two tiers, each tier consisting of two parallel rows of four cells each. Below these sixteen aboral cells is a ring of twelve larger cells, overlying and partially surrounding the four large oral cells, which still occupy the lower surface of the larva. The upper halves of these four oral cells, being surrounded by the ring of twelve intermediate cells, are thus enclosed by them within the segmentation cavity, and the latter is almost entirely obliterated at this stage (Pl. 24, fig. 55 *a*).

The Formation of Endoderm.—Pl. 24, fig. 57, illustrates a somewhat later stage than that just described. The small aboral cells and the ring of larger cells underlying these have divided, and the latter are now seen to enclose about two thirds of the four large central oral cells within the segmentation cavity. These four large oral cells have also again divided, but this time the plane of cleavage has been a horizontal one; and the four larger upper segments resulting from this division lie within the segmentation cavity, and represent the primitive endoderm. The four lower segments retain their original oral position.

The Ectoderm.—From this time onwards, cell division becomes less regular, and for a time at least, it takes place more rapidly in the transverse than in the longitudinal direction, so that the larva tends to become spherical in form. The small aboral cells divide repeatedly, forming the aboral ectoderm; while the ring of larger intermediate cells, which were shown to have been initially derived from the large oral cells, A $^{2}_{4}$, B $^{2}_{4}$, C $^{2}_{4}$, D $^{2}_{4}$, in like manner give rise to the oral ectoderm. A study of the living embryo and of sections (Pl. 24, figs. 53–60) shows that the cells of the aboral ectoderm tend to remain, throughout embryonic development, smaller than those of the oral ectoderm, the difference becoming more marked as development proceeds, but it is not possible at this, or at any later stage, to distinguish any definite ring of cells which can be correlated with the ciliated ring of the mature larva. The true origin of this structure will be dealt with later, but it may not be out of place to say here a few words in order to explain how it has come about that the existence of such a ring of cells has been supposed by Barrois and other authors to occur at this stage. It is true that observations made on the entire egg at this period, especially when it is viewed from the aboral surface, give somewhat the appearance of there being an equatorial ring of cells, but this appearance is a deceptive one. As stated above, an equatorial ring of twelve cells actually did exist at the thirty-two-cell stage, but the cells of this ring, as has already been described, have

divided to form the oral ectoderm. The cells of the oral ectoderm are all equal in size, though they are larger than those of the aboral ectoderm. A study of sections shows that the ring-like appearance seen at this stage, when viewing the egg from the aboral surface, is due simply to this difference in size between the cells of the oral and aboral surfaces causing the former to project out beyond the latter (Pl. 24, figs. 58–60). In later stages this appearance is enhanced by the development of the aboral groove or mantle cavity just above the line of junction of the two sets of ectoderm cells (Pl. 25, fig. 61). Barrois (2) was misled by this deceptive appearance, and, not having checked his observations on the living egg by the examination of sections, published figures (2, pl. xii, fig. 6), purporting to represent the larva at this stage, showing a prominent equatorial ring of large cells, while the remaining cells are represented as being of the same size both above and below this supposed ring.

The Mesendoderm.—Owing to the rapid growth of the oral ectoderm, the four central oral cells eventually become surrounded and enclosed in the segmentation cavity, thus forming, together with the four cells originally segmented off, eight mesendoderm cells in all. These eight cells divide repeatedly, and give rise to a solid mass of tissue, which in time comes to fill the segmentation cavity. Pl. 24, figs. 57–60, represent stages in this process. The asymmetrical position of the mesendoderm cells in Pl. 24, fig. 58, seems somewhat peculiar, and might well be considered to be abnormal, but the arrangement shown in this figure has been met with in all the sections examined, and must, therefore, be regarded as quite normal.

The Blastopore and Gastrulation.—The oral ectoderm remains for some time open at the point of enclosure of the mesendoderm cells, and this opening may be regarded as the blastopore (Pl. 24, figs. 58, 59, *Bl.*). All trace of the blastopore vanishes in later stages (Pl. 24, fig. 60). It is impossible to say whether the enclosure of the second set of mesendoderm cells be due to the pressure of the surrounding

cells—that is to say, whether it represents a process of invagination, or whether the oral ectoderm simply grows over and encloses the mesendoderm. In either case the process may be regarded as a form of gastrulation, but no trace of an archenteron has been observed.

Later Segmentation Stages.—At the stage represented in Pl. 24, fig. 60, except that the oral surface is slightly flattened, the embryo has become almost spherical in form. Aborally, it is covered by a layer of small, flat, ectoderm cells, while the oral surface is composed of large, high cells; and from these latter a mass of endoderm or mesendoderm cells projects into the segmentation cavity, which is still visible at this stage. A shallow groove, bounded below by a slight protuberance, is noticeable on the exterior of the larva rather above the equatorial line. A study of sections shows that this groove marks the junction of the oral and aboral ectoderm, and that it is due to the difference in the size of the cells in this region. It is to be again noted that there are no specially enlarged cells, such as have been described by Barrois, in this region.

Up to this stage in the development, the oral surface has been relatively larger and more convex than the aboral, but now cell division becomes more active on the latter surface, so that it in turn becomes relatively larger and more convex than the oral surface. Owing to the increased growth of the aboral surface, the slight groove and the protuberance already noticed now lie below the equatorial line, and in sections this groove is now seen to be the result, partly of the difference in size of the ectoderm cells at the line of junction of the oral and aboral series, and partly of an actual bulging out of the oral ectoderm (Pl. 25, fig. 61, *M.C.*). This encircling groove represents the initial stage of the aboral groove or mantle cavity.

The stage thus briefly described marks the close of the segmentation period. The larva now passes gradually from a spherical to an elongated form, which is typical of the mature larva. When viewed in section (Pl. 25, fig. 61), the

difference in size and character between the cells of the oral and aboral ectoderm is now seen to be very marked, and the mesendoderm forms a solid mass entirely obliterating the segmentation cavity.

Summary.—To sum up the results obtained from the foregoing study of the cell division:—

Four equal cells—A, B, C, D—result from the primitive cleavages. These four cells divide and give rise to eight cells, arranged in two series, four small upper cells, and four large lower cells, each of which series is destined to play a distinct part in the subsequent history of the cell layers. The four small upper cells, A^1_4, B^1_4, C^1_4, D^1_4, give rise only to the aboral ectoderm. The four large lower cells, A^2_4, B^2_4, C^2_4, D^2_4, give rise in part to the oral ectoderm, in part to a tissue, which may for the present be best termed "mesendoderm." The cells from which the oral ectoderm is derived are cut off from the four large oral cells by two successive vertical divisions at right angles to each other. The four large oral cells then divide a third time horizontally, and the four upper products of this cell division pass into the segmentation cavity, and there give rise to the primitive endoderm, or rather, mesendoderm. Owing to the rapid growth of the surrounding ectoderm, the four remaining oral cells also eventually become enclosed within the segmentation cavity, giving in all eight mesendodermic cells, which, by subsequent division, eventually form a solid mesendodermic mass.

A true blastopore, which does not close until after the formation of a considerable mass of mesendoderm, is present during the stages referred to.

The formation of mesoderm has not been actually observed; it seems probable, however, that the mesoderm is derived from the primitive mesendodermic mass at a later stage than those hitherto dealt with, and this point will be further discussed (p. 467) when dealing with the degenerating larva.

In view of the fact that the most essential of the larval organs are developed from the oral ectoderm, it seems of

especial interest to note that this tissue is from the first distinct from the aboral ectoderm; the difference in the relative sizes of the oral and aboral ectoderm cells, which is so marked in early stages, is noticeable throughout larval life.

Comparison with other Bryozoa.—As yet, comparatively little has been written on the early development of the Bryozoa; the most important papers on the subject being those by Barrois, Joliet, Repiachoff, Vigelius, Harmer, Braem, Prouho, and Calvet.

Barrois has published descriptions of the early stages of several Bryozoan larvæ, and among others he dealt with (2) that of Flustrella hispida. His observations, however, were made solely upon entire eggs and larvæ. His descriptions and figures are in entire agreement up to the thirty-two-cell stage with the general results described in the present paper, but he does not make any special mention of the lineage of these cells, and he was also unable to study the formation of the endoderm. As has already been pointed out, Barrois erroneously describes a later stage, in which both dorsal and ventral ectoderm are said to be composed of small equisized cells, the two series being separated by an equatorial ring of large cells (2, pl. xii, fig. 6), and he figures this ring as being present in all subsequent stages. Had Barrois sectioned any of his material, instead of relying solely on external appearances, he would have seen that no equatorial ring of single cells, such as he described, is present at any of the stages figured.

In another paper (3) Barrois describes the enclosure, in Schizoporella unicornis, of four primitive endoderm cells by epiboly. He also describes the formation of two bands of mesoderm, which at a later period fuse with the endoderm to form a single mesendodermic mass.

Repiachoff (23), in a paper on Tendra zostericola, also describes the endoderm as originating by the enclosure and division of four large oral cells. He, however, states that the process is followed in Tendra by the formation of an

archigastrula with an opening to the exterior: no such stage occurs in Flustrella hispida.

Vigelius (**25**) studied the early stages in the development of Bugula, and noted the presence of four large dorsal cells within the segmentation cavity and the subsequent division of these to form the endodermic mass.

Harmer (**11**), in his paper on Alcyonidium, mentions the presence of a blastopore as occurring in that form.

Prouho, in a paper (**20**) on Flustrella hispida, makes no mention of the early stages of this form. In a later paper (**21**), however, he describes the formation of endoderm in the Cyphonautes larva. He states that at the thirty-two-cell stage the embryo is flattened along an axis perpendicular to the plane of the first segmentation, the four oral cells being larger than the other cells and especially rich in yolk; these four large cells subsequently become enclosed by the rapid growth of the ectoderm. In the case of Alcyonidium albidum, Prouho records that the endodermic cells each divide into two before becoming enclosed by the ectoderm, and he defines the blastopore in this case as the point at which the ectodermic cells close over the four large cells.

Braem, in his account (**5**) of the embryology of Paludicella Ehrenbergi, points out that segmentation in the larva of this species is total and almost equal. At the eight-cell stage the segmentation cavity is visible, and the four upper cells are somewhat larger than the lower cells. The sixteen-cell stage is similar to that which occurs in Flustrella hispida. At the thirty-two-cell stage the embryo is spherical, and forms a typical blastula; the blastula has a large segmentation cavity, and the cells of the vegetative pole are larger than those of the animal pole and do not increase in number as rapidly. The four central cells of the vegetative pole then become surrounded by the ectoderm cells, and are pressed into the segmentation cavity. After their enclosure, these four cells segment to form other endoderm cells, which multiply and give rise to a many-celled archenteron opening to the exterior. A cell layer containing muscle fibres lies

between the ectoderm and endoderm, and Braem considers that this tissue may represent the mesoderm, and that it is possibly derived from the initial cells separated off from the original four large endoderm cells.

Calvet (8), in his general account of the embryology of Cheilostomes and Ctenostomes, makes the general statement that segmentation is equal and regular up to the thirty-two-cell stage; but, from the above description of the process in Flustrella hispida, this is obviously not invariably the case. Calvet describes the formation of endoderm as taking place in a manner similar to that in which it originates in Flustrella, and he arrives at the conclusion that the endoderm arises partly by endocytulation, partly by planulation.

The endoderm, therefore, appears to originate in a similar manner in Flustrella and in the few other Bryozoa in which its formation has so far been investigated.

The Development of the Larval Organs.

The formation of mesendoderm being completed, the first traces of larval organs soon appear in the shape of a two-fold invagination of the oral ectoderm, and this is followed soon after by a third invagination at a point anterior to the previous ones. Both ectoderm and mesendoderm cells have by this time begun to lose their definite cell outlines.

Ectodermic Organs.—The internal sac is the first organ to be formed. It arises as a median invagination of the oral ectoderm; and in Pl. 25, fig. 62, it is seen as an elliptical space (*I.S.*) communicating with the exterior by means of a narrow opening. The cells lining this sac are short and flat, and their nuclei lie close to the periphery. These cells are seen to contain large globules of a substance closely resembling the yolk spherules in their general appearance and in their reactions to staining reagents; and drops of this substance are also found to be exuding from many cells (Pl. 25, fig. 62 *g.*). This substance subsequently disappears entirely

from the cells of the internal sac (Pl. 25, fig. 65). The significance of its appearance will be discussed in a later section.

The second oral ectodermic invagination is destined to form an organ representing the pharynx; it occurs in front of that from which the internal sac arises, and appears at a slightly later period. The cells bounding its opening are large and high, their nuclei lie on the side nearest the opening, and the cells themselves are much vacuolated. The vacuoles (Pl. 25, figs. 62–64) in the walls of the pharynx are filled with a substance similar in appearance to that which has been described as occurring in the cells of the internal sac (Pl. 25, fig. 62), and, as in the latter case, this substance also entirely disappears at a later stage (Pl. 25, fig. 65). From its mode of origin, the pharynx is to be regarded as a true stomodæum.

The pyriform organ arises, at a somewhat later period than the internal sac and the pharynx, as an oral ectodermic invagination anterior to the latter organs. In Pl. 25, figs. 62, 63 *a*, the internal sac and the pharynx are shown well developed, while at this stage the pyriform organ is represented only by a slight invagination (*Py.*).

The aboral organ, the "calotte" of French authors, is at the stage figured in Pl. 25, fig. 62, already visible as a thickened mass (*Ca.*) of aboral ectoderm overlying the pharynx and provided with numerous nuclei. From this organ a delicate network of fibres and nuclei passes to the developing pyriform organ (Pl. 25, figs. 62, 63 *a*).

Organs of Mesendodermal Origin.—The three-fold ectodermic invagination leading to the formation of the internal sac, pharynx, and pyriform organ has the effect of compressing the mesendoderm into a solid mass, which lies in the posterior part of the larva with its anterior end overlying the inner end of the pharynx (Pl. 25, fig. 62, *Ed.*). This mesendodermal tract consists of a mass of yolk spherules with scattered nuclei and it rapidly loses all trace of definite cell structure. At a slightly later period, the mesendodermic mass becomes hollowed out and forms what,

from its origin, appearance, and position, there can be no doubt is a vestigial stomach (Pl. 25, figs. 63 *a-b*, 64, 65 *a–c*, *St.*). This supposed stomach is identical in its structure with that described by Harmer (**11**, p. 446, Pl. XXVII, figs. 1, 2) as occurring in the larva of Alcyonidium only, unlike the stomach in that case, communication with the exterior by the pharynx is never established. As is the case also in Alcyonidium, the stomach is lined simply by a protoplasmic mass in which nuclei and numerous yolk spherules are embedded. The lining epithelium shows no trace of any glandular character, and the organ itself remains entirely vestigial and disappears before free life commences.

In describing the development of the stomach in the Cyphonautes larva, Prouho (**21**) says: "La depression orale devient de plus en plus profonde, pendant que la région aborale devient de plus en plus conique . . . Les cellules de la masse endodermique, qui se sont un peu multipliées pendant que l'embryon subissait les modifications ci dessus, se desposent autour d'un axe et forment une masse, pleine, allongée, oblique, dont une extrémité vient s'appuyer contre le fond de l'invagination orale ; cette masse endodermique, définitivement rejetée à l'arrière de la larve occupe d'ores et déjà la position de futur estomac."

The organ which has been regarded as the stomach in the larva of Flustrella hispida has been shown by its origin and position to correspond to the endodermic mass which ultimately gives rise to the stomach in the Cyphonautes larva, and it is also closely comparable to the rudimentary stomach of the larva of Alcyonidium, except for the fact that it never communicates with the pharynx. The exceedingly slight development of the stomach in Flustrella is easily explained when the short duration of free larval life, and the correlated abundance of the supply of food yolk are taken into consideration.

According to Prouho (**20**) the organ which has been here termed the pharynx is to be regarded as a rudimentary intestine much less differentiated than that of Alcyonidium. But, as

we have seen, this structure is a true stomodæum, and from the above account it will be evident that the alimentary apparatus is much better developed in Flustrella than Prouho had supposed to be the case, in that it really comprises both stomach and pharynx, though both it is true are of a rudimentary character.

It will be remarked, when viewed in relation to the vestigial character of the stomach, that the considerable development of the pharynx is somewhat surprising; but may it not be that the pharynx has, in accordance with the exigencies of larval life, assumed another function? Mention has already been made of the globules which are present in the vacuoles of the cells of the pharynx. At about the time that these disappear, the larva becomes freed from the vitelline membrane, and at the same time the slimy, mucus-like substance already noted becomes very abundant around the embryos. Possibly, therefore, the pharynx has assumed a glandular function, and it may well be that this mucus-like substance has been derived from the globules previously contained in the pharyngeal cells. Attempts to prove, by treatment with Mayer's mucicarmine, that the globules in the pharynx and internal sac really consist of mucus, have so far given negative results; and this point must, therefore, for the present remain unsolved. The suggestion here put forward may also afford an explanation of the existence of the drops previously noticed as exuding from the cells of the internal sac.

In Pl. 25, figs. 63 *b*, 64, are shown two bands of tissue marked * which appear to be budding off from the sides of the mesendodermic mass. Their significance will be discussed later.

The Mature Larva.

In Pl. 25, figs. 65 *a–c*, the larva is represented at a stage shortly anterior to that at which degeneration commences. It is now enclosed in a chitinous bivalve shell, and has escaped from the vitelline membrane. From this point

onwards the development of the larval organs, with the exception of the stomach, has been already so fully described by Prouho (**20**) as to make detailed description unnecessary.

The internal sac has become much elongated, so that it now occupies the greater part of the interior of the larva. Its lining epithelium has become much thickened, and has already lost all trace of cell structure.

The pharynx has altered somewhat in appearance, owing to the loss of the large globules already described.

The pyriform organ is now fully developed. On the exterior two depressions are noted—an anterior depression (Prouho's "fossette supérieure") and a posterior one (Prouho's "fente ciliée"), and between these lies a tuft of cilia, the "papille du plumet vibratile." Internally, corresponding to the "fossette supérieure," is the "système glandulaire supérieure," which consists of a single mass of cells lying in the longitudinal axis of the larva, while, similarly corresponding to the "fente ciliée," are two masses of cells, which are placed in the transverse axis of the larva, one on either side of the posterior part of the "système glandulaire supérieure," and which represent the "système glandulaire inférieure."

The aboral organ (Pl. 25, fig. 65 *a*) at this stage is fully developed. It consists of a tuft of long cilia arising from the thickened patch of aboral ectoderm which has already been mentioned. This organ is best seen in the living larva, in which it is visible protruding between the two valves of the shell; and connecting the aboral organ and the pyriform organ is seen the neuromuscular cord. In the living larva the jerking movements of the neuromuscular cord are distinctly visible, but it has not been possible to draw any conclusions from these movements as to the functions of either the pyriform or the aboral organs. The structure of the aboral organ and neuromuscular cord have already been fully described by Prouho (**20**). As stated by this author the nerve-muscle tract, on reaching the pyriform organ, breaks into three strands, one of which passes to that organ

between the cells of the two glandular systems, the other two passing to the cells of the ciliated crown.

The ciliated crown (the "couronne" of French authors), first visible at this stage, does not consist of a single ring of cells as it is has been described in other Bryozoan larvæ, and as figured by Barrois (**2**) for Flustrella hispida, but of two or three rows of cells, as is shown in Pl. 25, figs. 65 *a–c* (*C.*). All trace of definite cell walls in the ectoderm has vanished by this time, but certainly the ciliated crown contains at least three series of nuclei, corresponding presumably to originally three rows of cells.

The cells from which the cilia originate lie rather below the aboral groove, and can at will be retracted within the valves of the shell (Pl. 25, fig. 66). Later on the ciliated crown certainly does have the appearance described by Prouho (**20**), of a single series of flat discoidal cells with long vibratile cilia, each imbedded in a cuticle, and prolonged below this into a triangular mass of protoplasm (Pl. 25, fig. 66); each of these cells contains a single large nucleus: this appearance does not, however, arise until quite late in larval life. No traces of the ciliated crown, or of any specially enlarged cells, are visible before the larva escapes from the vitelline membrane, despite Barrois' assertion to the contrary. It is evident, therefore, that in Flustrella the ciliated crown is formed by a series of cells, and that it is only late in larval life that these unite to form a single row of large cells.

The chitinous bivalve shell is developed as a secretion of the aboral ectoderm, and is closely adherent to the latter.

The aboral groove, which is now strongly developed, occupies its original position above the ciliated crown (Pl. 25, figs. 65 *a–c*, *M.C.*). It is best seen in transverse section.

The stomach, as a result of the growth of the larva, has become much more elongated, and, owing partly to this, partly to the absorption of food material, the yolk spherules surrounding the stomach have become much reduced in number.

The Degenerating Larva.

Shortly after the stage which has been briefly described above, the degeneration of the larval organs commences. The initiation of this process is shown in Pl. 25, fig. 66.

The internal sac becomes enormously thickened, and its lining ectoderm highly modified, especially near the opening of the sac to the exterior, where it now assumes a granular character. The pharynx gradually loses its cellular structure; the pyriform organ is at this stage still fully developed, as are also the aboral organ and neuromuscular tract. The ciliated crown, as has already been stated, consists at this stage of a single ring of large cells. The stomach has practically vanished, its position being marked only by a number of scattered yolk spherules and of nuclei lying between the internal sac and the aboral ectoderm.

Origin of the Mesoderm.—Among the above-mentioned scattered elements of mesendodermic origin, and apparently developed from them, occur fibres (Pl. 25, fig. 66, *M*), which are presumably muscular in nature. Others of these supposed muscular fibres occupy the former position of, and are probably developed from, the lateral bands of tissue previously noted as budding off from the stomach It is this mesendodermic mass of yolk spherules, nuclei, and fibres which Prouho (**20**) regards as representing the mesoderm in Flustrella hispida. He maintains that the mesoderm occurs as a distinct layer of cells lying beneath the aboral ectoderm, generally thickened at the aboral pole; that a similar membrane overlies the internal sac, and also that all the muscular elements of the larva are of mesodermic origin. But, as has already been shown, it is quite impossible at any early larval stage to differentiate the mesoderm from the general endodermic mass, and Prouho's so-called "mesoderm" is, therefore, undoubtedly not simply mesoderm, but endoderm, or perhaps rather mesendoderm, since it is, of course, possible that in this endodermic mass lie enclosed the elements of the future mesoderm, from which the muscles are

now formed. The two bands of tissue apparently budding off from the main mesendodermic mass have already been noticed; possibly these may represent the true mesoderm, which in that case would appear to develop only late in larval life. Prouho, owing to the fact that he only studied the later stages of larval life in which the stomach had already begun to disappear, was led to regard the whole mesendodermic mass as mesoderm, or as mesoderm containing some endodermic cells, but he himself suggests the necessity of a detailed study of the larva in its earlier stages in order to decide the correctness of his view.

The Alimentary Canal in Larval Ectoprocta.

The presence of an alimentary canal in Ectoproct larvæ has been described by Barrois, Vigelius, Repiachoff, Prouho, and Harmer.

Barrois (**2** and **3**) at one period regarded larval Ectoprocts as having an alimentary canal, owing to his mistaken supposition that the internal sac represented the stomach. Later on he saw reason to modify his views, but he pointed out and depicted (**2**, pl. vii, fig. 13) an invagination between the pyriform organ and the internal sac, which he regarded as the rudiment of a pharynx. He therefore believed that many Ectoprocts were originally provided with a digestive tube, and that in cases where no such system is formed the endoderm arises as in other Bryozoa where the digestive system is better developed, but that later on it degenerates to a mass of yolk spherules filling the interior of the embryo.

Vigelius (**25**) stated that in the early larval stages of Bugula, a slight split occurs in the mesendodermic mass filling the interior of the larva; this split he regards as representing a primitive stomach, which however, plays a purely passive rôle, and does not open to the exterior. Later on the supposed stomach vanishes, and the endoderm forms a simple cell mass.

Repiachoff (**23**) describes for Tendra zostericola the

formation of an archigastrula with a definite opening to the exterior: this communication becomes obliterated at a later stage. The fully-developed larva has a stomach which communicates with the exterior, similar to that which has been described by Harmer (**11**) as present in the developing larva of Alcyonidium.

Prouho (**21**) describes the presence of a functional alimentary canal in the "Cyphonautes" larva, the stomach being developed from the internal endodermic mass in the manner described above. In an earlier paper on Flustrella hispida (**20**), he regards the pharynx as a rudimentary digestive tube less differentiated than that of Alcyonidium, and representing either an attempt to form a digestive organ or a vestige of one which has vanished.

Harmer (**11**) found, in a species of Alcyonidium, a definite alimentary canal closely resembling that occurring in Flustrella hispida, but communicating by a narrow opening with the exterior. In the same paper he points out the probability that a similar structure occurs in Flustrella hispida.

From its endodermic origin, its position, appearance, and mode of development, and from the close agreement in structure with the stomach described in Alcyonidium, there can be no doubt that the larva of Flustrella hispida possesses a vestigial stomach, and that this and the associated pharynx must be regarded as vestiges of a digestive system in which degeneration has proceeded a stage further than it has in Alcyonidium, since no communication with the exterior is ever established at any stage.

General Summary.

The main points in the foregoing paper may be summarised as follows:—

(1) A "yolk nucleus" of the type described by Bambeke, as occurring in the egg of Pholcus, is present in the developing egg of Flustrella hispida.

(2) Segmentation and cell-lineage have been followed out in detail up to the 32-cell stage.

(3) The formation of the endoderm has been traced.

(4) The oral and aboral ectoderm are differentiated as early as the 16-cell stage, and remain quite distinct from that time onwards.

(5) The ciliated ring of the larva is formed by the coalescence of several originally distinct rows of cells, and not by the hypertrophy of a single row.

(6) A stomach, comparable to that of Alcyonidium, is present also in Flustrella.

References.

1. Bambeke, C. v.—"Contributions à l'histoire de la constitution de l'œuf," iii, "Recherches sur l'oocyte de Pholcus phalangioides (Fuessl.)," 'Arch. Biol.,' vol. xv, pp. 511–598, pls. xxiii–xxviii, 1898.
2. Barrois, J.—'Recherches sur l'Embryologie des Bryozoaires,' Lille, 1877.
3. Barrois, J.—"Mémoires sur les métamorphoses des Bryozoaires," i, "Métamorphose des Escharines," 'Ann. Sci. Nat.,' ser. 6, Zool., vol. ix, No. 7, 67 pp., pls. xiii–xvi, 1880.
4. Braem, Fritz.—"Untersuchungen über die Bryozoen des süssen Wassers," 'Bibliotheca Zoologica,' No. vi, Cassel, 1890.
5. Braem, Fritz.—"Die geschlechtliche Entwickelung von Paludicella Ehrenbergii," 'Zool. Anz.,' vol. xix, pp. 54–57, February, 1896.
6. Braem, Fritz.—"Die geschlechtliche Entwickelung von Plumatella fungosa," 'Zoologica,' Heft 23, Stuttgart, 1897.
7. Calkins, G. N.—"Observations on the Yolk-nucleus in the Eggs of Lumbricus," 'Trans. New York Acad.,' vol. xiv, pp. 222–230, 1895.
8. Calvet, L.—"Contributions à l'Histoire Naturelle des Bryozoaires Ectoproctes Marins," 'Trav. Inst. Zool. Montpellier,' ser. 2, No. 8, 1900.
9. Crampton, H. E.—"Studies upon the Early History of the Ascidian Egg." Part I.—"The Ovarian History of the Egg of Molgula manhattensis," 'Journ. Morphol.,' vol. xv, suppl., pp. 29–56, pl. iii, December, 1899.
10. Foot, K.—"Yolk-nucleus and Polar Rings," 'Journ. Morphol.,' vol. xii, pp. 1–16, pl. i, May, 1896.
11. Harmer, S. F.—"Sur l'embryogénie des Bryozoaires ectoproctes," 'Arch. Zool. Expt.,' ser. 2, vol. v, pp. 443–458, pls. xxvii, xxviii, 1887.

12. Henneguy, L. T.—"Le corps vitellin de Balbiani dans l'œuf des vertébrés," 'Journ. l'Anat. Physiol.,' vol. xxix, pp. 1–39, 1893.

13. Hincks, Thomas.—"Notes on British Zoophytes, with Descriptions of some New Species," 'Ann. Mag. Nat. Hist.,' ser. 2, vol. vii, pp. 353–362, pl. xiv, November, 1851.

14. Joliét, L.—"Contributions a l'histoire naturelle des Bryozoaires des cotes de France," 'Arch. Zool. Expt.,' ser. 1, vol. vi, pp. 193–304, pls. vi–xiii, 1877.

15. Jordan, E. O.—"The Habits and Development of the Newt (Diemyctilus viridescens)," 'Journ. Morphol.,' vol. viii, pp. 269–366, pls. xiv–xviii, May, 1893.

16. Korschelt, E., and Heider, K.—"Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere," "Allgemeiner Theil," pt. i, Jena, 1902.

17. Kraepelin, Karl.—"Die Deutschen Süsswasser Bryozoen" II, "Entwicklungsgeschichtlicher Teil.," 'Abhandl. Naturw. Ver., Hamburg,' vol. xii, 1892.

18. Mertens, H.—"Recherches sur la signification du corps vitellin de Balbiani dans l'ovule des mammifères et des oiseaux," 'Arch. Biol., vol. xiii, pp. 389–422, pl. xiv, 1895.

19. Ostroumoff, A.—"Zur Entwicklungsgeschichte der cyclostomen Seebryozoen," 'Mitth. Zool. Stat. Neapel,' vol. vii, pp. 177–190, pl. vi, April, 1887.

20. Prouho, H.—"Recherches sur la larve de la Flustrella hispida (Gray)," 'Arch. Zool. Expt.,' ser. 2, vol. viii, pp. 409–459, pls. xxii–xxiv, 1890.

21. Prouho, H.—"Contribution à l'histoire des Bryozoaires.," 'Arch. Zool. Expt.,' ser. 2, vol. x, pp. 557–656, pls. xxiii–xxx, 1892.

22. Redfern, Peter.—"Flustrella hispida and its Development," 'Quart. Journ. Micr. Sci.,' ser. 1, vol. vi, pp. 96–102, 1858.

23. Repiachoff, W.—"Ueber die ersten embryonalen Entwicklungsvorgänge bei Tendra zostericola," 'Zeitschr. wiss. Zool.,' vol. xxx, suppl., pp. 411–423, pl. xix, May, 1878.

24. Van der Stricht, O.—"La structure de l'œuf des Mammifères. 1re. Partie. l'Oocyte au stade de l'accroissement.," 'Arch. Biol.,' vol. xxi, pp. 1–101, pls. i–iii, December, 1904.

25. Vigelius, W. J.—"Zur Ontogenie der marinen Bryozoen," 'Mitth. Zool. Stat. Neapel,' vol. vi, pp. 499–541, pls. xxvi–xxviii, June, 1886.

26. Wallace, W.—"Observations on Ovarian Ova and Follicles in Certain Teleostean and Elasmobranch Fishes," 'Quart. Journ. Micr. Sci.,' n. s., vol. 47, pp. 161–213, pls. 15–17, 1903.

27. Wilson, E. B.—"On Protoplasmic Structure in the Eggs of Echinoderms and Other Animals," 'Journ. Morphol.,' vol. xv, suppl., pp. 1–25, pls. i, ii, December, 1899.

Postscript.—Since the above account of the yolk nucleus in the egg of Flustrella hispida was written, a paper (**24**) has appeared by O. van der Stricht on the yolk nucleus in the eggs of mammals. The account of the yolk nucleus given by this author appears in the main to confirm Bambeke's observations and views.

EXPLANATION OF PLATES 22—25,

Illustrating Mrs. R. M. Pace's paper on "The Early Stages in the Development of Flustrella hispida (Fabricius)."

Reference Letters.

A. Aboral surface of larva. *A.Ec.* Aboral ectoderm. *Bl.* Blastopore. *C.* Ciliated crown, corona, or "couronne." *Ca.* Aboral organ or "calotte." *Ci.* "Fente ciliée." *cr.* Crystalloid bodies. *C.T.* "Papille de plumet vibratile," ciliated tuft. *Ec.* Ectoderm. *Ed.* Endoderm, or mesendoderm. *Fo.* Follicle cells. *Fs.* "Fossette supérieure." *Fu.* Funicle. *G.* Chromatin granules. *g.* Globules in cells of pharynx and internal sac. *I.* Intestine. *I.S.* Internal sac. *M.* Muscle fibres. *M.C.* Aboral groove or mantle cavity. *Mc.* Mesenchyme lining of zoœcium. *N.* Nucleus. *n.* Nucleolus. *N.M.* Neuromuscular cord. *O.* Oral surface of larva. *Oe.* Œsophagus. *O.Ec.* Oral ectoderm. *Ol.* Oil globules. *Ov.* Ovary. *P.B.* Polar bodies. *Ph.* Pharynx. *Py.* Pyriform organ. *R.* Rectum. *S.* Shell. *S.C.* Segmentation cavity. *S.G.'* "Système glandulaire supérieure." *S.G.''* "Système glandulaire inférieure." *St.* Stomach. *T.* Testes. *T.S.* Tentacle sheath. *vc.* Vacuoles in the yolk nucleus. *V.M.* Vitelline membrane. *x.* Space or clear zone surrounding the yolk nucleus. *Y.* Yolk spherules. *Y.N.* Yolk nucleus. *Z.* Zoœcial cavity. Those tissue tracts which are possibly to be regarded as being destined to give rise to the mesoderm are marked by a small asterisk.

In the figures of segmenting ova, the first four cells formed have been lettered, merely for convenience of reference, "A," "B," "C," "D," and the daughter cells arising from these latter are distinguished by index numbers, a negative index indicating the generation to which a particular cell belongs, and a positive index its place in that generation; thus A^4_5 denotes the fourth cell derived from A in the fifth generation (see also p. 452).

All the figures, both of sections and of entire larvæ, have been drawn by the aid of the Zeiss camera lucida.

PLATE 22.

Fig. 1.—Transverse section of a young colony of Flustrella hispida, collected in March. The section is taken close to the apex of the colony, and shows the position of the testes on the lateral walls in the front of one zoœcium, and the ovary lying on the funicle at the back of a neighbouring zoœcium. × 65.

Fig. 2.—Section of a young ovary lying on the funicle, showing the follicle cells commencing to grow in among the young ova. × 225.

Fig. 3.—Section of an older ovary; the follicle cells are now seen to have increased in number. × 225.

Fig. 4.—Section of a young ovary showing four young ova, the walls of which are still unformed. The yolk nucleus is present in the form of small dark granules. × 400.

Fig. 5.—Section of a somewhat older ovary showing four ova around which the follicle cells have not yet developed. The dark granules which represent the yolk nucleus have increased in number, and in many cases are seen to lie in close contact with the membrane of the germinal vesicle. × 400.

Fig. 6.—Section of a young ovum more advanced than those shown in Fig. 5. The granules of the yolk nucleus are seen to lie in four groups, each containing two granules, and three of these groups are surrounded by clear spaces (*x*). × 400.

Fig. 7.—Section of a young ovum of the same age as that shown in Fig. 6. The granules of the yolk nucleus are grouped together and lie within a clear space. × 400.

Fig. 8.—Section of a young ovum somewhat more advanced than that shown in the preceding figure. The majority of the dark granules have become fused to form a single large yolk nucleus lying within a clear space (*x*) separated from the germinal vesicle. Two vacuoles have already appeared in the yolk nucleus. × 400.

Figs. 9—12.—A series of four sections through a slightly older egg, to show the difference in the appearance of the egg according to the point at

which the section is taken. Fig. 9.—A section of the egg showing the commencement of the clear region in which the yolk-nucleus generally lies. In Fig. 10 the section passes through the nucleus and nucleolus, and the clear space has assumed a hemispherical shape. In Fig. 11 the yolk-nucleus is shown as a well-developed crescentic body lying in the clear space visible in the preceding figures, and in close contact with the germinal vesicle; the section passes through only the upper part of the nucleus. Fig. 12.—The yolk nucleus is seen lying in a clear space. The germinal vesicle is no longer visible, but lies underneath the cap formed by the yolk nucleus. × 400.

Fig. 13.—A section of an ovum of about the same age as that illustrated in the preceding figures. The yolk nucleus has become rather more crescentic in form, and three large vacuoles are present and contain crystalloid bodies (*cr.*). × 400.

Fig. 14.—A section of an egg of the same age as that shown in fig. 10. The yolk nucleus is more markedly hemispherical in form, and the number of vacuoles is larger than at previous stages. × 400.

Fig. 15.—Section of an older egg. The yolk nucleus has now assumed the form of a ring surrounding, and in close contact with the germinal vesicle. An indication of approaching degeneration is seen in the reticulate structure of the yolk nucleus. × 400.

Fig. 16.—A section of a still older egg in which the degeneration of the yolk nucleus has commenced. The yolk nucleus has lost its regular outline, and shows a markedly reticulate structure, the meshes of the network staining more deeply than the interlying substance. The outlines of the surrounding space have also become somewhat irregular. × 400.

Fig. 17.—A section of an egg at a slightly later stage. The degeneration of the yolk nucleus has advanced considerably and is very marked. The body has completely lost its regular outline, and the boundary of the surrounding space is very irregular. × 400.

Fig. 18.—Section of an older egg, showing the complete disintegration of the yolk nucleus, which has now assumed the form of numerous minute, darkly-staining granules which lie in a loose ring around the germinal vesicle × 400.

Fig. 19.—Section of an egg of about the same age as that shown in fig. 18, but which exhibits a somewhat different method of fragmentation of the yolk nucleus. In this case the products of disintegration have assumed the form of dark, irregular patches lying within clear spaces, and forming an open ring around the germinal vesicle. × 400.

Figs. 20, 21.—Sections of eggs showing still other methods of fragmentation of the yolk nucleus. × 400.

Figs. 22, 23.—Sections of slightly older eggs in which the products of disintegration of the yolk nucleus have retreated towards the periphery of the egg, there forming an open ring of deeply staining patches, each of which lies within a clear space. × 400.

PLATE 23.

Fig. 24.—Section of an egg showing the first appearance of the yolk in the form of minute globules scattered in the protoplasm. All trace of the yolk nucleus has vanished. × 400.

Fig. 25.—Section of an egg in which the yolk is fully developed. The section was stained with iron hæmatoxylin, and the centres of many of the yolk spherules remained stained even after prolonged washing. × 400.

Figs. 26, 27.—Sections of eggs of about the same age as that illustrated in figs. 14 and 15, and which have been treated with osmic acid. The yolk nucleus is in immediate contact with the germinal vesicle, and the clear space in which it usually lies is not present; oil globules (*Ol.*) are present both in the yolk nucleus and in the surrounding protoplasm. × 250.

Fig. 28.—Section of an egg stained with osmic acid, in which yolk-formation is occurring. All trace of the yolk nucleus has vanished; oil-drops are scattered among the developing yolk, and are especially abundant towards the periphery of the egg; they may be distinguished by their darker colour. × 250.

Fig. 29.—Section through the germinal vesicle of a young egg. The chromatic network is well developed, and darkly staining nodules are present at its nodes. Lying at the edge of the germinal vesicle is a dark nodule (*Y.N.*) similar to those which occur at the nodes of the chromatin reticulum, and which is apparently in process of passing out through the membrane of the germinal vesicle to become one of the granules which will coalesce and form the yolk nucleus. × 650.

Fig. 30.—Section of the germinal vesicle showing the gradual attenuation of the chromatin network as maturation proceeds. × 650.

Figs. 31, 32.—Sections of the germinal vesicle in somewhat older eggs, showing the increased size of the germinal vesicle and the attenuation of the chromatin network. × 650.

Fig. 33.—Section of the germinal vesicle showing the thickening of the chromatin network which occurs in later stages. × 650.

Fig. 34.—Section of the germinal vesicle of an egg in which the formation of the polar bodies will shortly take place. The chromatin network is much thickened, and the substance between its meshes stains more darkly than at earlier stages. The nuclear membrane has become irregular. × 650.

FIG. 35.—Section of the germinal vesicle at a stage slightly later than that shown in fig. 34. The nucleolus has become relatively very large, the chromatin network is thicker, and the substance between the meshes stains still more deeply than at previous stages. × 650.

FIG. 36.—Section of the germinal vesicle in a mature egg before the formation of the polar bodies has taken place. The germinal vesicle has decreased in relative size and has assumed an amœboid form. All trace of the chromatic network has vanished. × 650.

FIGS. 37–42.—Series illustrating the primitive cleavage of the egg. × 65.

FIG. 43.—The segmenting egg at the four-cell stage. × 90.

FIG. 44.—The segmenting egg at the eight-cell stage. Lateral view. × 90.

FIG. 45.—The segmenting egg at the twelve-cell stage. × 90.
(*a*) Lateral view. (*b*) Aboral view.

FIG. 46.—Oral view in optical section of the larva at the twelve-cell stage, showing the formation of spindles in the four oral cells prior to the division which gives rise to eight oral cells. × 400.

FIG. 47.—Larva at the sixteen-cell stage. × 90.
(*a*) Oral view. (*b*) Lateral view. (*c*) Aboral view.

FIG. 48.—Oral view of the larva at the sixteen-cell stage, showing the formation of spindles in the four central oral cells prior to their division to form four new oral cells of the sixth generation. × 180.

FIG. 49.—Larva at the twenty-cell stage. × 90.
(*a*) Oral view. (*b*) Lateral view. (*c*) Aboral view. The four large oral cells are of the sixth generation; of the eight surrounding cells, four belong to the fifth and four to the sixth generation.

FIG. 50.—Larva at the thirty-two cell stage. × 90.
(*a*) Oral view. (*b*) Lateral view. (*c*) Aboral view.

PLATE 24.

FIG. 51.—Section of an egg in which the first polar body has been formed and the second is in process of formation; the first polar body lies outside the egg. × 250.

FIG. 52.—Section of a larva at the four-cell stage showing the formation of the nuclear spindles prior to the division to form the eight-cell stage. × 225.

FIG. 53.—Longitudinal section of the larva at the twenty-cell stage. × 225.

FIG. 54 *a*.—Transverse section of the larva at the twenty-cell stage, showing the formation of nuclear spindles in the aboral cells A_5^2, C_5^2, prior to division. The four oral cells belong to the sixth generation. × 225.

Fig. 54 *b*.—Transverse section of the larva at the twenty-cell stage, showing the formation of nuclear spindles in the aboral cells $A\frac{1}{5}$—$C\frac{1}{5}$, and in the oral cell $A\frac{3}{5}$, prior to division to form cells of the sixth generation. × 225.

Fig 55 *a*, *b*.—Transverse sections of the larva at the twenty-cell stage, but at a slightly later period than that illustrated in Figs. 54 *a*, *b*, the division of two of the aboral cells being now completed. × 400.

Fig. 56.—Longitudinal section of the larva at the thirty-two cell stage. × 225.

Fig. 57.—Longitudinal section of the larva showing the formation of the primitive mesendoderm cells by the division of the four large central oral cells. × 225.

Fig. 58.—Section of the larva showing the enclosure of the four large central oral cells within the primitive segmentation cavity, forming, together with the four cells previously enclosed, in all eight mesendoderm cells; a blastopore (*Bl.*) is represented. × 225.

Fig. 59.—Section of the larva at a slightly later stage than that shown in the previous figure. The difference in size between the cells of the aboral and oral ectoderm is noticeable; the eight primitive mesendoderm cells have divided, forming a mesendodermic mass which projects from the oral face into the segmentation cavity; the blastopore is still open. × 225.

Fig. 60.—Section of the larva at a stage subsequent to the closure of the blastopore. *M.C.* marks the position of a slight groove which is to be seen on the exterior of the larva, and the slight protuberance visible below this in the external view is due to the difference in size of the cells of the oral and aboral ectoderm, which meet along this line. The mesendoderm almost completely fills the segmentation cavity. × 225.

PLATE 25.

Fig. 61.—Section of an older larva in which the formation of the mesendoderm is completed, but in which the larval organs have not yet begun to form. The difference in size between the cells of the oral and aboral ectoderm is now very marked; the groove *M.C.* has deepened, and the oral cells below it are slightly protruded, giving rise to a raised ring on the external surface of the larva; this ring is, however, quite unconnected with the ciliated ring which develops later. The mesendodermic mass has by this time completely obliterated the segmentation cavity. × 225.

Fig. 62.—Longitudinal section of a young larva at the period when the larval organs are beginning to form. Three oral invaginations are observable; these will later develop into the internal sac, pharynx, and pyriform organ, respectively. Globules (*g.*) are seen to be exuding from the cells of the internal sac, and similar globules are also present within the cells of the internal sac

and pharynx. The neuro-muscular cord (*N.M.*) is in process of development. The mesendoderm forms a solid mass in the posterior part of the larva. Viewed from the left side. × 225.

Fig. 63 *a*.—Longitudinal section through the larva at a slightly later stage than that illustrated in Fig. 62, viewed from the left side. The mesendodermic mass has now become hollowed out to form the stomach. Viewed from the left side. × 225.

Fig. 63 *b*.—Transverse section through the region of the pharynx of a larva of the same age as that shown in Fig. 63 *a*. Two bands of tissue (*), which may possibly represent the true mesoderm, are budding off from the mesendodermic mass. × 225.

Fig. 64.—Transverse section of a larva older than that which is represented in Fig. 63; the plane of section passing through the stomach, internal sac, and pharynx. × 225.

Fig. 65 *a–c*.—Sections of mature larvæ.

a. Longitudinal section of a larva, viewed from the right side, in which the larval organs are all fully developed. The pharynx and internal sac have lost the globules which were present in earlier stages; the pyriform organ now consists of the "système glandulaire supérieure" (*S.G.'*) with the "fossette supérieure" (*Fs.*) and the "système glandulaire inférieure," (*S.G.''*) only the right side of which is visible: the "fente ciliée" is not shown. The stomach has become very elongated. The ciliated crown (*C.*) is now visible. (From a drawing by Dr. S. F. Harmer.)

b. Transverse section of the larva in a plane passing through the stomach and internal sac. The two bands of tissue which appear to be budding off from the mesendoderm are again noticeable. × 225.

c. Transverse section passing through the stomach and pharynx of the larva. × 225.

Fig. 66.—Transverse section of a larva in which degeneration has commenced. The walls of the internal sac have become much thickened and the protoplasm near the opening to the exterior has assumed a granular appearance. The ciliated crown now consists of a single row of large cells. The stomach has vanished, and the mesendoderm is represented only by scattered nuclei and yolk spherules, among which lie numerous muscle-fibres. × 225.

Researches on the Origin and Development of the Epiblastic Trabeculæ and the Pial Sheath of the Optic Nerve of the Frog, with illustrations of Variations met with in other Vertebrates, and some Observations on the Lymphatics of the Optic Nerve.

By

J. T. Gradon, M.A.,
St. John's College, Oxford.

With Plates 26, 27.

Introduction.

Just over a year ago I began to feel dissatisfied with Assheton's (1) conclusion that the cells of the optic stalk do nothing more than serve as a conductor for the fibres of the optic nerve.

As I was aware that Assheton's (1) opinion had been fully endorsed by Professor Ryder (8) in the embryological section of Norris and Oliver's 'System of Diseases of the Eye,' I thought it unnecessary to go any further into the literature of the subject before beginning the present researches, and unfortunately I had finished them before I found that the part of the epiblastic trabeculæ that I shall speak of as transverse, had been dealt with by W. Müller (6), Kölliker (5), Robinson (7), Studnička (9), and Froriep (3 a). But, as all of these well-known investigators have dealt with the transverse fibrils as though they were the whole epiblastic trabeculæ of the optic nerve, instead of being only a part of

its complex framework, it will be my aim in the present paper to describe its origin and development as a whole.

The subject will be dealt with under the following heads:

I. The Relation of the Optic Stalk to the Nerve-fibres.

In discussing Assheton's (**1**) contention that the first nerve-fibres, though lying along the posterior border of the stalk, are at first entirely outside it and separate from it, Robinson (**7**), after showing that this conclusion is altogether at variance with the observations of W. Müller (**6**) upon the lamprey, of Kölliker (**5**) upon rabbits, pigs, and calves, of Keibel (**4**) upon reptiles, and of Froriep (**3**) upon cartilaginous fishes, makes the following very important statement:—"If the condition which Assheton (**1**) found in the frog is present in mammals also, then it follows that the sustentacular framework of the optic nerve of man may consist, for the most part, like the framework of an ordinary cerebro-spinal nerve, of mesoblastic tissue surrounding and embedding the epiblastic nerve-fibres, but if Müller's and Kölliker's statements are well founded, then the sustentacular tissue of the optic nerve in man and mammals must consist chiefly of epiblastic tissue derived from the primitive epithelial cells of the optic

stalk; . . . this is a matter of some morphological, and certainly of pathological, importance."

All my specimens undoubtedly confirm the observations of the authorities quoted by Robinson (**7**), and his own statements, which are based upon observations made on human embryos, cats, ferrets, sheep, rabbits, rats, and mice, viz. that the ingrowing nerve-fibres lie within the membrana limitans externa, throughout the whole of their course in the optic stalk, and that they enter the stalk along the ventral wall; though Froriep (**3 a**) has lately stated that, in his specimens of rabbit embryos, the earliest bundles of nerve-fibres grow in higher up on each side of the ventral wall, and that the nuclei that lie above the ingrowing nerve-fibres are pushed up towards the lumen of the stalk, whilst those that lie below are pushed still further down, as the number of nerve-fibres increases.

In tadpoles of 6 mm. in length I have invariably found the earliest bundles of nerve-fibres, as they issue from the optic cup, occupying a central position just within the membrana limitans externa of the ventral wall of the stalk, and, as they approach the brain, getting more and more towards the posterior side of it, though in 8·5 mm. tadpoles they seem first of all to travel a little anteriorly for a very short distance, just after leaving the optic cup. These observations are in agreement with Froriep's (**3 a**), figs. 237—239, taken from tadpoles.

II. Cellular Segmentation.

Robinson (**7**) has referred to the difficulty of obtaining indications of definite cell-territories in the early stages of the embryonic optic stalk of the rat.

In tadpoles of 4·5 mm. in length cell limits are certainly recognisable (fig. 1), but in those of 6 mm. in length they can rarely be distinguished from the pigmented fibrils of the protoplasm that encircles the granules of food-yolk, or

takes up the position lately occupied by those that have been assimilated.

An inspection of fig. 2 will show that the entrance of the nerve-fibres along the ventral wall of the stalk produces a confluence and stretching of these delicate protoplasmic fibrils, and, at the same time, brings into prominence the connections subsisting between nucleus and nucleus.

Further ingrowth of the nerve-fibres resolves these fibrils into a complex framework of supporting elements which, from transverse, longitudinal, and horizontal sections, may be seen to radiate in every direction from the border of each nucleus of the stalk.

This intermediate arrangement of the condensed protoplasmic fibrils finally becomes differentiated with the multiplication of the nuclei of the stalk into a transverse, oblique, and longitudinal framework which, as we shall afterwards see, also provides a complete system of lymph channels throughout the interior of the optic nerve.

III. Obliteration of the Lumen of the Optic Stalk.

The obliteration of the lumen of the stalk has received considerable attention from previous observers. Assheton (1) ascribes it to pressure from the cartilaginous walls of the cranium, whilst Robinson (7) considers that this cannot be looked upon as an important agent, and concludes that the obliteration "is brought about by developmental changes in growth and relationship of the constituent parts of the stalk," and that "with these is associated the invasion of the optic nerve-fibres."

In fig. 12 we can see that pressure is exerted on the stalk by the cartilaginous walls of the cranium, and it is also certain that pressure produced by contact with the back of the eye is the cause of the very decided oval shape of the stalk at this point; in an 11 mm. tadpole (fig. 4), its shape, when free, is almost round. But there are probably several causes at

work—both within the stalk itself and outside it—in bringing about the obliteration of the lumen.

The pressure everywhere outside the stalk is evidently greater than that within its lumen for, although the first nerve-fibres lie just within the external membrane, the presence of the smallest bundle is enough to produce a certain amount of bulging of the upper border of the ventral wall into the lumen without in the slightest degree altering the regularity of the outline of the external membrane underneath it (fig. 2).

It is true that further ingrowth of nerve-fibres produces a considerable change in the outline of the stalk, as shown in fig. 3, but by this time, the lumen has almost been closed, and still further ingrowth of nerve-fibres at the sides, completes its obliteration, and, at the same time, restores the slightly longer axis of the stalk to the horizontal position (figs. 4 and 5).

IV. Period of Slow Growth, followed by one of Great Activity, consequent on the Formation of the Arachnoid Sheath and the Enclosure of the Subarachnoidal Lymph Space.

Between the stages shown in figs. 4—6, representing tadpoles from 11 mm. to 21 mm. in length, the diameter of the stalk increases only very slightly. This is due to the fact that there is scarcely any karyokinesis going on within the stalk, and the protoplasmic framework, which is now binding the nerve-fibres together, seems unable to accommodate itself to further expansion.

Meanwhile, the stalk is being continually more and more stretched between the eye and the brain, so that it is possible to obtain transverse sections of a 21 mm. tadpole that do not contain a single nucleus, only the protoplasmic fibrils proceeding from nuclei that lie in the preceding and succeeding sections.

As there are no blood-vessels inside the optic nerve of the frog, and very few capillaries on the pial sheath, it will be evident that, up to this stage, the nutrition of the stalk is at a very low ebb; there are, however, no indications of degeneration; in fact, it is possible to show a solitary instance of cell-division now and again where the fibrils of the trabeculæ are in contact with the delicate capillaries that have crept up the pial sheath from the pia mater of the brain (fig. 9).

But when we turn to a 27 mm. tadpole (fig. 7) it is evident that a remarkable change has taken place; mitosis is everywhere abundant, the number of cells has already greatly increased, and the total diameter of the stalk is considerably greater than it was in the preceding stage.

This sudden change coincides with the more complete enclosure of the subarachnoidal lymph space, which has come about through the formation of the arachnoid sheath. Before the dural sheath has had time to form, the arachnoid itself is directly connected with the ophthalmic artery by a band of connective tissue, by means of which transudation of lymph from the artery doubtless takes place.

The subarachnoidal space, which is evidently not sufficiently enclosed until this stage has been reached, is now filled with nutritive material, which passes through the pial covering, and then reaches every nucleus of the stalk by means of the elaborate system of tiny channels that follow the course of each fibril of the epiblastic trabeculæ (figs. 8 and 13, and *pc.* fig. 17).

Moreover, we may now find, among the meshes of the connective-tissue cells that join the ophthalmic artery and the optic nerve, numbers of lymph-corpuscles, all in various stages of cell-division, though I have only shown them in outline in fig. 7.

Development now proceeds very rapidly, but I have not thought it necessary to publish any drawings of stages between that shown in fig. 8 and the adult stage shown in fig. 10.

In the latter figure we can see the final arrangement of th

epiblastic trabeculæ from the sector that I have filled in with nuclei and fibrils; the other part of the drawing only shows the distribution of the nuclei and some of the thicker fibrils.

It will also be evident from this drawing that the stellate arrangement of the nuclei and the trabeculæ, found in a 32 mm. tadpole, does not persist; it is gradually lost in succeeding stages.

V. Pigmentation.

In the earliest stages of the stalk the fibrils of the protoplasm surrounding each granule of food-yolk, are pigmented, and can be traced perhaps separately.

But, when there has been a confluence of probably several of these, through ingrowth of nerve-fibres, it makes the condensed transverse fibrils, seen in sections transverse to the stalk, stand out in bold relief, and, when one has learnt what to look for by means of these sections, allows the condensed oblique and longitudinal fibrils to be seen, in longitudinal sections, without having recourse to special methods of staining.

But as soon as the increased flow of lymph, that we have ascribed to the enclosure of the subarachnoidal lymph space, takes place throughout the nerve, the trabeculæ, excepting sometimes a very short and thickened piece close to the nucleus, become completely depigmented.

This renders it afterwards very difficult to follow the delicate, oblique and longitudinal fibrils among the nerve-fibres.

But even in the adult state the amount of pigment which the thickened end of the fibril sometimes contains near its nucleus is sufficient to catch the eye when the rest of the fibril would easily escape notice.

I have not thought it necessary to publish drawings of longitudinal sections from stages later than that represented in fig. 14, as there is nothing further to show than a con-

tinual increase of the number of nuclei and fibrils of the trabeculæ, without any apparent increase in the thickness of the latter.

It clearly follows, from what has been said, that the cells of the optic stalk are spongioblasts, and that they, therefore, take no part in the production of optic nerve-fibres, which arise, according to the researches of Ramon y Cajal (2) and other well-known investigators, from neuroblasts, chiefly in the retina.

VI. Supplementary Remarks.

Even when there is not sufficient protoplasm surrounding the resting nuclei of the stalk to be represented in the drawings, it will be understood that there is still an extremely delicate layer of it covering them, and that the fibrils of the trabeculæ form the continuations of it. This thin sheet of protoplasm may, however, be distinctly recognised around the nuclei that are undergoing division (vide especially fig. 9).

The fantastic outlines of the nuclear walls are accounted for by the fact that each fibril of the trabeculæ is being stretched by continual ingrowth of nerve-fibres, and is, therefore, pulling its nucleus towards the point of its attachment.

In this connection it will be interesting to compare the nuclear outlines of the densely-packed optic nerve of the frog (fig. 8), with those of the much less densely packed optic nerve of the dog-fish (fig. 17).

The nerve-fibres contained in the optic nerve of the latter are so comparatively few in number, and the lymph channels so wide and numerous, that, when favourable transverse sections of it are viewed with a very low power, the nuclei themselves appear to form a well-defined framework, through being pulled into mere threads between the nerve-fibres.

In the frog, on the other hand, the lymph channels, though

numerous, are so comparatively narrow, and the nerve-fibres so densely packed around the nuclei, that the pull exerted on a nucleus by each fibril of the trabeculæ, can only result in the production of a short cone.

VII. Formation of the Pial Sheath, and its Relation to the Membrana Limitans Externa.

I have figured the formation of the pial sheath from the earliest stages to show how the mesoblastic cells that enter into the formation of its connective-tissue layer, gradually unite with the external membrane of the stalk or its later representatives—the ends of the epiblastic trabeculæ.

But the union is only apparent, for a regular system of lymph spaces is formed between the ends of the trabeculæ and the layer of connective tissue, which may be separated in sectionising (fig. 17).

I have referred to the scantiness of its vascularity in the frog in a preceding section, p. 484.

In rat embryos of 8 mm. in length Robinson (7) found the peripheral boundary of the stalk clearly defined, but was unable to demonstrate a distinct external limiting membrane.

In the frog there never is any doubt about the external limits of the stalk, though the boundary is naturally more delicate in a 6 mm. tadpole than in those of succeeding stages.

The optic stalk of the chick, containing a very great number of cells, shows a well-defined external limiting membrane, supported by numerous mesoblastic cells, when the nerve-fibres begin to grow in, on the fourth day of incubation.

VIII. Some Variations of Epiblastic Trabeculæ met with in the Developing Optic Nerve of the Mouse, the Trout, the Dog-fish, and the Chick.

Although I have selected the tadpole for tracing the complete development of the epiblastic trabeculæ, still we can

find in other embryos some interesting variations which assist us very materially in gaining a fuller comprehension of the subject.

Fig. 15, which represents a longitudinal section of the optic nerve of an embryo mouse of fourteen days, gives us a better idea of the longitudinal fibrils than we have been able to gain in considering the later stages of these fibrils in the frog, as they lie in the same plane for a much greater distance, and are, at the same time, rather thicker for a greater part of their length than those we have seen in the frog.

On the other hand, in fig. 16, representing a longitudinal section of the optic nerve of a developing trout (length of optic stalk ·5 mm.), we see fibrils that are extraordinarily thick, and consequently very easily seen near their nuclei, but undulating to such an extent that it is only possible to follow them a very short distance away from their nuclei.

In fig. 17 I have shown a transverse section of the optic nerve of a 33 mm. dog-fish, and on p. 486 I have compared the nuclear outlines of the densely packed optic nerve of the frog (fig. 8) with those of the much less densely packed optic nerve of the dog-fish, shown in this figure, so that I need only point to the difference in the arrangement of the nuclei themselves, though, as I have stated on p. 485, the stellate arrangement of the nuclei and the trabeculæ in the optic nerve of the tadpole is lost before the adult stage is reached.

Another point of difference between the frog and the dog-fish lies in the fact that the pial sheath of the latter is richly supplied with blood-vessels, though not represented in the drawing.

A transverse section of the optic nerve of an eight-day chick (fig. 18) shows certain peculiarities of trabecular formation; the arrangement of the nuclei is free, like that of the dog-fish, but the nuclear outlines more closely resemble those of the frog, due, in my opinion, to the same causes as those I have given to account for the peculiarities of these outlines in the frog.

I have shown one of the numerous capillaries that supply

the interior of the optic nerve of the chick with blood and lymph descending into it from the pial sheath which is richly supplied with blood-vessels, and the adjacent fibrils of the epiblastic trabeculæ may be seen in contact with it (fig. 18).

In conclusion, I deeply regret to say that since this article was written, the sudden death of the Linacre Professor of Comparative Anatomy has rendered it impossible for me to publicly express my thanks to him for allowing me to carry on my researches in ocular embryology in the Department of Comparative Anatomy at Oxford, and more especially for the kind interest that he always took in my work. But I gratefully avail myself of this opportunity of thanking Dr. J. W. Jenkinson, Assistant to the Linacre Professor, for kindly providing me with unlimited material and preparations for the purpose of the present article.

Summary.

We have seen that our trabeculæ are entirely epiblastic in origin, for we have shown that the entrance of the nerve-fibres along the ventral wall of the embryonic optic stalk produces a confluence and stretching of the protoplasmic fibrils of the epiblastic cells of the stalk, which result in a complex framework of supporting elements radiating in every direction from the border of each nucleus of the stalk, and that this complex framework afterwards becomes more or less differentiated into a transverse, oblique, and longitudinal trabeculæ with the multiplication of the nuclei of the stalk and without any admixture of mesoblastic cells, for we have also shown that the nerve-fibres lie, throughout the whole of their course, in the optic stalk, within the membrana limitans externa, on the outside of which we have followed the gradual formation of the connective-tissue layer of the pial sheath.

We have noticed the obliteration of the lumen of the stalk, and have ascribed it to various causes operating within the stalk itself and outside it, though chiefly to the ingrowth of nerve-fibres.

We have seen that, in the development of the optic nerve of the frog there is a period of slow growth, followed by one of great activity, and we have felt justified in ascribing this sudden outburst of activity to a greatly increased flow of lymph into it by means of the elaborate system of minute channels that follow the course of each fibril of the epiblastic trabeculæ, and consequent upon the formation of the arachnoid sheath and the enclosure of the subarachnoidal lymph space.

We have therefore shown that the cells of the optic stalk perform the following three functions:

1st. They conduct the nerve-fibres, which, in their turn, resolve the constitution of the cells of the stalk, so that they—

2nd. Provide the nerve-fibres with a supporting framework which—

3rd. Provides the whole interior of the optic nerve with an elaborate system of minute lymph channels.

Alphabetical List of Literature referred to.

1. Assheton, R.—"On the Development of the Optic Nerve of Vertebrates, and the Choroidal Fissure of Embryonic Life," 'Quart. Journ. Micr. Sci.,' vol. 34, 1892.

2. Cajal, Ramon y.—"Sur la fine structure du lobe optique des oiseaux, et sur l'origine réele des nerfs optiques," 'Monthly International Journal of Anatomy and Physiology,' vol. viii, parts 9 and 10, 1891.

3. Froriep, A.—"Ueber die Entwickelung des Sehnerven," 'Anat. Anz.,' vi Jahrg., 1891.

3a. ——— "Entwickelung des Auges," 'Handbuch der vergleichenden und experimentellen Entwickelungslehre der Wirbeltiere," O. Hertwig, Bd. ii, Abt. 2, Jena, 1905.

4. Keibel.—"Ueber die Entwickelung des Sehnerven," 'Deutsch. Medizin Wochenschr.,' xv Jahrg., 1889.

5. Kölliker.—"Zur Entwickelung des Auges und Geruchsorgans menschlicher Embryonen," 'Festchrift der Universität Zürich dargebracht,' Würzburg, 1883.

Kolliker.—"Entwickelungsgeschichte des Menschen und des höheren Thiere," Leipzic, 1879.

6. Müller, W.—"Ueber die Stammesentwickelung des Sehorgans der Wirbeltiere," Leipzic, 1874.

7. Robinson, A.—"On the Formation and Structure of the Optic Nerve, and its relation to the Optic Stalk," 'Journ. Anat. and Physiology,' vol. xxx, April, 1896.

8. Ryder, J. A.—"Development of the Eye," 'System of Diseases of the Eye,' Norris and Oliver, vol. i, London and Philadelphia, 1897.

9. Studnička, F. K.—"Untersuchungen über den Bau des Sehnerven," 'Jenaische Zeitschr. Naturw.,' Bd. xxxi, 1898.

EXPLANATION OF PLATES 26 AND 27,

Illustrating Mr. J. T. Gradon's paper, "Researches on the Origin and Development of the Epiblastic Trabeculæ and the Pial Sheath of the Optic Nerve of the Frog."

Alphabetical List of Reference Letters for all the Figures.

Br. Brain. c.c. Cartilage of the cranium. *c.c.* Connective tissue cells between the ophthalmic artery and the optic nerve. e. Eye. *e.* Outline of pigmented epithelium of retina. *e.m.* Membrana limitans externa. *i.m.* Membrana limitans interna. *l.* Lymph-corpuscle. *l.c.* Lymph channel. *l.s.* Lumen of optic stalk. *m.a.* Mesoblast of the arachnoid sheath. *n.f.* Optic nerve fibres. *n.s.* Space occupied by nerve fibres, not represented. *o.n.c.* Interior capillary of optic nerve. *p.c.* Capillary of pial sheath. *p.p.* Pigmented protoplasm. *p.s.* Pial sheath. *sa.s.* Subarachnoid space. *tr.l.* longitudinal, *tr.o.* oblique, *tr.t.* transverse trabeculæ. *y.* Granules of food-yolk.

Fixing agent: Aceto-corr. subl. Stain: Borax carmine + picro-indigo-carm.

All the figures have been drawn with the Abbe camera.

The terms transverse and longitudinal apply to the optic stalk.

Figs. 1 to 8 are transverse sections taken from tadpoles, the lengths of which are given below.

They are all taken from the distal fourth of the stalk, except Fig. 2, which is taken from the proximal fourth.

They show the gradual formation of the transverse trabeculæ and the pial sheath.

The nerve fibres have only been represented in some of the figures, but they will be understood to occupy the spaces between the trabeculæ.

The resting nuclei of the stalk, except in Fig. 16, have been outlined only, and all the nuclei of cells entering into the formation of the pial sheath have been shaded.

The top of the page represents "dorsal."

PLATE 26.

Fig. 1.—4·5 mm. × 800. (See previous page.)

Fig. 2.—6 mm. × 800.

Fig. 3.—8·5 mm. × 800.

Fig. 4.—11 mm. × 800.

Fig. 5.—15 mm. × 800.

Fig. 6.—21 mm. × 800.

Fig. 7.—27 mm. × 800.

Fig. 8.—32 mm. The nerve fibres and lymph channels are shown in part of the drawing only. × 500.

Fig. 9.—Oblique transverse section, from the same series as Fig. 6, taken close to the brain. × 800.

Fig. 10.—From a transverse section of the optic nerve of an adult frog. Taken 160 μ from the eye. Only a sector has been filled in with the transverse trabeculæ. × 500.

Fig. 11.—Horizontal section from an 8·5 mm. tadpole; showing fibrils of the transverse, oblique, and longitudinal trabeculæ. × 800.

PLATE 27.

Fig. 12.—Longitudinal section, from a 15 mm. tadpole. Taken near the brain. × 800.

Fig. 13.—Peripheral longitudinal section, from a 29 mm. tadpole. Taken midway between the eye and the brain. × 800.

Fig. 14.—Longitudinal section, from a 32 mm. tadpole. × 800.

Fig. 15.—Longitudinal section, from a 14 day embryo mouse. × 500.

Fig. 16.—Longitudinal section, from a developing trout. Body length not known; length of stalk ·5 mm. × 500.

Fig. 17.—Transverse section from a 33 mm. dog-fish. The two large spaces between the pial sheath and the membrana limitans externa, here represented by the epiblastic trabeculæ, are due to sectionising, and they show that the pial sheath forms a separate layer around the optic nerve. × 500.

Fig. 18.—From a transverse section of the optic nerve of an 8-day chick. × 800.

Piroplasma muris, Fant., from the Blood of the White Rat, with Remarks on the Genus Piroplasma.

By

H. B. Fantham, B.Sc.Lond., A.R.C.S.,

Derby Research Scholar, University College, London; and Demonstrator in Biology, St. Mary's Hospital Medical School.

With Plate 28.

Contents.

I. Introductory.

Among the Hæmosporidia few genera are of greater interest to-day than Piroplasma. Its complete life-cycle is still unknown, yet "piroplasmosis"[1] is a dreaded malady which attacks many mammals, including man. In 1893 Smith and Kilborne (46) published their epoch-marking monograph on P. bigeminum, the parasite of Texas fever in cattle. According to Koch, this ranks as one of the three great dis-

[1] We owe this useful term to Lignières.

coveries in the ætiology of protozoal diseases, the other two being the discovery of the human malarial parasite by Laveran in 1882, and the Trypanosome of "Nagana" by Bruce in 1895.

Since 1893 other species of Piroplasma have been notified in horses, dogs, and sheep in various parts of the world. Recently Wilson and Chowning (**50**) have described P. hominis (Manson), the pathogenic agent of Rocky Mountain or Spotted Fever, while the parasite of "kala-azar" has been referred by Laveran and Mesnil to this genus.

Some months ago I had three white rats (Mus rattus, albino variety) affected with ulcerations on the ears and tail, and alopecia. On further examination all of these white rats were found to be suffering from piroplasmosis; one died almost immediately, another lived but a short time, while the third and last one died towards the end of November, 1905. Unfortunately pressure of work precluded my devoting much time to the examination of these rodents when they first came into my possession, and I was only able to give undivided attention to the last one, having, perforce, to be content with a partial examination of the others. Attempts at inoculation of infected blood from diseased into healthy white rats were unsuccessful, and the strain has thus been unfortunately lost. Under these circumstances, and in view of my non-success, up to the present, in procuring other white rats suffering from piroplasmosis, I have thought it might be of interest to publish my results on the morphology of the parasite, hoping later on to continue my researches, if possible, on fresh material.

Preparations of the blood of infected white rats were exhibited by me before the Zoological Society of London on December 12th, 1905 (**12**). I then proposed for the parasite the specific name of muris,[1] from its habitat. I would then

[1] It might, perhaps, be urged that "muris" at once suggests "mouse," whereas the parasite occurs in "rats." However, I have followed the well-established custom of naming the species of the parasite after the genitive of the generic name of the host.

designate the parasite described in this paper as Piroplasma muris.

II. Technique.

Fresh blood-films, obtained from the tail or ear, were examined from time to time, and in some cases a few red blood-corpuscles exhibited, in their interior, small, bright, usually ovoid bodies with dark contour, which occasionally showed slight motility. The change of position inside the corpuscle was usually from near the periphery towards the centre and back again to the periphery, and it sometimes set up slight rotation of the corpuscle. No especial change of shape was noticed. The small size of the intra-corpuscular or endoglobular bodies increased the difficulty of observation in freshly-drawn blood.

Most of the observations hereafter recorded were, however, made on fixed and stained preparations of thin smears or films of blood from the peripheral circulation, and on smears made from certain of the internal organs as soon after death as possible. Many of these were stained for some time with various modifications of the Romanowsky method, especially a combination of the methods of Laveran and Plimmer, using Bleu Borrel, erythrosin, and tannin orange. I also obtained good results with Leishman's stain, which possesses the added advantage of simplicity; and with an adaptation of Giemsa's stain, using a 1 per cent. aqueous solution of azur ii, together with a 0·1 per cent. aqueous solution of erythrosin,[1] mixed on the slide after fixation with pure methyl alcohol. Leishman's stain is useful in that it imparts to the erythrocyte-cytoplasm a pink colour, which affords—quite easily—a contrast with the blue cytoplasm and red chromatin of the enclosed para-

[1] The respective quantities were:—1 drop of azur ii to 2 drops of erythrosin, diluted with 5 to 8 drops of distilled water. The preparation may afterwards be stained with a dilute solution of tannin orange. The azur ii may be used first, followed for a short time by erythrosin, with good results.

site. Laveran's method gives the best results when successful, though it is a little difficult to manipulate and somewhat uncertain. Methyl alcohol fixes more sharply and rapidly than ethyl (absolute) alcohol.

Other blood-films and smears of organs were fixed with a mixture of mercuric chloride (two parts) and absolute alcohol (one part), or with osmic acid, and then stained with a dilute acidulated solution of Delafield's hæmatoxylin followed by eosin. The staining is slow, at least twenty-four hours being necessary, but the fixation is superior to that obtained with alcohol alone.

I also used, on a few occasions, a slightly alkaline solution of methylene blue, after fixation with absolute alcohol. Portions of liver, kidney, and spleen were fixed in formalin, embedded in paraffin and sectionised. Affixed to slides, sections of these organs were placed in a dilute aqueous solution of Leishman's stain for about twelve hours (*vide* Christophers [**4**] and Graham Smith [**14**]), then treated with a dilute solution of acetic acid (1 volume of acid to 500 of water) for a short time till pink, washed in distilled water, rapidly dried, then immediately moistened with xylol and mounted in balsam. Unfortunately, formalin is not a satisfactory fixative for these tissues, causing shrinkage.

Most of the observations hereafter recorded were made on material stained by the Laveran-Plimmer or Leishman methods.

III. Occurrence of the Parasite in the White Rat.

The parasites were rarely met with in the peripheral circulation, judging from observations on blood-smears from the tail of infected rats or from scrapings of the ulcers on the ears. On an average about 1 per cent., or rather less, of these erythrocytes were infected.

In smears of the internal organs—as the liver, kidneys, spleen, bone-marrow, lung, heart-muscle, and brain—the

parasites were more numerous. They were most plentiful in red corpuscles occurring in the capillaries of these organs, as seen in sections, especially in the kidneys, liver, and spleen (fig. 23, dilated capillary of liver).

Extra-corpuscular stages of the parasite, free in the blood-plasma, occurred in groups, probably resulting from the disintegration of the corpuscle host (fig. 21). Such groups were noticed sometimes in the peripheral circulation, more frequently in spleen blood.

Leucocytes were relatively rather more numerous than usual in the smears above mentioned, especially in those taken from the sores on the ears.

IV. Morphology of the Parasite.

In the red-blood corpuscles of the mammalian host ovoid or pear-shaped organisms [1] were noticed, which, after adequate staining by modifications of the Romanowsky method, showed a definite contour, blue cytoplasm, and a red or purple chromatin body, without any trace of pigment. Such characters are diagnostic of the genus Piroplasma.

These endoglobular bodies may be centrally placed in the blood-corpuscle, but more usually they are rather peripheral in position. They represent the trophozoite stage of the parasite, and may occur either singly (figs. 1 to 3) or in pairs (figs. 6 to 9) within the erythrocytes. Double, and even multiple, infection may be observed, as a dividing trophozoite together with a single pyriform trophozoite, or two trophozoites each in process of division (fig. 10), may be seen simultaneously inside blood corpuscles. In the smaller, and apparently younger forms, the internal chromatin body is somewhat flattened and peripheral in position (fig. 1). The chromatin body ("nucleus" or "karyosome" of various authors) is, indeed, seldom quite central in posi-

[1] The chromation of the parasite stains purple with azur ii alone; this is a test for a parasite (cf. Koch and Theiler), in contra-distinction from an artefact.

tion, but usually polar (figs. 2 and 3), that is, nearer to the rounded or blunt end of the pyriform trophozoite.

The smaller ovoid forms of the parasite measure 0·5 μ to 1·5 μ in diameter, while the pear-shaped forms are from 2 μ to 3 μ long, and from 1 μ to 1·5 μ broad.

Four pyriform bodies are sometimes seen in the red corpuscles of the peripheral circulation (fig. 10), but rarely more than four. In the spleen six and eight small pyriform bodies may occur in an erythrocyte (fig. 13). In some cases in the spleen they are rather more irregular in shape than strictly pyriform, affording examples of the so-called "amœboid" trophozoites (figs. 14 and 18) known to occur in other species of Piroplasma, and first described by Piana and Galli-Valerio in the case of P. canis.

In one case a vermiform trophozoite was noticed with a chromatic appendage. This had been fixed and stained towards the conclusion of the act of entering a red blood corpuscle (fig. 19). Flagellate forms of P. canis have been described by Bowhill and Le Doux (**2**), of P. equi by Bowhill (**1**), and of the Piroplasmata of cattle by Lignières (**30**) and others. The suggestion that such flagellate forms may possibly be microgametes seems to me premature and doubtful in the present state of our knowledge, as the "flagella" described by Bowhill and Le Doux are beaded, and may really be only pseudopodia.

Another vermiform or gregariniform, but entirely intracorpuscular, trophozoite, containing a chromatic dot attached to an irregular rod-shaped portion of chromatin, is shown in fig. 16.

The cytoplasm of P. canis is described by Nuttall (**41**) as "vacuolated or trabecular." In the case of the smaller organism, P. muris, the protoplasm is hyaline, and apparently finely granular, though it is very difficult to observe the finer structural details of so small an object through the wall of the enclosing blood corpuscle.

A clear zone of protoplasm often occurs around the chromatin body in the case of some of the large trophozoites

(fig. 17), while a distinct vacuole, more or less polar in position, may occur in other forms (figs. 1, 2, and 5).

The outer border of some of the larger forms of the parasite often takes up the stain more intensely than the more central cytoplasm, and so appears of a distinctly blue tint after Romanowsky staining.

Usually there is only one chromatic dot in each ovoid, pyriform, or amœboid body (figs. 2, 3, and 17). Occasionally two chromatic dots are seen (figs. 4 and 15, note also fig. 18), while in one case, as already mentioned, there was a chromatic appendage, somewhat flagellum-like, protruding from the body of the parasite, and even outside the erythrocyte host (fig. 19). The chromatin body averages 0·3 μ to 0·5 μ in diameter, and may be irregular in outline.

A few remarks seem necessary respecting the chromatin of the trophozoites of P. muris and other Piroplasmata. In view of the recent researches of Schaudinn and others on the "vegetative" and "reproductive" differentiations of the chromatin in parasitic Protozoa one may well hesitate nowadays to use indiscriminately the terms "nucleus" and "karyosome."[1] Laveran (**24**), in 1901, used the term "karyosome" to designate the chromatic body of the trophozoite of P. equi. However, since all the species of Piroplasma are comparatively small, it is difficult, in the present state of our knowledge, to be quite precise in naming the chromatin body or dot occurring in a trophozoite of Piroplasma, and it would seem best simply to refer to such structures as "chromatin bodies" or "chromatic dots."

No bacillary (**25**) or rod-like forms were seen, types which are characteristic of P. bigeminum in the blood of immune Bovines (**48**), and are also common in the case of P. parvum (**48, 49**).

The **mode of multiplication**[2] of the trophozoite is, as

[1] Siedlecki has lately (Oct., '05) written on the "Significance of the Karyosome," putting forward somewhat different views ('Bull. Acad. Sc. Cracovie,' 1905, No. 8, pp. 559-81).

[2] This mode of multiplication, which is endogenous, is in this genus simple,

in other Piroplasmata, by the primitive process of binary fission (figs. 6 and 8). Each "merozoite" or daughter trophozoite formed from a dividing trophozoite ("schizont") may, in turn, similarly divide. I am sorry that, in view of the smallness of this intra-corpuscular parasite, I am unable to give cytological details of the process of binary fission other than observing that the "chromatin body" of the ellipsoidal trophozoite (schizont) divides into two parts, arranged at the poles (fig. 5), and that the twin merozoites, when longitudinally constricted apart (fig. 6), remain attached for a time by their pointed ends (fig. 8). However, in the case of erythrocytes enclosing several parasites the numbers so enclosed are usually multiples of two (vide figs. 10 and 13, but exception fig. 12).

I have not yet seen examples of multiple fission forming "rosette" stages, as figured by Laveran (**24**), in the case of P. equi, or "cross" stages of four, as mentioned by Koch (**21**) in the case of P. parvum.

Large extra-corpuscular, sausage-shaped "gamete" forms have been figured by Nuttall and Graham-Smith (**41**, Pl. 9, figs. 59—62) in the case of P. canis. Stephens and Christophers (**47**, Pl. 3, fig. 10), too, have figured a pair of large intra-corpuscular forms, containing much chromatin, as possible "gamete" forms of P. bovis. In each case it is only tentatively suggested that such are "gametes" (perhaps more strictly "gametocytes"), but the correctness of these and similar interpretations has not yet been established. (Cf. Minchin [**37**, pp. 269–70] on Hunt's "crescents," Lignière's "gametocytes" and Doflein's views, where he remarks:—"The relations of the various phases hitherto observed, and their true rôle in the life-cycle is, at present,

so it might be considered unnecessary to use the term "schizogony" for simple binary fission, or "schizont" for a trophozoite so dividing, as the trophozoite is not here sporulating into many daughter forms, but usually into two only, which themselves, perhaps, need not be specially termed "merozoites." Another view, however, is that schizogony is here witnessed in its simplest form, and this seems the better view to take.

. . . purely conjectural.") Nor do these easily fit in with Koch's recent researches on P. bigeminum in the tick (**21**).

In sections of the internal organs, as the liver and kidneys, infected corpuscles are seen to be numerous in the capillaries (fig. 23). Endogenous reproduction of the parasite would appear to be especially prevalent in these parts.

Regarding the **sporogony** of P. muris, I have, unfortunately, no observations, nor, indeed, have any well-authenticated details been published by any observer on the exogenous reproduction of any species of Piroplasma, with the possible exception of a preliminary note by Lingard and Jennings (**33**) on Piroplasmata in Mammals and even birds and lizards (!), wherein figures of what purport to be sporogony ("sexual") stages are given. This account by Lingard and Jennings, although avowedly preliminary, is, unfortunately, somewhat condensed and disconnected, and so not very clear. Information on the sporogony of many, indeed most, of the Hæmosporidia is still wanting.

With respect to the dissemination of piroplasmosis among white rats little can at present be stated. I carefully searched the infected rodents for ticks, but found none. Lice were abundant on one rat, and fleas also occurred, but no clearly-defined further stages of the Piroplasma were seen in the internal organs of these insects. The "intermediate" (invertebrate) host is probably a tick, as has been shown in all other cases of true piroplasmosis hitherto examined. The three rats infected with P. muris and discussed in this memoir were, I understand, obtained from two separate sources in the East-end of London, but as to the manner in which they became infected in the first instance I have no information.

Very few examples of phagocytosis of the parasites were observed. One or two instances of apparently free parasites being engulfed by leucocytes were noticed (fig. 22), but no cases of leucocytes actually destroying infected erythrocytes could be detected. The paucity of examples of phagocytosis has been emphasised by other observers of Hæmosporidia.

V. Note on Piroplasmosis in the White Rat.

As I am not a medical man I would crave indulgence for deficiencies in the diagnosis or setting forth of symptoms in the following outlines of piroplasmosis as exhibited by white rats.

These rodents, in whose blood P. muris was found, at once attracted attention by the presence of pronounced ulcers on the ears. There were also smaller sores on the tail, and sometimes slight ones on bald patches on the body, and in one case on slight swellings near the anus and the snout. The bald patches, from which the fur had quite disappeared, were variously distributed in the different rats; there was a marked patch devoid of fur on the necks of the rodents examined. The body temperature of the rats, determined per rectum, was at times above the normal (for example, readings of 102° F. and 101·6° F. were obtained), and indicated an irregular fever. After death in one case a yellowish discoloration of the skin and some tissues was noticed, apparently due to biliary fever. There was also slight anæmia, and a relative increase in the number of leucocytes, with enlarged spleen. Before death the rats became emaciated, and showed gradually increasing loss of appetite. Some solid bile was found in the bile-ducts, and in one case the urine was dark coloured (hæmoglobinuria).

From the comparatively long time two of the infected rats lived while suffering from the disease (two to five months), and the fewness of the parasites in the peripheral circulation, the cases perhaps approached the chronic type.

A few nucleated red cells occurred in the blood, but none of these were noticed to be infected, while erythrocytes containing many parasites were sometimes slightly enlarged (that is, greater than 7 μ in diameter), and when stained were pale in colour, sometimes approaching a slight blue tint after the use of Leishman's stain.

It has already been mentioned that the parasites were

more numerous in the internal organs, such as the liver spleen, kidneys, heart-muscle, lung, and bone-marrow, especially in the capillaries of these, which were enlarged. In the liver, and to some extent in the kidneys, the outlines of the cells were not easily apparent or were even broken down, the cytoplasm was ill-defined, and the nuclei of the hepatic cells were often hypertrophied (fig. 23).

Probably most, if not all, of the symptoms outlined above are those of piroplasmosis, judging from published accounts of cases of the disease in other mammals.

It would be interesting to determine if the disease is periodic; possibly it occurs in the spring or early summer. Information is also required, as already remarked, regarding the invertebrate host, probably a blood-sucking Arthropod, which may be concerned in the spread of the disease.

Further, the disease may not be strictly limited to the white rat, that is, the albino variety, but will perhaps be found in black (Mus rattus) and brown (M. decumanus) rats.

Cultures of infected blood, made by adding sodium citrate and a little citric acid to freshly drawn blood, showed no further stages or development of the parasite, even after several days.

VI. Systematic; the genus Piroplasma.

Summarising briefly some of the more important characteristics of the parasite[1] already described, we notice the usually ovoid or pyriform shape of the trophozoite, generally with a single well-marked chromatin dot, multiplication by binary fission into two merozoites, the absence of melanin pigment, and the cytozoic habitat within a red blood-corpuscle during the endogenous stages. From these features it may be concluded that the parasite is a Hæmosporidian,

[1] The sizes of the various forms of the parasite are given on pp. 498 and 508.

belonging to the order Acystosporea[1] on account of its simple body form, and to the genus Piroplasma on account of its ovoid or pyriform shape and simple fission in schizogony. Since it occurs in the white rat and is apparently confined to rats, I have proposed the new specific name muris.

The other well-authenticated species of Piroplasma[2] (Patton, 1895), as mentioned by Laveran (**23**) and others, are—

(1) P. bigeminum (Smith and Kilborne, 1893), the parasite of Texas fever in the Bovidæ, which has since been observed in most parts of the world. This species is sometimes called P. bovis (as by Nuttall [**40**], and Stephens and Christophers [**47**]). The correct name of the species is doubtful. Judging from the illustrations of the relative sizes of the parasite and its corpuscle host, as figured by Smith and Kilborne (**46**) in cases from Texas, and by Stephens and Christophers (**47**) in cases from Madras, there would seem to be more than one species. Lignières (1900) also thought there were two species of P. bigeminum (P. bovis) in Argentina, and Nuttall (**40**) has emphasised this point. Some authorities, again, apply the name P. bovis to the parasite of bovine hæmoglobinuria in Europe, spread by Ixodes reduvius, separating it from P. bigeminum, which latter name is restricted to the parasite of Texas Fever (Tristeza, Redwater).

(2) P. parvum, separated by Theiler (**48, 49**) in 1904, as a distinct species from the former, and found in Bovidæ suffering from East Coast Fever (Tropical Bovine Piroplasmosis, "Rhodesian Redwater" [**16, 18, 19, 20**]). It also occurs in Transcaucasia (**11**).

(3) P. canis (Piana and Galli-Valerio, 1895), occurring in

[1] The distinction between the sub-orders Acystosporea and Hæmosporea is not now so sharp as formerly considered, since the discovery of intermediate hosts in the case of several Hæmogregarines, and the finding of Hæmogregarina gerbilli by Christophers in a mammal.

[2] The synonymy of the generic name "Piroplasma" is given by Minchin (**37**, p. 269). Probably the strictly correct name, by priority, is "Babesia," though the name "Piroplasma" is almost universally used.

"malignant jaundice" in dogs in South Africa, India, Senegambia, Italy, and France (**13, 34, 39, 40, 41, 51**).

(4) P. ovis (Starcovici, 1892) in sheep in Hungary, Roumania, Italy, and Germany ("Carceag" [**38**]).

(5) P. equi (Laveran, 1899), the pathogenic agent of biliary fever in horses in South Africa and Italy. The same or a closely allied species occurs in donkeys in South Africa (Dale [**8**]).

(6) A species, apparently unnamed, has been described by P. H. Ross (**44**) in 1904 from monkeys (Cercopithecus) in Africa.

(7) P. hominis (specific name due to Manson in 1903, though the parasite was first described by Wilson and Chowning) in cases of "Tick" or "Spotted Fever" in man in the Rocky Mountains.

(8) P. donovani (Laveran and Mesnil [**27, 28**]), for the Leishman-Donovan bodies (**9, 10**) found in cases of kala-azar and Oriental sore in man in India, Arabia, China, Egypt, and Tunisia. There is doubt as to the accuracy of placing these bodies in the genus Piroplasma (see below).

(9) Lühe (**33 a**, p. 201) mentions a little known, and apparently unnamed, species of Piroplasma found by Ziemann (**53**) in the Cameroons in the blood of sheep, goats, horses, and donkeys ("Tier-Malaria").

P. muris, as I have found and measured it, seems distinctly smaller than P. canis, and apparently slightly smaller than the type species, P. bigeminum, though the sizes of the latter, as given by different observers from various localities and cases, vary somewhat. Indeed, this variation in size seems to apply to many of the species of Piroplasma, according to case, locality, and observer, perhaps due to the smallness of the parasite and consequent difficulty in precise measurement, as well as to differences in fixation and staining.

The genus Piroplasma **stands distinctly apart from the other Hæmosporidia.** It may be that the Hæmosporidia, as at present understood, is really a heterogeneous group, which will ultimately have to be broken up. Laveran (**22**), one of

the founders of this group of the Sporozoa, divided it in 1901 into three great genera, namely, Hæmamœba, Hæmogregarina, and Piroplasma. Some authorities, although allowing the correctness of the basis of this arrangement, would recognise more genera (vide Schaudinn's monograph on the "Tertian Parasite" and Minchin [**37**, p. 265]). However, the classification and nomenclature of the Hæmosporidia is still in a confused state, indeed few groups of the animal kingdom are so involved from this point of view. Laveran, in a recent essay (**23**), returns to this matter, and reiterates his former classification, giving also a list of recognised species to date (October, 1905). The species which Laveran enumerates under the genus Piroplasma have just been set forth above, and, in addition, P. H. Ross's species from Cercopithecus (**44**). The species P. donovani, for the Leishman-Donovan bodies of kala-azar, is open to discussion.

To consider this point (the systematic position of the Leishman-Donovan bodies) at length is hardly within the purview of this paper. Some of the more important debatable points may, however, be very briefly set forth, to show the connection, or otherwise, of these bodies with the genus Piroplasma.

The Leishman-Donovan bodies are endocellular in habitat, occurring in spleen cells, endothelial cells, leucocytes, and possibly in erythrocytes. Their occurrence in the latter (erythrocytes) is not now generally held, and the first observations of them in this position have been variously interpreted. These bodies are piroplasmoid in shape, but are bounded by a perfectly definite external layer, more marked and consistent than in a Piroplasma, and possess two well-marked chromatic bodies, differentiated in character, as well as an internal "tail." In view of these differences Ross (**45**) has proposed for the parasites found in cases of kala-azar a new and separate genus Leishmania.[1]

[1] Containing two species, L. donovani (from Kala-azar) and L. tropica (from Delhi boil or Oriental sore).

Rogers (**42, 43**) and others (**4, 5, 6**) have obtained flagellated organisms from cultures of the Leishman-Donovan bodies. These flagellates are obtained in an essentially artificial medium, namely, by mixing infected spleen blood with sodium citrate and slightly acidifying with citric acid. In nature flagellate stages of these bodies, probably similar in character to those obtained in artificial media, might occur in the alimentary canal of an Arthropod, but have not as yet been observed. It would seem, then, a little premature to refer these flagellates, developed in citrate cultures, to the genus Herpetomonas, as the "Herpetomonas of kala-azar," Rogers (**43**).[1]

Apparently flagellates have not yet been obtained from cultures of the similar Cunningham-Wright bodies of Oriental sore (**7, 35, 52**).

A true Piroplasma possesses only one[2] chromatin body, and no typically flagellated stages are yet known in its life-history.

Koch (**21**) has recently published some short, but stimulating observations, on stages of P. bigeminum in the gut of ticks just gorged with infected bovine blood, and in tick eggs, observed in German East Africa. He states that the Piroplasmata in blood-corpuscles taken into the alimentary canal of ticks already, or very soon, show division of their chromatin into two, and that radial processes are developed from the parasite after it leaves the blood-corpuscle. Similar radiate forms are mentioned in the case of P. parvum. Later, copulation stages (probable zygotes) of the Piroplasmata are seen in the alimentary tract of adult ticks. Large pear-shaped forms of the parasite are described from tick eggs. I have myself seen similar forms in eggs of ticks infected with P. canis. There are no recorded observations of the parasites in larvæ and nymphs of ticks.

[1] Unfortunately Rogers, in his paper, writes of the "group Hepatomonas," apparently in mistake for the genus Herpetomonas.

[2] See Addendum for remarks on Lühe's researches, and the presence of a blepharoplast in P. canis.

Graham-Smith (**15**) has recently (October, 1905) recorded an intra-corpuscular parasite from the erythrocytes of moles. Although at first thought to be piroplasma-like, yet apparently the parasites do not belong to the genus Piroplasma, according to their discoverer, but are "longer or shorter rods of irregular shape" occasionally even devoid of chromatin. Graham-Smith does not appear to have named them yet.

It is interesting to note that a rodent, Spermophilus columbianus, is said to be concerned in the spread of human tick fever in the Rocky Mountains. Wilson and Chowning (**50**) give reasons for thinking that this Spermophilus is a third host of Piroplasma hominis, and consider that it is really the normal or true host of the parasite. In the Columbian Spermophile the P. hominis is non-pathogenic, and the human subject would seem to be not the true host but one in which the parasite lives with, perhaps, some difficulty, and wherein it consequently sets up pathogenic reactions resulting in human "spotted" or "tick fever." With this may be compared the action of Trypanosoma brucei, which is non-pathogenic in the "wild game" of South Africa, its true hosts, but is pathogenic or hurtful to the imported horses not indigenous to the country; similarly T. lewisi is non-pathogenic in the rat, which is apparently its true or natural host.

VII. Summary of Results.

The parasite described in this memoir occurs in the blood and certain organs, as the liver, spleen, kidneys, lung, heart-muscle, and bone-marrow of white rats, three of which came under my observation, but only one of them lived long enough to allow of continued study, and that only for a comparatively short time, too short to allow of observation on the methods of cross-infection.

The parasites are intra-corpuscular in habitat, occurring in the erythrocytes or hæmatids of the host, and belong to the

order Hæmosporidia, of the class Sporozoa. They were not found to be numerous in the peripheral circulation, but occurred in greater numbers in the internal organs above mentioned.

The trophozoites are ovoid (fig. 3) or pear-shaped (figs. 1, 2, and 7), the former varying from 0·5 μ to 1·5 μ in diameter, the latter being from 2 μ to 3 μ long and 1 μ to 1·5 μ broad, and devoid of melanin pigment (fig. 8). There is usually only one chromatin body or dot which may be peripherally or centrally placed, more usually near one end. A clear zone of protoplasm often surrounds this chromatin body (fig. 17), and a vacuole (fig. 5) may occur in the cytoplasm of the parasite. Pairs of trophozoites often occur in a host-corpuscle, but single trophozoites are also not infrequent.

Some so-called "amœboid" trophozoites (fig. 14) were seen in the spleen.

Endogenous multiplication takes place inside the rat's red blood-corpuscle by simple fission. Double infection (fig. 10) of a blood-corpuscle may occur, while free ovoid forms of the parasite have also been seen in the plasma (figs. 20, 21). Sometimes four parasites may be found in a corpuscle of the peripheral circulation, and as many as six or eight in corpuscles in the spleen (figs. 13, 14).

Some of the pathological effects in the white rats, very probably directly due to this parasite, were ulcers on the ears, alopecia, emaciation, anæmia, biliary fever, enlarged spleen, etc., and in each case death resulted.

From the foregoing characteristics the parasite may be placed in the genus Piroplasma. A short account of my exhibit of this parasite before the Zoological Society of London appeared in the 'Proc. Zool. Soc.,' 1905 (**12**), where I proposed the new specific name of muris, from its occurrence in a member of the Muridæ. I would, then, call this parasite Piroplasma muris.

The appended list of literature cannot be set forward as in any sense complete. To compile a complete list would need

long searching of zoological, medical, veterinary, and even general scientific journals, taking full advantage of the several catalogues of scientific literature now published, and even then allowing a margin for the rapid growth of the literature on this and allied subjects. A full bibliography of Piroplasma canis up to 1904 is given by Nuttall (**40**), together with references to many papers on other Piroplasmata. A complete list of papers relating to P. donovani, if it really be a Piroplasma, would also be difficult to compile, and even more difficult to collect and read. I only enumerate the more important papers relating to the systematic position of the Leishman-Donovan bodies. Since the intermediate host of P. muris has not yet been determined, I have not given many references to literature on ticks. Nevertheless, I hope that in the following I have not omitted any important papers on Piroplasma, although I have only enumerated the papers more or less directly referred to in the text.

In conclusion, I would take this opportunity of thanking Professor Minchin for the pleasure and help I have derived from attending his recent course of lectures on the 'Parasitic Protozoa,' which has been of use to me in writing the latter part of this paper, and for general help at all times.

March, 1906.

ADDENDUM.

Since writing the foregoing, there have appeared important works on Piroplasma by Lühe (**33a**, **33b**), wherein the generic name of Babesia is preferred (see my footnote, p. 504). Having worked recently on P. canis, the largest species of Piroplasma, under a magnification of 3000 diameters, Lühe states that the pyriform trophozoites only are endoglobular, and that, in addition to the "principal nucleus," there is a small chromatic dot nearer the pointed end comparable to the blepharoplast of a Trypanosome (cf.

my figs. 4 and 15, also figs. 11 and 18). I have only been able somewhat hurriedly to look over again some of my preparations of this smaller species, P. muris, but without obtaining any new observations. Lühe enumerates the various species of Piroplasma hitherto recorded, and discusses them in detail. From him I have inserted Ziemann's parasites (**53**) as No. 9 in my list on p. 505. Space does not admit of further discussion of Lühe's valuable treatise (**33a**) on the Hæmatozoa, which should be consulted in the original.

June, 1906.

VIII. References to Literature.

1. Bowhill, T. 1905.—"Equine Piroplasmosis, or 'Biliary Fever,'" 'Journ. Hygiene,' v, pp. 7-17, pls. i—iii.
2. Bowhill, T., and Le Doux, C. A. 1904.—"A Contribution to the Study of Piroplasma canis—Malignant Jaundice of the Dog," 'Journ. Hygiene.,' iv, pp. 217-18, pl xi.
3. Bruce, D. 1905.—"Stock Diseases of South Africa," Presidential Address to Physiological Section, Brit. Assoc.—'Nature,' lxxii, pp. 496-503.
4. Christophers, S. R. 1904.—"On a Parasite found in Persons suffering from Enlargement of the Spleen in India," Preliminary Report, 'Sci. Mem. Officers Med. and San. Depts., Govt. of India,' new series, No. 8, 17 pp., 2 pls.
5. Christophers, S. R. 1904.—[Same title.] Second report, op. cit., No. 11, 21 pp., 2 pls.
6. Christophers, S. R. 1905.—[Same title.]. Third Report, op. cit., No. 15, 14 pp., 1 pl.
7. Cunningham, D. D. 1885.—"On the Presence of Peculiar Parasitic Organisms in the Tissue of a Specimen of Delhi Boil," 'Sc. Mem. by Med. Off., Army of India,' pt. 1.
8. Dale, T. H. 1903.—"Pyroplasmosis of the Donkey," 'Journ. Comp. Path. and Therap.,' xvi, pp. 312-19.
9. Donovan, C. 1904.—"Human Piroplasmosis," 'Lancet,' 1904, ii, pp. 744-50, 1 coloured plate.

10. Donovan, C. 1905.—"Human Piroplasmosis," 'Lancet,' 1905, i, pp. 155-6.

11. Dschunkowsky, E., and Luhs, J. 1904.—"Die Piroplasmosen der Rinder," 'Centrbl. Bakter.' (1), xxxv, orig. pp. 486-92, 3 pls.

12. Fantham, H. B. 1905.—"Piroplasma muris, n. sp.," Note on a microscopic exhibit, 'Proc. Zool. Soc.,' 1905, ii, p. 491.

13. Galli-Valerio, B. 1904.—"Die Piroplasmose des Hundes," 'Centrbl. Bakter.' (1), xxxiv, ref. pp. 367-77.

14. Graham-Smith, G. S. 1905.—"Canine Piroplasmosis, III, Morbid Anatomy," 'Journ. Hygiene,' v, pp. 250-67, 2 charts and 2 pls.

15. Graham-Smith, G. S. 1905.—"A New Form of Parasite found in the Red Blood Corpuscles of Moles," 'Journ. Hygiene,' v, pp. 453-9, pls. 13, 14.

16. Gray, C. E., and Robertson, W. 1902.—"Red Water in Rhodesia," 'Agric. Journ., Cape Good Hope,' xxi, pp. 435-58.

17. Hunt, J. S.—"Progress Report on the Reproductive Forms of the Micro-organism of Tick Fever, etc.," 'Queensland Agric. Journ.,' ii, 3, pp. 211-20.

18. Hutcheon, D. 1903.—"Virulent Red Water in the Transvaal," 'Agric. Journ., Cape Good Hope,' xxiii, pp. 39-60.

19. Koch, R. 1903.—"The Rhodesian Cattle Disease," 'Agric. Journ., Cape Good Hope,' xxiii, pp. 33-39.

20. Koch, R. 1903.—"On Rhodesian Red Water or African Coast Fever" (4 reports), 'Journ. Comp. Path. and Therap.,' xvi, pp. 273 and 390.

21. Koch, R. 1905.—"Vorläufige Mitteilungen über die Ergebnisse einer Forschungsreise nach Ostafrika," 'Deutsch. med. Wochenschr.,' No. 47, 15 pp.

21a. Kossel, H., Schütz, Weber, and Miessner. 1903.—"Ueber die Hämoglobinurie der Rinder in Deutschland," 'Arbeit. a. d. Kaiserl. Gesundheitsamte,' Bd. xx, 1, 77 pp., 3 pls.

22. Laveran, A. 1901.—"Essai de classification des Hématozoaires endo-globulaires," 'C. R. Soc. Biol.,' liii, p. 798.

23. Laveran, A. 1905.—Hæmocytozoa, essai de classification," 'Bull. Inst. Pasteur,' iii, No. 20.

24. Laveran, A. 1901.—"Contribution à l'étude de Piroplasma equi," 'C. R. Soc. Biol.,' liii, p. 385.

25. Laveran, A. 1903.—"Sur la Piroplasmose bovine bacilliforme," 'C. R. Ac. Sci.,' cxxxvi, pp. 648-53, 18 text-figs.

26. Laveran, A. 1903.—"Au sujet du rôle des tiques dans la propagation des piroplasmoses," 'C. R. Soc. Biol.,' lv, pp. 61-63.

27. Laveran, A., and Mesnil, F. 1903.—"Sur un Protozoaire nouveau (Piroplasma donovani, Lav. et Mesn.) parasite d'une fièvre de l'Inde," 'C. R. Ac. Sci.,' cxxxvii, pp. 957-61, 17 text-figs.

28. Laveran, A., and Mesnil, F. 1904.—"Nouvelle observations sur Piroplasma donovani, Lav. et Mesn.," 'C. R. Ac. Sci.,' cxxxviii, pp. 187-9.

29. Laveran, A., and Nicolle, M. 1899.—"Contribution à l'étude de Pyrosoma bigeminum," 'C. R. Soc. Biol.,' li, pp. 748-51, 15 figs.

30. Lignières, J. 1901.—"Sur la 'Tristeza,'" 'Ann. Inst. Pasteur,' xv, pp. 121-8, pl. 6.

31. Lignières, J. 1903.—"La piroplasmose bovine," 'Arch. Parasit.,' vii, pp. 398-407, pl. 4.

32. Lingard, A. 1904.—"Can the 'Piroplasma bigeminum' find a habitat in the human subject?" 'Centrbl. Bakter.' (1), xxxvi, orig., pp. 214-16, 1 pl.

33. Lingard, A., and Jennings, E. 1904.—"A Preliminary Note on a Pyroplasmosis found in Man and in some of the Lower Animals," 'Ind. Med. Gaz.,' xxxix, pp. 161-5, 3 pls.

33a. Lühe, M. 1906.—"Die in Blute schmarotzenden Protozoen und ihre nächsten Verwandten," in Mense's 'Handbuch der Tropenkrankheiten,' Leipzig, Bd. iii, "Babesia," pp. 193-202, Taf. 8.

33b. Lühe, M. 1906.—"Zur Kenntnis von Bau und Entwicklung der Babesien," 'Zool. Anzeiger,' xxx, Nr. 1, pp. 45-52.

34. Marchoux, E. 1900.—"Piroplasma canis (Lav.) chez les chiens du Sénegal," 'C. R. Soc. Biol.,' lii, pp. 97-8.

35. Mesnil, F., Nicolle, F., and Remlinger, P. 1904.—"Sur le Protozoaire du bouton d'Alep," 'C. R. Soc. Biol.,' lvii, pp. 167-9.

36. Mettam, A. E. 1905.—"A Note on Bovine Piroplasmosis," 'Journ. Hygiene,' v, pp. 271-3.

37. Minchin, E. A. 1903.—"Sporozoa," in Lankester's 'Treatise on Zoology,' pt. i, fasc. 2, pp. 150-360.

38. Motas. 1903.—"Sur le rôle des Tiques dans le développement de la piroplasmose ovine (Carceag)," 'C. R. Soc. Biol.,' lv, pp. 501-3.

38a. Motas. 1902.—"La piroplasmose ovine, 'Carceag,'" 'C. R. Soc. Biol.,' liv, pp. 1522-4.

39. Nocard and Motas. 1902.—"Contribution à l'étude de la Piroplasmose canine," 'Ann. Inst. Pasteur,' xvi, pp. 256-90, pls. v, vi.

40. Nuttall, G. H. F. 1904.—"Canine Piroplasmosis, I," 'Journ. Hygiene,' iv, pp. 219-52, 2 pls.

41. Nuttall, G. H. F., and Graham-Smith, G. S. 1905.—"Canine Piroplasmosis, II," 'Journ. Hygiene,' v, pp. 237-49, with pl. 9.

42. Rogers, L. 1904.—"On the Development of Flagellated Organisms (Trypanosomes) from the Spleen Protozoic Parasites of Cachexial Fevers and Kala-azar," 'Quart. Journ. Micr. Sci.,' 48, pp. 367-77, pl. 25.

43. Rogers, L. 1906.—"Further Work on the Development of the Hepatomonas [Herpetomonas] of Kala-azar and Cachexial Fever from Leishman-Donovan Bodies," 'Proc. Roy. Soc.,' 77, B. 517, pp. 284-93, pl. 7.

44. Ross, P. H. 1905.—"A Note on the Natural Occurrence of Piroplasmosis in the Monkey (Cercopithecus), " 'Journ. Hygiene,' v, pp. 18-23, 3 charts.

45. Ross, R. 1903.—"A New Parasite of Man (Leishmania)," 'Thompson Yates Lab. Rep.,' 5, ii, pp. 79-82, 1 pl.

46. Smith, Th., and Kilborne, F. 1893.—"Investigations into the Nature, Causation, and Prevention of Texas or Southern Cattle Fever," '8th and 9th Ann. Rep., Bureau of Anim. Industry,' pp. 177-304, with 10 pls. Washington, U.S.A.

47. Stephens, J., and Christophers, S. R. 1904.—"The Practical Study of Malaria and other Blood Parasites," 2nd edit.; "Piroplasma," pp. 332-7, pl. 3; "Ticks," pp. 337-49.

48. Theiler, A. 1904.—"The Piroplasma bigeminum of the Immune Ox," 'Journ. Roy. Army Med. Corps,' iii, pp. 469-88.

49. Theiler, A. 1904.—"East Coast Fever," 'Journ. Roy. Army Med. Corps,' Dec., 1904, 22 pp.

50. Wilson, L. B., and Chowning, W. M. 1904.—"Studies in Pyroplamosis hominis ('Spotted Fever' or 'Tick Fever' of the Rocky Mountains)," 'Journ. Infect. Diseases,' i, pp. 31-57, 2 pls.

51. Wright, J. A. 1905.—"Canine Piroplasmosis: IV. On certain Changes in the Blood," 'Journ. Hygiene,' v, pp. 268-70, 3 charts.

52. Wright, J. H. 1903.—"Protozoa in a case of Tropical Ulcer ('Delhi sore')," 'Journ. Med. Research, Boston,' x, pp. 472-82, 4 pls.

53. Ziemann. 1903.—"Vorläufiger Bericht über das Vorkommen des Texasfiebers der Rinder in Kamerun und weiteres über die Tsetsekrankheit sowie über die 'Tiermalaria,'" 'Deutsch. med. Wochenschr.,' Jahrg. 29, Nr. 16, pp. 289, 290.

IX. EXPLANATION OF PLATE 28,

Illustrating Mr. H. B. Fantham's paper on "Piroplasma muris, Fant., from the Blood of the White Rat, with Remarks on the Genus Piroplasma."

The figures were all carefully outlined with camera lucida, under Zeiss' 3 mm. homog. immersion lens, apert. 1·40, and compensating ocular 18 (except in the case of Fig. 23).

The scheme of colouring adopted is approximately that of Leishman's stain, within the limits of the two colours used, blue and pink. Other modifications of the Romanowsky method used have, for the sake of uniformity and simplicity, been also thus represented, the tint of the cytoplasm of the erythrocyte only needing to be sometimes modified in such cases.

The magnification is in all cases approximately 1950 diameters, except where otherwise stated.

Fig. 1.—Pyriform trophozoite, young, with peripheral chromatin and vacuole.

Fig. 2.—Typical pear-shaped trophozoite.

Fig. 3.—Ovoid trophozoite.

Fig. 4.—Pyriform parasite with two chromatic dots.

Fig. 5.—Trophozoite ("schizont") in process of longitudinal division, with chromatin bodies at the poles and well-marked vacuole.

Fig. 6.—Typical longitudinal fission of parasite in red blood corpuscle.

Fig. 7.—Two daughter trophozoites ("merozoites"), bigeminate.

Fig. 8.—Two pear-shaped parasites, still connected by a thin strand of protoplasm at their pointed ends.

Fig. 9.—Two ovoid forms, probably resulting from a simple binary fission of the parent parasite.

Fig. 10.—Two pairs of parasites in a red blood corpuscle, the pairs lying partly over each other. The members of the pairs are still connected, though at different stages of separation. This is probably a case of double infection of the blood-corpuscle host.

Fig. 11.—Three intra-corpuscular parasites; possibly a fourth behind the heart-shaped smaller pair. Two chromatin bodies occur in each of the members of the heart-shaped pair.

Fig. 12.—Three small parasites in a small blood corpuscle from the spleen.

Fig. 13.—Six intra-corpuscular parasites inside a corpuscle from the spleen.

Fig. 14.—Six "amœboid" parasites from the spleen.

Fig. 15.—Pear-shaped trophozoite, with somewhat pointed apex at the broader end, and two chromatin bodies, from tail blood.

Fig. 16.—Gregariniform trophozoite with rod-like, drawn out chromatin body, perhaps preparing for division.

Fig. 17.—Rather large, somewhat spherical trophozoite, with chromatin body lying in clear zone of protoplasm. From spleen blood.

Fig. 18.—"Amœboid" trophozoite, with a single pseudopodium in which lies a chromatin dot. From spleen blood.

Fig. 19.—Pyriform parasite with chromatic appendage still protruding from the erythrocyte. Probably a "flagellate" form, but no bulb or bead seen on the appendage. This may be a somewhat abnormal form of parasite, as no other similar one was observed, though it was quite distinct.

Fig. 20.—Bigeminate pair of free parasites from tail blood.

Fig. 21.—Group of free parasites in the blood plasma.

Fig. 22.—Leucocyte probably containing the remains of degenerate, infected blood-corpuscles, or remains of free parasites. Only the red chromatin masses of the parasites are left.

Fig. 23.—Portion of section of liver of infected white rat, showing dilated capillary containing many infected red blood-corpuscles and several (three) leucocytes, with large nuclei. × 1000 approx., somewhat diagrammatic.

THE MARINE BIOLOGICAL ASSOCIATION

OF THE

UNITED KINGDOM.

——:o:——

Patron—HIS MAJESTY THE KING.

President—Prof. E. RAY LANKESTER, LL.D., F.R.S.

——:o:——

THE ASSOCIATION WAS FOUNDED "TO ESTABLISH AND MAINTAIN LABORATORIES ON THE COAST OF THE UNITED KINGDOM, WHERE ACCURATE RESEARCHES MAY BE CARRIED ON, LEADING TO THE IMPROVEMENT OF ZOOLOGICAL AND BOTANICAL SCIENCE, AND TO AN INCREASE OF OUR KNOWLEDGE AS REGARDS THE FOOD, LIFE CONDITIONS, AND HABITS OF BRITISH FOOD-FISHES AND MOLLUSCS."

The Laboratory at Plymouth

was opened in 1888. Since that time investigations, practical and scientific, have been constantly pursued by naturalists appointed by the Association, as well as by those from England and abroad who have carried on independent researches.

Naturalists desiring to work at the Laboratory

should communicate with the Director, who will supply all information as to terms, etc.

Works published by the Association

include the following:—'A Treatise on the Common Sole,' J. T. Cunningham, M.A., 4to, 25/-. 'The Natural History of the Marketable Marine Fishes of the British Islands,' J. T. Cunningham, M.A., 7/6 net (published for the Association by Messrs. Macmillan & Co.).

The Journal of the Marine Biological Association

is issued half-yearly, price 3/6 each number.

In addition to these publications, the results of work done in the Laboratory are recorded in the 'Quarterly Journal of Microscopical Science,' and in other scientific journals, British and foreign.

Specimens of Marine Animals and Plants,

both living and preserved, according to the best methods, are supplied to the principal British Laboratories and Museums. Detailed price lists will be forwarded on application.

TERMS OF MEMBERSHIP.

ANNUAL MEMBERS	£1	1	0	per annum.
LIFE MEMBERS	15	15	0	Composition Fee.
FOUNDERS	100	0	0	„ „
GOVERNORS (Life Members of Council)	500	0	0	

Members have the following rights and privileges:—They elect annually the Officers and Council; they receive the *Journal* free by post; they are admitted to view the Laboratory at any time, and may introduce friends with them; they have the first claim to rent a table in the Laboratory for research, with use of tanks, boats, etc.; and have access to the Library at Plymouth. Special privileges are granted to Governors, Founders, and Life Members.

Persons desirous of becoming members, or of obtaining any information with regard to the Association, should communicate with—

The DIRECTOR,
The Laboratory,
Plymouth.

New Series, No. 200 (Vol. 50, Part 4). **Price 10s. net.**

Subscription per volume (of 4 parts) 40s. net.

NOVEMBER, 1906.

THE QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE.

EDITED BY

E. RAY LANKESTER, M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND OF THE ACADEMY OF THE LINCEI OF ROME, AND OF THE AMERICAN ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE ROYAL ACADEMY OF BELGIUM; HONORARY MEMBER OF THE NEW YORK ACADEMY OF SCIENCES, AND OF THE CAMBRIDGE PHILOSOPHICAL SOCIETY, AND OF THE ROYAL PHYSICAL SOCIETY OF EDINBURGH, AND OF THE BIOLOGICAL SOCIETY OF PARIS, AND OF THE CALIFORNIA ACADEMY OF SCIENCES OF SAN FRANCISCO; FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE AMERICAN PHILOSOPHICAL SOCIETY;
DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE FULLERIAN PROFESSOR OF PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN;
LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

FELLOW AND TUTOR OF TRINITY COLLEGE, CAMBRIDGE;

AND

SYDNEY J. HICKSON, M.A., F.R.S.,

BEYER PROFESSOR OF ZOOLOGY IN THE OWENS COLLEGE, MANCHESTER.

WITH LITHOGRAPHIC PLATES AND TEXT-FIGURES.

LONDON:
J. & A. CHURCHILL, 7 GREAT MARLBOROUGH STREET.
1906.

Adlard and Son, Impr.,] [London and Dorking.

CONTENTS OF No. 200.—New Series.

MEMOIRS:

With Title, Contents, and Index to Vol. 50.

On the Structure of the Nephridia of Dinophilus.

By

Cresswell Shearer,

Trinity College, Cambridge.

With Plates 29 and 30.

Schmidt (**22**) was the first to draw attention to the nephridia of Dinophilus in 1848, which he simply mentions in D. vorticoides as two longitudinal vessels. They seem to have escaped further attention till Korschelt (**11**) in 1882 again mentioned their presence in D. apatris. It is to Harmer (**8**), however, that we owe the most complete description of the nephridia. In 1889 he described five pairs in D. tæniatus, a species found abundantly in the tidal pools of Plymouth Bay. Since then they have been studied by Schimkewitsch (**23**) in D. vorticoides from the White Sea. This last author investigated them by means of the intra vitam method of staining with methylene blue, but was able to add little to Harmer's account of their structure.

I have long suspected from both Harmer's and Schimkewitsch's descriptions that the nephridia of Dinophilus would finally prove to be closed internally by flame-cells similar to those Goodrich (**5**) has described under the name of solenocytes, in certain Polychæts, Amphioxus, and the Actinotrocha larva of Phoronis. Only during the present season, however, have I been able to verify this, and to definitely determine that they are furnished with typical solenocytes.

This is, I think, a point of some morphological interest.

It not only adds a very primitive Annelid form to the already large class of animals possessing nephridia of this primitive type, but the presence of solenocytes on all four or five pairs of nephridia in Dinophilus shows, I think, that in the primitive Annelids not only the head-kidney and the immediately following segments, as in Polygordius, were furnished with solenocytes, but the nephridia of all the segments; and that while in some Annelids they have been retained on the nephridia of most of the segments, others have lost them or retained them only on the nephridia of the anterior segments. Moreover, the presence of solenocytes in Dinophilus is of interest on account of the many relationships this group shows with that of the Platyhelminths. Weldon (**24**) has pointed out the similarity of the muscular œsophageal appendage of Dinophilus to the pharynx of Planarians, and Harmer (**8**) has already called attention to the median position of the generative pore and the method of fertilisation of the male D. tæniatus as showing a certain affinity to Platyhelminths. The crawling swimming movements of Dinophilus and its manner of feeding is also suggestive of some relationship to this group. As will be seen from consulting figs. 12, 13, and 15 of the present paper the ventral surface of Dinophilus is definitely thickened into a crawling pad as in the Turbellaria.

The resemblance of the nephridial canals of Dinophilus to the excretory canal of the flame-cells of Thysanozoon is unmistakable; with the exception that one is furnished with solenocytes and the other is not, there is little difference between the two. The granular walls of the organ graduating into a delicate tube-like canal is found in each, and their whole appearance is remarkably the same in both cases. From this resemblance I think it is not impossible to look for the discovery of solenocytes in some of the higher Platyhelminths. Their discovery in this class will then definitely establish the homology of Platyhelminth protonephridia with the solenocyte-bearing nephridia of Annelids and the kidneys of Amphioxus. It may then be possible to trace a

consecutive series of changes in the form of the nephridia from the more primitive flame-cells of the Turbellaria to the complicated organs of Polychæts, and so establish a definite connection between the nephridia of these two groups, which at present would seem to be separated distinctly from one another.

Although I made a careful search for Dinophilus at Plymouth during the latter part of June, 1903, the season was too late, and the adults had already disappeared from their usual haunts. Until the present season I have had no opportunity of revisiting Plymouth. This year, however, no difficulty was experienced in obtaining an abundant supply of material, although limited to the male form. Towards the latter part of April I examined a considerable quantity of Dinophilus material daily without observing a single female. This apparent absence of the female I am unable to account for, except on the ground that the breeding season may have been passed, as young worms of all sizes were observed in numbers, and the males seemed to have already discharged their spermatozoa.[1] Both sexes disappear entirely at Plymouth, I believe, towards the end of May or the first part of June. During July and August they are not to be found at Plymouth.

This disappearance of Dinophilus during certain months has already been noticed by Hallez (**7**), Weldon (**24**), and Harmer (**8**). Weldon (**24**) observed the adults of D. gigas in April undergoing degeneration after the discharge of the sexual products, and concluded that the worms periodically died off after the breeding season. That this can hardly be the case has been shown by Schimkewitsch (**23**) in D. vorticoides, where the females continue to live long after they have laid their eggs, and in fact pass through several breeding periods during the course of the year. In an American species Moore (**17**) has found that the worms have the power of forming capsules and of encysting themselves. On being

[1] Harmer (**8**) states that about April 18th the females were still carrying their eggs.

placed in small watch-glasses of sea water the worms after a short time throw out a considerable quantity of mucus, which hardens about them forming a firm capsule, coiled up inside of which the worm can be plainly seen. The capsule soon loses its bright red colour, when it is quite indistinguishable against any small piece of alga to which it may be attached. This year towards the end of the season I noticed a number of males settling on small pieces of sea-weed and coiling up; they remained in this position for several days, when they seemed to have secreted a certain amount of mucus about themselves. These worms may possibly have been undergoing encystment; I was unable, however, to keep them long enough to determine their ultimate fate. As the worm during the encapsulated condition easily escapes observation as the result of its minute size and lack of colour, it can readily be understood how this encystment might account for their periodical disappearance. The mucus thrown out by the worm is evidently derived from the clear large cells that have been frequently noticed in the epidermis. These cells answer to all the usual staining reactions for mucus. They recall similar cells seen in a large number of Turbellaria, and this power of throwing out mucus to form a capsule is perhaps another point of resemblance between the two groups. In Histriobdella,[1] a form closely related to Dinophilus, and usually classed with it in the group of the Archiannelida, I have observed that the female in laying its eggs surrounds each egg with a mass of mucus which subsequently hardens about it, securely fastening it in a stalked capsule to the eggs of the lobster on which this animal lives parasitically. Inside this capsule the embryo undergoes its entire development, only emerging in the adult state. Among the Turbellaria a number of forms also lay their eggs in somewhat similar fashion, as, for instance, Leptoplana, Plagiostomum,[2] and numerous parasitic forms.

[1] I follow Haswell (9) in retaining the name Histriobdella instead of Foettinger's (4) modification Histriodrilus.

[2] See Bresslau (2), figs. 73 and 74.

Another point of resemblance between these groups is that in the majority of marine and fresh-water Turbellaria development is simple and direct, as in Dinophilus and Histriobdella. While it is true other members of the Archiannelida, as Protodrilus, Polygordius, and Saccocirrus, may possess larval stages, these forms are more nearly related to the Polychætæ than to either Dinophilus[1] or Histriobdella. Saccocirrus is undoubtedly a Polychæt, and can hardly be considered at all an Archiannelid, as Goodrich (**5**) has shown. Protodrilus is also evidently closely related to the same class, for young larvæ I obtained at Naples in 1903 plainly showed the presence of a well-marked ciliated ring, and in external appearance bear a certain resemblance to young Nereis larvæ.[2] The larval form of Polygordius is of course well known. Moreover, Haswell (**9**), in a paper on Histriobdella, has advanced strong reasons for including Dinophilus and Histriobdella in one class separate from that of Polygordius and Protodrilus. The great difference of metamerism in the two cases, the head segments, the relation of the brain commissure to the mouth, and the great difference of the reproductive organs in the two groups, shows that their affinity is remote. On the other hand, Dinophilus and Histriobdella show more relationship with one another in the possession of a distinct head, a nervous system consisting of a metamerically arranged series of ventral ganglia, an alimentary canal essentially similar in both forms, and a close resemblance in the reproductive organs, especially in the male. I agree therefore with Haswell in grouping Histriobdella and Dinophilus in one class separate from Polygordius, Protodrilus, and possibly Ctenodrilus. Regarding this last it is hard to say anything until something has been determined of its life history, as so far it has

[1] Nelson (**19**) claims that Dinophilus shows a remarkable resemblance in its cleavage stages to Polychæts, and thinks that in this respect it cannot be considered a primitive form.

[2] See Pierantoni's (**20**) figs. 1 *a* and 1 *b*.

only been observed reproducing (Zeppelin **27**) by fission. Possibly Ctenodrilus may be intermediate in position between Polygordius and Dinophilus, but the possession of distinct setæ sacs would seem to denote that it is a degenerate Polychæt. Eisig (**3**) has also expressed the opinion that Histriobdella is a very degenerate form and not an Archiannelid, and it is possible that all the members of this class are after all very degenerate forms, in which their simple structure is by no means primitive. This would seem to be borne out by a number of features in their development. In Dinophilus and Histriobdella, certainly the most interesting forms of this group, the direct mode of development affords unfortunately little evidence of their affinity, and what can be obtained from this source seems to point to their being degenerate forms, as Eisig holds to be the case.

I. General Description of the Nephridia.

The nephridia of Dinophilus can be satisfactorily examined by placing the living worm on a glass slide and covering it with a cover-glass to hold it and prevent excessive movement. The most convenient method of doing this is to support the cover-glass by a little soft wax which prevents the worms from being crushed too much, but at the same time allowing of their being compressed as required. The structure of the nephridia can then be readily investigated under an oil-immersion lens. As the nephridia frequently run through the testis, which forms the largest part of the body tissue in the male (see fig. 14) it is sometimes necessary to examine the preparation from both sides in order to follow a particular nephridium throughout its entire course. For this reason I soon discarded ordinary glass slides for making my preparations, and adopted big cover-glasses such as those used for making large embryological slides; the thinness of the glass allows of the preparation being focussed under high power lenses from either side as required.

The normal orange pigment of the worm gives a yellow tone to the light passing through the preparation when placed under the microscope, which rendered the finer details of the structure of the nephridia more visible; the outlines of the transparent solenocytes being more clearly defined in a yellow than in a pure white light.[1] For the study of the head-kidney of transparent Trochophore larvæ I have found the employment of a yellow glass screen most useful, and in Dinophilus the yellow pigment serves much the same purpose.

Although I placed the worms in various solutions of methylene blue and indigo-carmine I never succeeded in getting the nephridia to take up these colours, as they readily do in the White Sea species. While the nervous system and the sensory cells of the epidermis absorb the colour by the methylene blue method of impregnation, the nephridia remain entirely unstained.

Lang has remarked on the difficulty of obtaining good preparations of the flame-cells of Platodes with living material on account of the inhibiting action of the cover-glass on the flagella of the flame-cells. Much the same difficulty is experienced with Dinophilus; as soon as the worms are compressed the action of the flagella in the nephridial canals is at once stopped, being only resumed some time afterwards, and then in only one or two of the nephridia at the most. It is impossible to get a preparation in which all the nephridia are to be seen at once active; sometimes it will be the first, sometimes it will be the third, but rarely two consecutive nephridia are seen active, and it is thus difficult to determine accurately the relations of one nephridium to another when they have to be observed from different preparations. This is especially so with regard to the terminal portion of the nephridium, where it is a canal so delicate as to be almost indistinguishable except under the most favourable conditions.

[1] Although blue violet light has greater resolving power than yellow light, it remains a fact nevertheless, that the solenocytes are more readily seen in a yellow light.

As the following observations apply only to the male worm, I have never observed the fifth nephridium which, according to Harmer (**8**), is present in the female. Schimkewitsch (**23**) has confirmed this observation on the White Sea species, so there can be no doubt of the presence of this fifth pair in the female. Nelson (**19**), in an American species obtained like Korschelt's (**11**) from an aquarium tank, has been unable to find any trace of the nephridia; this is the more remarkable on account of their almost constant presence in other species. They can so readily be observed in D. tæniatus that it seems almost impossible they could have been overlooked if present in this American species. In D. gyrociliatus,[1] D. vorticoides and D. tæniatus there are five pairs of nephridia in the female, and this would seem to bear out Harmer's suggestion that possibly the body in the genus Dinophilus is composed of five metameres. What we know of the disappearance of nephridia and the apparent ease with which they can be dropped from various segments, as, for instance, in the anterior segments of the Arenicola larva, the presence of more than one pair to a segment, as in Capitella, and their very erratic behaviour in the development of Oligochæts, renders it doubtful whether they offer trustworthy evidence as an index to the number of segments, and their total absence may possibly exemplify this in the case of the American species of Dinophilus. Again, Ctenodrilus, a form usually classed with the Archiannelids, possesses only one pair of nephridia although the animal is plainly divided at least into seven metameres.[2] On the other hand, in Histriobdella, according to Foettinger (**4**), there are five pairs in the female as in Dinophilus. Harmer has emphasised this as indicating another point of relationship between them. I have, however, re-examined the nephridia in Foettinger's

[1] D. gyrociliatus according to Repiachoff (**21**) is identical with D. apatris of Korschelt's (**11**) paper.

[2] I have confirmed Kennel's (**10**) and Zeppelin's (**27**) observations on a species of Ctenodrilus found at Naples, and there is only one pair of these structures present.

species, and I cannot find more than four pairs in the female.[1] It is certain their number is not five, as in Dinophilus. Haswell (**9**), in Stratiodrilus, which is almost identical with Histriobdella, finds four nephridia in the female. It is plain in Dinophilus, the number of nephridia are remarkably constant, while the same cannot be said of the number of metameres.

As already stated the nephridia in the male consist of four pairs placed very much in the positions Harmer has indicated —that is, the head of the first nephridium is very slightly behind the second pre-oral ring of cilia on a level with the anterior margin of the muscular pharynx organ, the second at the anterior end of the stomach, the third about the middle of the stomach, while the fourth lies at the posterior end of the stomach close at its junction with the intestine. Their ducts run outwards and round the segments to open on the ventral surface not very far from the median line, not in the segment to which they belong as stated by Harmer (**8**), but in the subsequent following segment as in Annelids. This point is difficult to exactly determine as the field of vision is so limited under an oil immersion objective (with which it is necessary to examine this terminal part of the canal), that it is thus hard to tell where the segment septa lie in relation to the end of the nephridial canals. A number of successful preparations, however, seemed to clearly show the canals running some distance backwards into the next segment before ending under the epidermis, and I think I am quite right in saying that the canals end in the segment following that to which they belong, as in Annelids. The closed internal ends of the canals bearing the solenocytes project slightly into the irregular space surrounding the gut—the so-called body-cavity (fig. 17). This space is of variable dimensions, being lined by no distinguishable membrane, and is traversed in many

[1] As in Dinophilus they project into the so-called body-cavity where they appear to end as a single flame-cell. In every respect they resemble the structure of the head-kidney of the Trochophore larva, the whole organ of which is quite comparable to a single very enlarged solenocyte.

directions by irregular strands and muscular fibres. About the gut it forms a sort of sinus in which a few large orange-brown granules are constantly seen (see fig. 3, *e.gr.*); these move up and down it from one end of the stomach to the other as the worm twists and bends. Roughly, the contour of the space follows the general form of the external segmentation, sending prolongations towards the surface at the end of each segment, which augment the marked pseudo-metameric appearance of the animal when seen under the microscope. That this space cannot be strictly regarded as a cœlomic cavity is amply testified by the fact that the spermatozoa are seen enclosed in an entirely different set of spaces lined with a definite membrane, by no chance ever being seen in this space or any of its numerous prolongations into the general mass of the testis tissue (fig. 4, *sp.m.*). Also by the fact that it is traversed in all directions as already mentioned by numerous muscle-strands and cells which denote its primitive blastocœlic nature. It seems to me this space is directly comparable to the great blastocœlic cavity of the head-segment of the Polygordius larva, into which the larval nephridia project with their solenocytes, but with which their canals do not communicate, and to the blastocœlic collar space of the Actinotrocha larva, into which their larval organs project under similar conditions; the space that subsequently gives rise to the circular blood-vessel ring of the adult in this animal.

In Dinophilus this cavity sends two prolongations from the corners of the stomach forward into the head region, these run outward to terminate in two small triangular enlargements beneath the skin a short distance behind the eyes (fig. 14, *blc.*1). These spaces are shown partially on either side of the œsophagus (fig. 15). Into these the heads of the first pair of nephridia bearing the solenocytes project, the solenocytes standing out on the end of the nephridial canal into the lumen of the space like bristles from a brush (fig. 1). The protoplasmic parts of the solenocytes are but imperfectly seen on account of their transparent nature, and their presence

is only shown by the refractive tubes which make them look not unlike a number of pins inserted on the ends of the nephridial canals. With proper lighting, however, and the use of a coloured glass screen their actual outline is brought into view, when they are seen to be pear-shaped bodies inserted on the end of the blind nephridial canal, being attached by a delicate hyaline tube piercing the nephridial wall, each tube having a long flagellum in its interior which passes into the lumen of the nephridial canal and down almost the whole length of this canal (fig. 2). I can find no evidence of the canal itself being ciliated as Harmer (**8**) has described. On the first nephridium I have counted at least thirty solenocytes, but their number is possibly double this as it is impossible to accurately count them on account of the active movements of the worm.

Collectively they form the "triangular body" or "ciliated appendage" mentioned by Harmer (**8**) as being inserted into the distal extremity of the nephridial canal. They by no means form really a triangular body, as they can be frequently seen to be spread out fan-like, and to be composed of a large number of separate solenocytes. As the preparation is compressed, however, sometimes the solenocytes become separated into two distinct masses, and this would seem to happen more frequently in the case of the first nephridium than with any of the others (fig. 1). This appearance has undoubtedly given rise to Harmer's (**8**) supposition that the ciliated appendage is sometimes bifid, that this condition is merely artificial being temporarily assumed, and that the solenocytes form a single mass on the end of the canal can be easily ascertained with a little careful examination. The space into which these structures project, like the main portion surrounding the gut, usually contains a few small brown granules. These, during the movements of the animal under compression are frequently forced in among the solenocytes, where they lodge as if stuck amongst the hairs of a brush. Sometimes they are seen to be forced directly against the end of the nephridial canal of the point where the solenocytes are

attached, and from the fact that they are never seen to enter the canal itself, being soon swept free again into the general space of the cavity, demonstrates that the end of the canal is not open. And this is also borne out by the appearance of the end of the canal itself, which shows no trace of any such opening. A drawing is shown in fig. 1 of the first nephridium under compression, in which the solenocytes are seen divided into these two groups as just mentioned.

Under normal conditions the organ is not so spread out as shown in this figure. The head of the nephridium projects into the body-cavity space so as to look outwards and backwards, lying remarkably close under the epidermis. If for convenience of description we follow Harmer (**8**) in dividing the nephridium into three portions; then the solenocyte body being the first part, the large thick-walled part of the canal marked nep. c. 2 in the figure will form the second part, while the fine delicate duct into which this rapidly passes composes the third part (nep. c. 3).

The second portion of the first nephridium is shorter and less developed than the corresponding section in other nephridia. It reaches its greatest development in the third nephridium. Its walls quickly widen out, and are not so granular or marked with orange pigment as in the case of the following organs. These granules are so placed as often to give it the appearance of being composed of numerous cells: of being an inter-cellular instead of an intra-cellular canal. This condition is again more marked in the case of the third nephridium. The third portion of the nephridium is remarkable for its uniform diameter, which remains the same till it almost reaches its point of termination, where it then narrows down to a very fine duct. It forms by far the longest portion of the nephridium, running backwards and ventralwards close under the skin, to terminate in the basement membrane of the epidermis close to a conspicuous vacuole just over the line that marks the anterior border of the following segment (fig. 9). In sections of fixed material I have been unable to trace the course of the nephridial

canals. Schimkewitsch (**23**) shows these in some of his transverse sections. I have, however, never been successful in seeing them in sections. In sections like those shown in figs. 12—15, taken from material treated with cocaine and carefully fixed in Hermann's fluid, and afterwards treated with Zenker's fluid, no trace of the nephridial canals could be found.

II. The First Nephridium.

The position and general course of the canal of the first nephridium has been very correctly given by Harmer (**8**), indicated in his figure (Pl. 10, fig. 15). The main portion of the nephridium lies in a plane slightly dorsal to the wall of the pharynx when the animal is flattened slightly. The testis extending into the head region is pierced by the canal which terminates ventrally to it, but probably some distance lateral to the median line. Throughout its course it is seldom seen to undergo any variation in size during the movements and contractions of the worm, which suggests that its walls are composed of some fairly firm substance, which does not allow of the canal being readily compressed and the lumen of the canal obliterated. In none of the nephridia can any trace of an actual opening of the canal on the surface be seen, and in all cases they would appear to terminate at the basement membrane of the epidermis. The closest examination fails to reveal any traces of an external pore. The point at which the canal terminates, and up to which the flagella in its interior can be traced, is but a very short distance from the surface, careful measurement showing it to be less than ·004 of a mm., yet the external surface is perfectly intact. The terminal point of the nephridium is well shown in fig. 7, which represents a portion of the margin of a preparation under high magnification. The canal is plainly seen to end at the limiting membrane of the epidermis opposite a large vacuole into which sometimes the ends of the flagella are seen beating, not, however, under normal condi-

tions, but only when the preparation has been pressed considerably out of shape. In calling these clear spaces vacuoles I do so through lack of a more suitable term; they are in all probability filled with fluid, as in preserved material they appear (as in fig. 13) as a series of narrow chinks, while in the living state they are seen as large refractive spaces as shown in fig. 7. Böhmig (**1**) in Triclada maricola shows the excretory canals terminating in the basement membrane beneath the epidermis, much as they do in Dinophilus. In his fig. 19, Pl. 20, he shows this very clearly, and this figure might very well do to illustrate the condition in Dinophilus. In this Triclad the epidermis also contains numerous clear vacuoles, the walls of which lie close against the terminal point of the excretory canals. In spite of much time devoted to this point in Dinophilus I have been quite unable to determine anything further regarding the relationships of the nephridial canals to the vacuoles or whether these vacuoles in turn open on the exterior. This point is of some interest in connection with Korschelt's (**11**) observation of the probable presence of a fine system of anastomosing canals in the basement membrane with which the nephridial canals may possibly connect, although he could not succeed in D. apatris in establishing any such connection. This is well shown in Korschelt's (**11**) fig. 29, but no similar system is to be found in the case of D. tæniatus. Korschelt points out the resemblance of this network to the system of canals into which the ducts of the flame-cells of Polyclads open. These in turn open on the exterior in two dorsal pores. By certain improper focussing, however, of the muscle-strands which run irregularly throughout the body, an appearance is obtained of a series of anastomosing canals looking not unlike the picture shown in Korschelt's (**11**) fig. 29, and possibly it is this Korschelt has taken for a system of canals; that this network is really made up of anastomosing muscle-fibres can readily be determined by proper focussing. When it is considered how notoriously difficult it is to see the external openings of the ducts of the head-kidney in the Polygordius larva, the

dorsal pores of Polyclads, and also the openings of the larval excretory organs of the Actinotrocha larva, it may be claimed justly that I have adduced, perhaps, no conclusive evidence for their absence in Dinophilus, to this I can only say that after prolonged investigation of this point I can find no evident traces of their presence. It is, perhaps, worthy of note that Schimkewitsch (**23**) in the White Sea species also can find no evidence of external openings to the nephridia by the methylene-blue method of impregnation.

In several preparations the canals could be traced down to these vacuoles which appeared closed, but careful examination of the external surface over the vacuole seemed to show the presence of a slight depression. In this depression a number of granules were adherent to the external surface, these moved backwards and forwards as if disturbed by the escape of fluid from the interior of the vacuole. Frequently they were noticed to have a peculiar dancing movement. I could never see distinct evidence, however, of the escape of any fluid, and the granules were themselves never seen to be detached or actually washed from their places as one might suppose would take place if any fluid was being discharged from the vacuole. Close examination of the surface of the vacuole when the movement of the granules was most evident failed also to reveal traces of any external pore. In some cases the granules seemed to dance round one particular point in the middle of the surface of the vacuole, but close examination of this under a Zeiss 2 mm. oil-immersion objective combined with 12 m. and 18 m. compensating ocular, with careful lighting, failed to show the presence of any opening. I am inclined to think the fluid escapes from the vacuole by osmosis through its wall, and this escape of fluid accounts for the movement of the granules which themselves may be of an excretory nature. They are frequently orange coloured like the granules of the blastocœlic space, though very much smaller, and are probably deposited on the external surface from the slowly excreted fluid of the vacuole as it reaches the exterior.

The solenocytes of the first nephridium are quite like those of the following nephridia in appearance, although a number of observations have led me to believe that they are somewhat finer and more pin-head-shaped in outline. Unlike the solenocytes of Polychæts, it is difficult to see the lumen of their solenocyte tubes, although their flagella can be traced a little way up the narrow refractive stalk which attaches the solenocyte to the end of the nephridial canal; neither can the end of this tube be seen projecting into the nephridial canal as in some Polychæts. In the head of each solenocyte is a clear refractive dot; this is so regular in shape and size as to preclude its being a nucleus. Each solenocyte sends a single flagellum down the canal of the nephridium, so that the accumulated flagella of all the solenocytes form a mass which almost fills the lumen of the canal. The waves of ciliary motion starting in the solenocytes travel progressively down to the ends of their flagella. As already mentioned, I believe each flagellum extends the length of the nephridial canal (fig. 6). In cases when the ciliary motion has almost ceased and the movements of the flagella are consequently slow, the wall of the canal is exposed from time to time as the flagella move from side to side; it is then seen to be bare without any trace of the insertion of cilia (fig. 5). At the same time the individual lashes of the flagellum can be distinctly seen, and in some instances traced to their respective solenocytes. As each lash beats backwards and forwards in the canal it causes its solenocyte to vibrate with it in the body-cavity space, and as the waves of motion pass down the flagellum in a metachronous manner the solenocyte vibrates backwards and forwards at each wave. As the solenocytes are inserted on the ends of the nephridial canals in all possible directions with regard to one another their vibrations are not necessarily in the same plane. Sometimes it happens that they are arranged so that a large number of them beat in the same plane, when the whole mass moves together; usually, however, this is not the case, each solenocyte has its own separate motion, and they all appear to beat independently of

one another. As the preparation gradually dies they are the first to stop, while their flagella in the nephridial canal still continue to make a few sluggish movements. Under compression they seldom remain active longer than fifteen minutes, at the end of which time their action has become quite slow. They never show any tendency to unite together for mutual support in the body-cavity as in some Polychæts, but always remain separate. Their protoplasm is clear and remarkably free from granules, and is, for this reason, highly refractive. As already mentioned, the solenocytes of the first nephridium seem somewhat finer than those of the following segments, and in several cases their ends appear as if slightly flattened and hook-shaped; these are smooth, and never throw off protoplasmic processes into the body-cavity as in Polygordius. There is also considerable variation in size between those attached to the margin and those attached to the centre of the end of the nephridial canal, the latter being much longer and more decidedly pear-shaped in form; their long stems densely packed together afford considerable support to the mass of solenocytes. Beyond the refractive granule mentioned (figs. 2 and 5) as sometimes distinguishable in their heads, no obvious evidence of the presence of a nucleus is visible in the living state, nor in the second portion of the nephridium, the thick granular-walled part, could I ever distinguish the presence of nuclei. It will be seen from Meyer's (**16**) figure of one of the nephridial canals of D. gyrociliatus (fig. 10) that a conspicuous nucleus is present on one side of the canal. I have never observed any such nucleus in D. tæniatus.

I have never seen excretory matter passing down the nephridial canals in the form of granules, and they would seem to excrete clear watery fluid alone. Whatever function the so-called body-cavity performs, it is certain that the light orange fluid filling it must at least play a considerable rôle in the areation and the removal of waste products from the body tissues among which it ramifies. In the removal of this fluid by osmosis the solenocytes, with their hyaline tubes,

undoubtedly take a great part. Their action is probably selective. If we take the average length of a solenocyte tube to be one hundredth of a millimetre, and allow sixty solenocytes for each of the eight nephridia, a number very much below their number in the case of the second, third, and fourth pairs of nephridia, we get a total length of 4·8 mm. of solenocytes for the whole animal, which represents a considerable area for osmotic exchange in an animal whose total length is under one millimetre. It is thus evident that a considerable amount of fluid could be rapidly excreted from the body-cavity by means of the solenocyte tubes. The nephridial canals, however, never show the presence of fluid passing down their interior, and the process of excretion is possibly a very slow one.

III. The Second Nephridium.

The second pair of nephridia occupy the corners of the body-cavity space opposite the anterior end of the stomach (figs. 3 and 4). Into the triangular ends of these spaces their solenocytes project from the ends of the canal portions of the nephridia. The solenocytes are more numerous than in the case of the first nephridium, and, in fact, the whole nephridium is better developed. Numerous granules are seen, as in the case of the first nephridium, frequently lodging among the solenocytes. The end of the canal towards the body-cavity in this case is distinctly closed, its walls being marked out by dense masses of deep orange pigment arranged in irregular masses along the first part of its course. The granules in the wall are large and refractive, and render the nephridium readily visible even when the flagella in its interior are not in motion. About the middle of the testis this portion of the nephridium graduates into the third part at the point at which the canal turns to run through the testis in a ventral direction (see fig. 4). The canal then continues its course ventral to the testis and slightly backwards into the next segment, to terminate in

the basement membrane of the epidermis near the median line on the ventral surface of the worm. On the course of this portion of the nephridium the canal sometimes widens into lacuna-like spaces in the living worm under compression. A similar space is shown by Meyer (**16**) on all of the nephridia in D. gyrociliatus (fig. 9). In the enlarged figure (fig. 10) this space is again seen very much as it looks in D. tæniatus (fig. 16) under compression. The walls of this space are remarkably thin and uniform in thickness, and have a transparent appearance, the masses of orange pigment seen about the rest of the canal being usually wanting in the walls of the spaces or lacunæ. Through the middle of these lacunæ the flagella, which, as I have mentioned, can be traced down from the solenocytes, beat passing on and through and down the nephridial canal (fig. 6). Meyer shows these spaces lined with cilia. I think, however, they are not so lined in D. tæniatus, there being considerable grounds for believing that the flagella beating in the spaces where they spread out somewhat produce a false appearance of ciliation. It is difficult to describe this in some instances, where these spaces, on the contrary, would seem to be furnished with cilia.

A more important difference between the canals of D. gyrociliatus and D. tæniatus is that in D. tæniatus they are never folded, as shown in Meyer's (**16**) fig. 10. The lacunar appearance of these spaces is greatly increased by the worms being under some compression when examined, and they may be in great part produced by this compression. While Meyer figures these spaces on all five pairs of nephridia in D. gyrociliatus, I have observed them only in one instance on the first nephridium and on the second, but almost constantly on the third, in about five hundred preparations examined altogether. Meyer (**16**) also shows a well-marked external pore. It must be remembered that in D. gyrociliatus, from Repiachoff's (**21**) figures and descriptions, as well as in the two figures given by Meyer, the body-cavity space is much better developed than in D.

tæniatus, and the testis in the male is a much smaller structure; this allows room for the nephridial canal becoming coiled, and therefore more complex in structure. In D. tæniatus the muscular system is also better developed, and this probably plays some part in effecting the arrangement of the nephridial canals.

In one instance the canal of the second nephridium could be traced back into the next segment to about the level of the head of the third nephridium, and so to the middle of the segment. This relationship, however, may be somewhat incorrect. It has to be remembered that the worm during examination is sometimes able to crawl a little bit, in doing which under the cover-glass the proper position of the dorsal side of one segment above its ventral surface is displaced. When the preparation is viewed vertically from above, what is ventral does not correspond always to what is dorsal as belonging to the same segment, and so relationships like these established on living material may sometimes be incorrect. In saying, therefore, that the nephridia of the first and second pairs almost overlap, it must be kept in mind that this is applied to living material observed under somewhat abnormal conditions. As the canal passes through the testis it frequently passes close to a number of the large vesicles in which the spermatozoa are seen actively moving; these, however, are always distinctly walled off from the canal, and even under great compression never rupture or discharge into the nephridial canal. In every case the spaces containing the spermatozoa are always completely shut off from the body-cavity; while they are seen in almost every other part of the body, they are never seen in the body-cavity or in any of its connecting spaces.

IV. The Third Nephridium.

The third nephridium is the most characteristic of all the nephridia (figs. 5, 8, 10, and 16). It is situated in the wall of the body-cavity space about opposite the middle point of

the stomach. Here its solenocytes project into the lumen of the space, as in the case of the former organs. In this nephridium all three parts of the structure reach their fullest development, the solenocytes being most numerous, the second portion being large, and the third portion remarkable for its great length (fig. 10). The thick granular wall of the second portion immediately catches the eye on the first examination of the preparation, its dark red pigment and its hyaline granular walls render it striking. The greater part of its canal lies dorsal to the main mass of the testis. It pierces this to run ventral to it about its outer third, terminates near the median line on the ventral surface as in the previous organs, and like them in the segment following that to which it properly belongs. The course of this nephridium is shown in fig. 10. It terminates in the basement membrane, as shown in fig. 7.

V. The Fourth Nephridium.

The head of the fourth nephridium is situated opposite the posterior end of the stomach about its junction with the gut. It lies for the most part ventral to the testis, running backwards and outwards to terminate on the ventral surface near the median line. Its solenocytes project into the posterior prolongation of the corners of the body-cavity space, and are somewhat difficult to see on account of the stomach usually folding over and hiding them when the animal is compressed. It is more feebly developed than the third nephridium, and from the denseness of the testis to which it lies ventral its canal is difficult to follow. It terminates beneath the surface of the epidermis in a manner similar to the other nephridia. The second portion of the nephridium is not marked out by the masses of red pigment as in the previous cases, its walls are but slightly granular. The canal passes among several large clear spaces crowded with spermatozoa. With these it never shows any connection.

VI. The Fifth Nephridium.

The fifth nephridium I have had no opportunity of examining personally through lack of material as already stated. According to Harmer (**8**) this nephridium is situated in the fifth segment of the female "on the ventral side of the intestine (behind the cæcal end of the stomach)." In general structure it resembles the other nephridia just described. In the male Harmer suggests it is represented by the vesicula seminalis, and this view I wish to discuss under the present section. This suggestion of Harmer's concerning the vesiculæ has found considerable favour among subsequent investigators, so that it is important to consider the grounds on which it is based. Schimkewitsch (**23**) thinks it highly probable that the seminal vesicles of the male represent the modified fifth pair of nephridia of the female, while in the female the nephridia of the sixth segment are represented by the oviducts. Haswell (**9**) in the allied form of Histriobdella states that "in the fourth segment the nephridia are probably represented in the female by the oviducts, in the male by the vasa deferentia."

The grounds which Harmer (**8**) has advanced in support of this contention are partly anatomical and partly embryological. The anatomical reasons are—that the seminal vesicles in the male occupy the position of the fifth pair of nephridia of the female; in the second place these vesicles are furnished with ciliated funnels, opening into the cavity of the testis, which resemble the ciliated appendage of the nephridium (which Harmer considered probably opened in the primary body-cavity by funnel-like apertures); thirdly, cases occur among Annelids in which we know the nephridia are transformed into genital ducts and functions as such in the adult animal.

The embryological reasons are derived from the study of the immature vesicles of young male worms. One of these is shown in Harmer's (**8**) figure 5, and concerning which

figure he states, p. 14, "the vesiculæ seminales were in their definitive position in the fifth body segment, and their identification as vesiculæ was rendered sufficiently certain by the fact that they contained ripe spermatozoa. The vesiculæ were arranged in an obliquely transverse position, their outer portions ending blindly at the level between the two ciliated rings of the fifth segment, their inner ends opening into the cavity of the testis. A part of the vesicula immediately succeeding the internal aperture was lined with long cilia; the next part of the tube contained a small mass of spermatozoa. The penis was well developed, and obscure indications of a duct leading from the vesicula to the penis was observed; the existence of the duct was not, however, completely proved. The resemblance of the young vesicula seminalis to an ordinary nephridium was manifest not only in its shape and position, but still more conspicuously by the fact that its walls contained an orange pigment, exactly resembling that so commonly found in the walls of the excretory tubes."

Numerous stages between this form and the mature condition were observed. "The final form is acquired by the gradual distension of the originally subcylindrical tube by spermatozoa, this distension being accompanied by an alteration in the direction of its axis, the result of which process is that the end which, in the young vesicula, is external, is situated in the adult condition in front, the whole organ having now acquired an antero-posterior direction. The funnel during the above changes will naturally come to be situated near the posterior end of the organ." "It must be especially noted that the funnel of the vesicula is in a position corresponding with that of the ciliated appendage of an ordinary nephridium, and that the original external aperture of the modified nephridium was probably (in the phylogenetic history of the organ) at the opposite end of the tube, which ultimately becomes the blind anterior end of the vesicula. The relations of the outer ends of the young vesicula to the ciliated rings of the fifth segment further

support this conclusion." Thus, while the nephridia project into the space of the body-cavity, the modified nephridia of this segment (the fifth pair of the female) open into the cavity of the testis. However, it seems to me there are many difficulties in the way of accepting this origin of the seminal vesicles.

I have already called attention to the closed nature of the spaces of the testis tissue and the fact that they are entirely separated from the primary body-cavity about the gut (fig. 4, *s. p. m.* and fig. 11, *m. v.*), and Dinophilus would seem to be one of the few animals in which both the primary and secondary body-cavity exist alongside of one another at the same time. Repiachoff (**21**) at some length has considered the relationships of these two cavities in Dinophilus, and has clearly pointed out how the secondary body-cavity of Annelids is probably represented in Dinophilus by the spaces in the testis tissue. These towards the posterior end of this structure fuse together to form the large and roomy cavity of considerable size well shown in Schimkewitsch's fig. 43 *b* (**23**). It is lined by a definite epithelium, while the primary body-cavity about the gut possesses no such lining membrane.[1] As the nephridia in Dinophilus are related to the primary body-cavity alone it is necessary to suppose on the basis of Harmer's theory that those of the fifth segment have lost their connection with this structure, and acquired openings into the testis cavity. I have also shown that the nephridia do not open into the primary body-cavity, but are closed; therefore the funnel-like openings of the vesiculæ seminales are new structures that have been developed since this relationship has been established, and cannot have been transferred, as Harmer thought, from the primary body-cavity.

The resemblance of the male reproductive system of Dinophilus to that of the Turbellaria is so similar in many respects, although the reproductive system in these forms is

[1] Repiachoff (**21**) has described a peritoneal lining to the outer wall of the gut in D. gyrociliatus, but no such lining is present in D. tæniatus.

further complicated by their hermaphroditic condition, I think it would be as just to assume that in them the vesiculi and vasa differentia also represent modified portions of the nephridial system. In the Turbellaria either the male or the female sexual organs are readily reducible to the conditions presented in Dinophilus. This, connected with the changes necessary in the position of the relative parts of the nephridium in order to make it agree with the observed growth of the vesicles in young worms as described by Harmer (**8**) renders it probable that they are not modified nephridia. It is true, as Montgomery (**18**) has pointed out, no hard or fast distinction can be drawn between the blastocœl and cœlom as morphologically distinct spaces; since their presence as separate cavities is dependent to a large extent on the form of cleavage and gastrulation, which often differs so greatly in closely allied forms. While realising no great importance can be attached to the separation of these spaces, nevertheless in Dinophilus this separation is so well marked that it is hard to suppose that the nephridia of the fifth segment could readily lose their connection with one space, and acquire openings into that of the other in the manner Harmer supposes without showing more obvious evidence of this change. This view will certainly need more conclusive evidence in its favour than has so far been advanced for it, and it is possible ultimately it will prove to be wrong.

VII. Summary.

In the present paper it has been shown that the nephridia of Dinophilus are of the primitive solenocyte-bearing type so frequently found in Annelids. In the male there are four such pairs of nephridia whose solenocytes project into the primary body-cavity or blastocœlic space about the gut. The shape and general position of the nephridia is the same as Harmer (**8**) has already described for this species. The terminal portion of the nephridial ducts, however, probably

end beneath the skin, just over the division line of the segment to which they properly belong. The nephridia are furnished with typical solenocytes, and their canals are definitely closed, and do not open into the primary body-cavity. They are not ciliated, but the flagella of the solenocytes beating down the length of the canals give them the appearance of being ciliated. The presence of solenocytes in Dinophilus is a point of considerable morphological importance on account of the relationship this worm shows with lower forms, especially the Turbellaria. Their discovery in Dinophilus, on the other hand, in the absence of our knowledge of their presence in lower forms may be held to indicate close affinity with the more highly developed Annelids, and especially the Polychæts.

Literature cited in Text.

1. Böhmig, Ludwig.—"Tricladenstudien I, Triclada maricola," 'Zeit. f. Wiss. Zool.,' vol. 1806, p. 344.
2. Bresslau, E.—"Beiträge zur Entwicklungsgeschichte der Turbellarien I, Die Entwicklung der Rabdocölen und Alloiocölen," 'Zeit. f. Wissen. Zool.,' vol. lxxvi, p. 213, 1904.
3. Eisig, H.—"Die Entwicklungsgeschichte der Capitelliden," 'Mitth. Z. Stat. Neapel,' Bd. xiii, p. 1, 1898.
4. Foettinger, A.—"Recherches sur l'organisation de Histriobdella homari," 'Archives de Biologie,' vol. v, p. 435, 1884.
5. Goodrich, E. S.—"On the Nephridia of the Polychætæ," Part 3, 'Quart. Journ. Micr. Sci.,' vol. 43, p. 715, 1900.
6. Goodrich, E. S.—"On the Structure and Affinities of Saccocirrus," 'Quart. Journ. Micr. Sci.,' vol. 44, 1901.
7. Hallez, P.—'Contributions à l'Histoire Naturelle des Turbellaries,' Lille, 1879, p. 155.
8. Harmer, S. F.—"Notes on the Anatomy of Dinophilus," 'Journ. of Marine Biol. Assoc.,' N.S., vol. i, pp. 1—25, 1889.
9. Haswell, William A.—"On a New Histriobdellid," 'Quart. Journ. Micr. Sci.,' vol. 43, p. 299, 1900.
10. Kennel, J. V.—"Über Ctenodrilus pardalis," 'Arbeit. a. den. Zoologisch. Zootomische Inst. in Würzburg,' vol. v, p. 373, 1882.

11. Korschelt, Eugen.—"Über Bau und Entwicklung des Dinophilus apatris," 'Zeit. Wiss. Zool.,' vol. xxxvii, p. 315, 1882.

12. Lang, A.—"Der Bau von Grunda segmentata und die Nerwandlschaft der Plathelminthen mit Coelenteraten und Hirudineen," 'Nat. Zool. Stat. Neapel,' vol. iii, p. 187, 1881.

13. Lang, A.—"Die Polycladen (Seeplanarien) des Golfes von Neapel und der angreuzenden Meereabschnitte," 'Fauna Flora. Golf. Neapel,' Monograph xi, p. 678, 1884.

14. Lang, A.—"Beiträge zu einer Trophocöltheorie Jenaischen," 'Zeit. f. Nat. Wiss.,' vol. xxxviii, p. 1, 1903.

15. Lankester, E. Ray.—'A Treatise on Zoology,' part ii, chap. 2, "The Enterocœla and the Cœlomocœla," 1900, p. 1.

16. Meyer, E.—"Studien über den Korperbau der Anneliden," 'Mitt. a. d. Zool. Stat. zu Neapel,' vol. vii, 1886-7 (pl. xxvii, figs. 9 and 10).

17. Moore, Anne.—"Dinophilus gardineri (sp. nov.)," 'Biological Bulletin,' No. 1, p. 15, 1899.

18. Montgomery, T. H.—"On the Modes of Development of the Mesoderm and Mesenchyme with reference to the supposed Homologues of the Body-cavities," 'Journ. of Morph.,' vol. xii, p. 355, 1897.

19. Nelson, J. A.—"The Early Development of Dinophilus: a Study in Cell-linage," 'Proc. Acad. Nat. Sci. Philad.,' p. 687, 1904.

20. Pierantoni, Umberto.—"Sullo Svilluppo del Protodrilus e del Saccocirrus," 'Mitt. a. d. Zool. Station Neapel,' vol. xvii, p. 575, 1906.

21. Repiachoff, W.—"On the Anatomy and History of the Development of Dinophilus gyrociliatus," O. Schmidt, Odessa, 1886. (The paper is in Russian: a translation was kindly lent me by Dr. Harmer.)

22. Schmidt, Oscar.—'Neue Beiträge zur Naturgeschichte der Wurmer,' Jena, 1848.

23. Schimkewitsch, W.—"Zur Kenntnis des Baues und der Entwicklung des Dinophilus vom Weissen Mere," 'Zeit. Wiss. Zool.,' vol. xlix, p. 46, 1895.

24. Weldon, W. F. R.—"On Dinophilus gigas," 'Quart. Journ. Micr. Sci.,' 1886, p. 109.

25. Wilhelmi, J.—"Untersuchungen über die Excretionsorgane der Susswassertricladen," 'Zeit. Wiss. Zool.,' vol. lxxxi, p. 544, 1906.

26. Vejdovsky, F.—"Zur Hämocöltheorie," 'Zeit. f. Wissen. Zool.,' vol. lxxxii, p. 80, 1905.

27. Zeppelin, Max Graf.—"Über den Bau u. die Theilungsvorgänge des Ctenodrilus monastylos, Nov. Spec.," 'Inaugural Dissertation,' Leipzig, 1883, p. 1.

DESCRIPTION OF PLATES 29 & 30,

Illustrating Mr. Cresswell Shearer's paper on "The Structure of the Nephridia of Dinophilus."

Lettering.

*blc.*1. Blastocœlic space of the head segment. *blc.*2. Blastocœlic space of the trunk region. *bas.mem.* Basement membrane of the epidermis. *e.gr.* Excretory granules of the blastocœlic spaces. *fgl.* Flagella of the nephridial canal. *flg.* Flagellum of a solenocyte. *gr.s.* Granule in the head of the solenocyte. *Gt.* Gut. *msc.* Muscle fibres. *m.v.* Limiting membrane of the vesiculæ. *nep.c.*2. Second portion of the nephridium. *nep.c.*3. Third portion of the nephridium. *pig.str.* Pigment cells. *phr.* Pharynx. *phr.o.* Pharynx organ. *sep.d.* Line of division between segments. *sol.* Solenocytes. *sp.m.* Sperm reservoirs or spaces of the testis. *Stm.* Stomach. *Tes.* Testis. *vac.* Vacuoles of the epidermis. *v.c.* The ventral thickened crawling surface of the worm. *vesc.* Vesicula seminalis.

PLATE 29.

Figs. 1—12 were drawn under a 2 mm. oil-immersion obj. with oc. 6, except where otherwise indicated. They were drawn from living preparations, although no attempt has been made to exactly portray the appearance of the living protoplasm, which is simply roughly indicated as granular. The sections are from material treated with cocaine and fixed in Hermann's fluid and Zenker's fluid.

Fig. 1.—A portion of the first nephridium, somewhat unduly compressed and flattened. Obj. 2 mm., oc. 12.

Fig. 2.—A solenocyte of the third nephridium. Obj. 2 mm., oc. 18.

Fig. 3.—The second nephridium. Several granules being seen in the blastocœlic space about the gut.

Fig. 4.—The second nephridium.

Fig. 5.—The first and second portion of the third nephridium.

Fig. 6.—A portion of the flagella of the third nephridium.

Fig. 7.—The termination of the canal of the third nephridium in the basement membrane of the epidermis. Obj. 2 mm., oc. 18.

Fig. 8.—Head portion of the third nephridium.

Fig. 9.—Terminal portion of the third nephridium.

Fig. 10.—The third nephridium.

Fig. 11.—A transverse section through the region of the vesiculæ.

Fig. 12.—A transverse section through the region posterior to the stomach, showing the testis surrounding the gut.

Fig. 13.—Transverse section through the region of the junction of the gut with the stomach.

Fig. 14.—Longitudinal coronal section in a plane slightly below the middle of the stomach.

Fig. 15.—Transverse section through the region of the pharynx.

Fig. 16.—The third nephridium.

PLATE 30.

Fig. 17.—A general diagrammatic figure showing all the nephridia.

Contributions to our Knowledge of the Anatomy of Notoryctes typhlops, Stirling.

Part III.—The Eye.

By

Georgina Sweet, D.Sc.,
Melbourne University.

With Plate 31.

Introduction.

The work, of which this paper records a part, has been done in the Biological Laboratory of the University of Melbourne, for the use of which I am indebted to Professor Baldwin Spencer. To his kindness I owe all the material used, together with much criticism and advice, and assistance in obtaining literature, without reference to which this research would have been incomplete. I wish also to thank Mrs. H. R. Elvins, who had commenced this work on the eye, but was prevented from proceeding with it, for the use of her series of sections and sketches.

Part I of these Contributions, dealing with the Nose and Jacobson's Organ, and Part II dealing with the Blood Vascular System, were published in the 'Proceedings' of the Royal Society of Victoria in 1904.

The Structure of the Eye and associated parts.

The degenerate eye of Notoryctes has been previously referred to by Dr. E. C. Stirling, who has noted concerning

it [**10**, pp. 22, 23], that "it is not visible exteriorly," has "no bony orbit," and "consists of a small circular pigment spot" —and three years later, a longer reference was made to it [**11**, pp. 159, 180] giving its position in the head and its general characteristics, concerning which Dr. Stirling writes: —"I cannot be absolutely certain of its relations, or of the morphological value of its constituent parts." As the result of careful study of some seven or eight heads, I am able not only to corroborate Dr. Stirling's observations so far as they go, but also to record a quantity of additional information which will, I think, make clear the homologies of this very interesting relic, forming at the same time a striking study in degeneration and convergence. Considerable variations are met with in the different points of structure, as might be expected in a degenerate organ, and the following description takes note of the more important of these variations, while at the same time, it is based on the most constant features. It is a matter for regret that the embryo of this animal has not yet been obtained, as no doubt considerable light would be thrown on the degeneration of the eye by the study of its early stages.

In seeking to trace the homologies of the eye of Notoryctes typhlops, in the following account of its structure, I have refrained from making comparisons or contrasts with other forms, except in such cases as seem of assistance or special interest.

Position.

The whole eye, with its gland, the nerve, blood-vessels, muscles, etc., is situated at a distance of 13 mm. from the anterior end of the snout, deeply seated beneath the temporalis muscle, and often covered with a mass of connective tissue (contrast Typhlops, where the ordinary subcutaneous tissue is thinner [**6**, p. 119], and compare Siphonops [**6**, p. 105] and Typhlomolge [**2**, p. 51]). In addition to this the dermis and epidermis always pass over this region unaltered in general structure and thickness, in contrast to Pro-

teus, in which it is thicker [**6**, p. 75], and Siphonops [**6**, p. 105] and Typhlops [**6**, p. 117], in which it is thinner. From the exterior, therefore, even after shaving off the hair (contrast Scalops [**8**, p. 335] and Talpa [**6**[1], p. 13]), there is no sign whereby the presence of an eye may be detected (compare Myxine [**6**, p. 49], Troglichthys rosæ [**1**, p. 578], Amblyopsis [**1**, p. 560], Typhlichthys [**1**, p. 570], and Rhineura [**4**, p. 535] ; and contrast Zygonectes [**1**, p. 548], Chologaster [**1**, p. 549], Siphonops [**6**, p. 102], Proteus [**6**, p. 72], Typhlops [**6**, p. 116], and Typhlotriton [**3**, p. 34], Scalops [**8**, p. 335], and Talpa [**6**[1], p. 13], in which latter three it can be found by the eye cleft). Of those forms in which the deeply-seated position of the eye is comparable to that of Notoryctes, may be mentioned Myxine, Amblyopsis, Rhineura, and especially Troglichthys, in which, as here, its wall is in contact with the skull.

There are, however, to be found in Notoryctes in the head region, in common with the region of the modified "ischiotergal" patch, curious organs formed as modifications of the epidermis. These are presumably tactile in function, and will be described in detail in Part IV of these Contributions.

The eye, which is not much more than a hollow ball of pigment, lies within the anterior wider end of a fibrous cone-shaped capsule (fig. 1, *f.c.*), 5·25 mm. to 5·7 mm. long, 1·14 mm. to 1·75 mm. in vertical diameter, and ·7 mm. to ·87 mm. in transverse horizontal diameter. This capsule is attached by its small posterior end to the bony wall of the skull, and passes forwards close against the periosteum of the lachrymal bone, being only partially open in front. This is not to be confused with the fibrous capsule mentioned by Dr. Stirling [**11**, p. 180], as will be seen later. Structures comparable to this conical capsule are found only in Chologaster papilliferus [**1**, p. 550] and Amblyopsis spelæus [**1**, p. 560], in each of which cases orbital fat is found inside with the eye and eye muscles; in Notoryctes, fat has only been seen in one single eye, and that in small quantities. In Siphonops [**6**, Taf. vii, figs. 66 and 67] a somewhat similar fibrous cone

covers the front of the eye, immediately beneath the skin, instead of lying beneath the muscle and connective tissue as in Notoryctes.

Muscles.

In the posterior small end of the fibrous capsule are to be found the bands of muscle (fig. 1, *m.*) which represent the degenerated eye-muscles, being attached posteriorly to the wall of the skull. There are four constant muscles usually lying beneath the eye mass. Often there is a fifth slip of muscle, separated off from the upper and most external one of these, which very early becomes attached to the pyriform fibrous capsule around the eyeball. The other four continue forwards for a varying distance (fig. 1), sometimes stopping behind the level of the pigment ball, sometimes, but rarely, extending to the level of its anterior border. Invariably, however, they end in a more or less broken or irregular fashion in the outer fibrous capsule, or rarely within it, amongst the connective tissue, which is variable in quantity.

Variations are met with in the proportionate length of this hinder muscular region. Most frequently it is merely one sixth of the total length, in which case the gland mass is larger. In several instances the muscles are one half to two-thirds the length of the capsule, the gland being then proportionally smaller. At the extreme posterior end of the capsule there enters an arterial twig from the internal carotid, which appears to supply these muscles. At the anterior part of this muscular region there leaves the capsule a large vein, which runs downwards and outwards to join the facial vein.

These muscles are, in contrast to those of Mammalia generally, of the non-striated kind, resembling them in size, shape, character of the nuclei, and apparent absence of sarcolemma. As far as can be seen those which are external and inferior in the present condition are, as a rule, larger and run the farthest forward, in other words, degenerate last.

It is probable, however, that the present positions of these muscle slips are not indicative of their homology with the muscles of a normal eye. They are very variable both in length, position and size, and having changed their insertion from the true eye capsule to the abnormal fibrous cone, and lost their striated character, it seems not unreasonable to suppose that they may have lost their typical positions relative to each other. The condition of degeneration of these muscles may be compared with that in Amblyopsis [**1**, p. 561] and Troglichthys [**1**, p. 579], and contrasted with that in such forms as Scalops, Proteus [**6**, p. 77], Typhlotriton [**3**, p. 35], Siphonops [**6**, p. 100], Typhlops [**6**, p. 124], Talpa [**6**, p. 30], where they are well developed, or with such as Typhlichthys [**1**, p. 571], Typhlomolge [**2**, p. 52], and Rhineura [**4**, p. 537], where they are still more degenerate than in Notoryctes, and even sometimes absent altogether.

In Proteus, although the six muscles are present, the individual fibres are, according to Kohl, in an embryonic condition, not having yet developed into striated fibres. In Typhlops and Talpa also he considers that the unstriated fibres often found mingled with others are evidence of the imperfect development of the muscles, while in Myxine [**6**, p. 51; and **6**[1], p. 195] the masses of connective tissue in the place of the recti are accepted as being the predecessors of the true muscles, and not degenerate eye muscles. I cannot, however, but think that in Notoryctes at all events we have eye-muscles which have degenerated in company with the remainder of the eye, unless, indeed, we are to think that these fibrous bands are neither the relics nor the forerunners of the normal eye-muscles, but are new developments in connection with the abnormal conical capsule.

Glands.

At about the level of the vertical plane of the front of the muscle region there appears the enormous development of

glandular material (figs. 1—4, *l.g.*), which, as will appear later, is undoubtedly the representative of the lachrymal or Harderian glands, probably both, of normal forms. The histological structure of this mass, which often occupies most of the conical capsule, shows it to be a true serous gland, comparable exactly to the parotid of the same and other animals. In one instance, in which the whole head had been cut in a series of transverse sections, on one side there was seen an even greater development of glandular material outside and posterior to the conical capsule, and extending immediately beneath the dermis. It was, however, connected anteriorly with the gland inside, with which its duct also communicated. In two other eyes, which had been removed for sectioning purposes, there was a similar large development of gland outside the capsule. In these specimens the conical capsule is more or less undefined and irregular in its posterior portion, but is normally definite anteriorly. In harmony with the broken character of the outer capsule in the two latter cases, and the overflow of the gland beyond the capsule to which it is usually confined, the muscle bands appear to lie in a special mass of connective tissue which is here strongly developed, but anteriorly they end, one or sometimes two, in the conical outer capsule, and the others in the connective tissue outside the capsule instead of inside it. These are really the most degenerate cases.

Embedded in the gland mass within the cone lies the pyriform remnant of the eye proper (figs. 1, 3, 4, pl. 31), generally ·87 mm. to ·96 mm. behind the front of the tube. It always lies closely apposed to the bony wall, from which it is only separated by the periosteum and the conical capsule. The gland mass does not enter this part of the cone (figs. 1 and 4); it appears to do so in fig. 3 to a small extent, but in the succeeding sections it is quite absent here also. In front of the eye a varying amount of gland is present, and also sometimes a small fibrous band, running straight, forwards and outwards, for a very short distance from the pigment wall, or a line of pigment in a similar position. The main feature of

this part, however, is the large irregular sac (figs. 1 and 2, *c.s.*), the hinder wall of which is in close contact with the median anterior and antero-lateral part of the pyriform fibrous capsule (*p.c.*), which is here thinner though more compact than elsewhere. The size of this sac varies as follows: Length (antero-posterior) ·7 mm. to ·52 mm., vertical diameter ·52 mm. to ·42 mm., horizontal diameter ·42 mm. Into its cavity there open some four to six ducts (*l.g.d.*) from the large lachrymal gland, while from it anteriorly there pass off two ducts, the small and outer of which (fig. 2, *e.d.*) runs obliquely outwards and forwards for a variable distance, ·31 mm. to ·79 mm. long, and ends blindly in the dermis near the skin. In the case of the shorter of these outer ducts the pigment in its wall is so thick as to obscure its minute structure. The larger and inner of the ducts, however, descends obliquely forwards and inwards, and then passes through a definite opening in the skull wall, which is visible in the dried skull (contra Dr. Stirling [**11**, p. 162]), near the lachrymal notch, sometimes higher or lower.

The sac (*c.s.*) in front of the eye is lined by somewhat columnar epithelium, continuous with that of the two ducts leading from it, that of the exterior blind duct (*e.d.*) becoming, however, more cubical and thick-walled in structure. The sac also is covered with a thick layer of circular muscle-fibres, which becomes much thinner both where the sac wall is in contact with the eyeball, and over that part of the duct outside the skull wall, and is almost lost on that part inside the bone. In both sac and duct there is often to be found a quantity of secretion. The duct then descends from its entrance, as a definite tube through the skull wall, obliquely downwards and forwards close to the wall of the skull in a distinct groove (rarely absent) formed by the angle of the bony floor of the lateral cavity of the nose and its external side wall. It is separated from the olfactory mucous membrane by a more or less developed glandular mass, of which more has been said when dealing with Jacobson's organ in Part II of these Contributions. In

this position, where it lies dorsal to the ophthalmic nerve, the duct is definitely flattened, and is lined by columnar epithelium with very darkly-staining nuclei, the inner border of the cells of which appears slightly cuticular, while it is surrounded by concentric fibres. The duct soon comes to lie nearer to the middle line, and then sinks with a small artery, a veinlet, and a small bundle of nerve-fibres from the ophthalmic nerve, into a depression in the dorsal surface of the maxillary bone. This becomes enclosed anteriorly forming a canal [**12**, fig. 5, *n.l.d.*] by which the duct now more cylindrical in shape, and its accompanying structures may enter the inferior meatus of the nose. About the level of the posterior end of the organ of Jacobson it runs in a groove in the bony jaw, which forms the lateral boundary of this part, and it is here flattened and still lined by columnar epithelium. Its greatest diameter (vertical) is, in this region, ·25 mm. It then descends again into a canal [**12**, fig. 4, *n.l.d.*] in the bone, which it leaves opposite the Stenonian canals to lie near the bony floor of the nasal cavity [**12**, fig. 3, *n.l.d.*] with its artery and veins between the premaxillary bone and the cartilage which forms the floor of the nose in this anterior region. Immediately behind the connection of the ali-nasal cartilage with the ventral cartilages it runs towards the middle line embedded in a gland mass, becoming more and more cuticular inside and quite flat (·25 mm. in greatest vertical diameter), until it opens into the nasal furrow by an oblique aperture, on the under surface of the small primary lateral ridge or "concha" found near the external opening of the nose.

With regard to the general course of this naso-lachrymal duct, for such it undoubtedly is, we may see by reference to Klein's descriptions of this structure in the guinea-pig [**7**, p. 224] and rabbit [**7**, p. 567], that it is here very similar to what he has described in those forms, as also its accompanying artery and veins. He, however, has described the epithelium as a stratified columnar layer, the columnar cells being next the lumen of the duct, and more cubical cells outside this. I find an identical appearance of nuclei, but consider that there

is only one layer of ordinary columnar cells which are cut obliquely, so that there appear to be two or three layers of nuclei, whereas there is but a single layer of cells.

It is interesting to compare the state of development of this gland mass, conjunctival sac, and ducts in other forms. Thus we find in Typhlomolge [**2**, p. 52], whose eye that of Notoryctes resembles in many points of structure, that there is no sign of a conjunctival sac, nor is there any glandular structure connected with the eye. In Rhineura likewise [**4**, p. 536] there is no conjunctival sac, but Harder's gland is well developed; its secretion, on the other hand, is poured directly into the tear-duct, and so into the nasal cavity. In Siphonops [**6**, p. 101—105] we have a very well-developed gland, and also a conjunctival sac, into which, in one case, Dr. Kohl found the lachrymal glands emptying, and from which the naso-lachrymal duct passed off through the surrounding tissues and nasal wall, to open into the nasal cavity far forwards, as in Notoryctes. More generally, however, in Siphonops, the gland tubes open directly into the naso-lachrymal duct, while in Typhlops [**6**, p. 119—121] they open into a duct which leads into the back of the mouth cavity.

The relations of the glands, which are apparently true Harderian and lachrymal glands are similar in Typhlops to those in Notoryctes, as also the presence of blind ducts ending in the subcutaneous tissues.

In Scalops, Talpa, and Typhlotriton we find instructive stages in the closing up of the conjunctival sac, intermediate between that of Notoryctes and the normal eye. In both Scalops [**8**, pl. xviii, figs. 4, 7, 8; and pl. xix, fig. 16] and Talpa [**6**[1], Taf. i, figs. 3, 4, 5] the eyelids have closed over to such an extent that the only connection between this pre-corneal space and the surface of the body is by an open though small canal, which is of very little if any use for the passage of light rays.

In Typhlotriton [**3**, p. 41, figs. 1 and 1 *a*], the lids are merely overlapping slightly, a shallow groove indicating the position of the eye cleft. The comparatively small amount

of degeneration in Talpa is also indicated by the fact that the Meibomian glands connected with the eyelids are still functional though small, indicating that the closing over of the lids is but recent [6[1], p. 26]. Although Scalops is undoubtedly "much more degenerate in all its parts" than Talpa [8, p. 361], Notoryctes leaves Scalops far behind in this matter.

Bloodvessels.

The arteries supplying the eye region are very large in proportion to the size of the eyeball, to cope no doubt with the demands of the large lachrymal glands. They are derived as usual from the external carotid and facial arteries, with a twig from the internal carotid artery entering among the muscles at the apex of the conical capsule. As previously stated the large vein empties itself into the facial vein.

Nerves.

The second or optic nerve is discussed in connection with the eyeball itself.

Of the third or motor oculi, and fourth or trochlear nerves, I can find absolutely no trace in any specimen, either in connection with the eye or the brain.

The sixth or abducens is equally wanting, though in one animal there was to be seen extending through some two or three transverse sections (of the whole head) a slight swelling on the ventral surface of the medulla oblongata, near the middle line, just where one might expect to find the abducens leaving the brain, though I could find no sign in its structure of its being the root of a nerve.

The gland mass receives its innervation from a branch of the ophthalmic nerve, which it leaves in company with the nasal branch of that nerve, again proving the relationship of this gland with the lachrymal gland of the other forms.

The muscles, now no longer accessory optic structures,

and so receiving no innervation from the normal source, come to derive their nerve supply from a twig of the lachrymal branch of the ophthalmic nerve, viz. that which supplies the lachrymal gland.

The following data from one typical eye will help to give a better idea of the relative sizes of the parts described :

Length of whole conical tube	. .	5·25 mm.
Vertical diameter of conical tube (in front)	.	1·14 ,,
,, ,, ,, (greatest)	.	1·75 ,,
,, ,, ,, (behind eye)		1·40 ,,
Horizontal ,, ,, (in front)	.	·7 ,,
,, ,, ,, (greatest)	.	·87 ,,
,, ,, ,, (behind eye)		·75 ,,
Distance of eye from front of tube	.	·96 ,,
Length of eye . .	.	·96 ,,
Vertical diameter of eye .	. .	·87 ,,
Horizontal diameter of eye	. .	·85 ,,
Length of conjunctival sac (antero-posterior)		·7 ,,
Vertical diameter of sac .	. .	·37 ,,
Horizontal ,, .	. .	·42 ,,

On comparison with Talpa and Scalops [**8**, p. 337] it will be seen that the eye of Notoryctes is slightly bigger than that of Scalops, though distinctly smaller than that of Talpa.

The Eyeball.

In size this varies from ·96 to 1·1 mm. long, ·61 to 1·05 mm. in vertical diameter, and ·52 to ·87 mm. in horizontal diameter.

It is enclosed completely in a tough pear-shaped capsule (figs. 1, 3, 4, *p.c.e.*), consisting of closely layered fibres with scattered nuclei, and occasionally with small elongated patches of granular pigment in its inner part.

Rarely one could detect a small bloodvessel running in this layer. It would seem that this must be regarded as a sclero-choroid, the boundary between the two membranes

being indistinguishable. In two eyes a small oval nodule of hyaline cartilage (fig. 4, *c*, *n*), containing some six cells, is to be found lying in the sclero-choroid, just on the inner side of what ought to be the exit of the optic nerve. In another case a similar though smaller nodule was found lying in front of the eye, a little to its outer side. The structure of this sclero-choroid is very much like that of Rhineura [**4**, p. 537] among others. As in that also this layer is prolonged proximally, forming a sheath which must represent the covering of the optic nerve, being connected posteriorly to the skull along with the conical capsule. Small bars of cartilage comparable to the nodule are also found in Rhineura and Amblyopsis [**1**, p. 563].

Pigment Epithelium.

Immediately within this sclero-choroid lies the conspicuous hollow ball of pigment, dense and thick-walled anteriorly and exteriorly, as well as above and below, and very thin on its inner side and posterior end. At first, in the absence of embryonic material, one is led to consider this as a much thickened choroid, but, on comparison with other forms in which developmental changes can be followed, it is seen to be probably the greatly changed pigment epithelium of the retina, though there is nothing in the irregular, broken, and jumbled masses of granular pigment to suggest such a well-defined cell-layer as the retinal pigment. No trace of cell-structure can be seen enclosing the pigment, though, in occasional gaps between the masses, small oval cells can be seen without any granules within them. There are never any processes inwards or outwards. It will be noted that, as in Troglichthys [**1**, p. 581], and Typhlomolge [**2**, p. 53, and pl. 3, figs. 1, 6, and 7] the pigment is much denser in front, where it might be expected to be absent, and very thin or wanting posteriorly, where it ought to be thicker. In this these three forms differ from all other known degenerate vertebrate eyes. The irideal region is much reduced

insomuch that, as a rule, no remnant of iris epithelium, pupil, lens, vitreous humour, or hyaloid membrane can be seen, the unbroken wall of pigment forming the front of the eye. In this condition the eye of Notoryctes is almost exactly comparable to that of Troglichthys, which Eigenmann claims as the most degenerate of all vertebrate eyes. In the less degenerate specimens, however, structures are present which appear to be the remnants of those parts. Thus in one eye, a little to the outer side of what may be taken as the optic axis, is a slight gap, in which the pigment epithelium is much less dense than around it. This gap is tubular, sloping obliquely outwards and forwards; in it posteriorly can be seen a double layer of cells with oval and flattened nuclei, lying, not edge to edge, but obliquely against one another. These are continuous internally on either side, with a similar layer sometimes two or three cells thick instead of one, and extending over the anterior region of the eye. In one or two other eyes these flattened cells are present in a less definite manner over this anterior region, but more restricted in extent, and not being connected with any gap. There can be no doubt, I think, that this imperfect gap represents the last vestige of a pupil piercing the greatly-developed pigment layer (pars iridis), the two walls of the gap being, in that case, the edges of the iris. Similar vestiges have been found by Eigenmann in Amblyopsis [**1**, Taf. xi, fig. 9, Taf. xiii, figs. 31 and 38, Taf. xiv, fig. 40], in Typhlichthys [**1**, Taf. xiv, fig. 48], and in Troglichthys [**1**, Taf. xiv, figs. 54 and 56], and Typhlomolge [**2**, plate 3, figs. 6 and 7]. On the other hand, the iris and lens are fairly well developed in such forms as Petromyzon [**6**, Taf. ii, figs. 18 and 19], Chologaster [**1**, Taf. xi, fig. 4], Siphonops [**6**, Taf. vii, fig. 67], Typhlops [**6**, Taf. viii, fig. 84], and Talpa [**6**[1], Taf. iii, figs. 27 and 28]. In Proteus [**6**, Taf. vi, fig. 59] the lens is absent in the adult, but the iris is readily distinguishable, though reduced.

In Typhlomolge [**2**, pp. 53, 54] Eigenmann considers the pigment filling up the pupil as being of choroidal origin.

There is, however, nothing in Notoryctes to indicate that it is any different from the rest of the pigment of the eye, i. e. it is uveal in character. Apparently the edges of the pupil have fused almost completely. Rhineura [**4**, p. 537], like the more degenerate cases of Notoryctes, is even more reduced than these forms since there, even this rudiment of the irideal epithelium has gone. The lens was but rarely seen in Rhineura, while in Typhlomolge it has gone altogether. In respect of the iris Scalops [**8**, pl. xviii, fig. 7, and pl. xix, fig. 9] apparently resembles Rhineura, but in the mole the lens is well marked, though only consisting of cells.

Vitreous Humour, Hyaloid Membrane, Retina, and Optic Nerve.

With regard to these, there seem to be three stages in reduction in Notoryctes. I. The first and most highly developed state found in this "mole" is that of which one section is shown in figs. 1 and 4. In it the retina shows a division into outer nuclear and outer molecular layers, and less defined inner nuclear and inner molecular layers. The section drawn shows fibres coming from a group of cells at the anterior end, evidently the remnant of the ganglion cell layer, and to this extent is reminiscent of Amblyopsis [**1**, Taf. xiii, fig. 34], Typhlichthys [**1**, Taf. xiv, fig. 46], Troglichthys [**1**, Taf. xiv, figs. 54 and 56, and **6**, Taf. viii, fig. 77], and Proteus [**6**, Taf. v and vi, figs. 54, 55, 59]. In the next sections to that shown in the figure the outer molecular layer is not visible, the inner molecular layer being, on the other hand, more sharply defined. In the sections ventral to that drawn there appears a longitudinal split (the vitreous cavity) separating the fibres into two layers, and bounded by a thin membrane with elongated nuclei, evidently the hyaloid membrane. Anteriorly the split spreads in a Y-shaped manner, its anterior face being formed by the flattened cells of the uveal layer of the iris, before described (cf. Typhlichthys [**1**, Taf. xiv, fig. 42], Typhlomolge [**2**, plate iii, figs. 2, 6, 7], and

Proteus [**6**, Taf. vi, fig. 59]). In this eye there was no choroid split nor pupil visible. The fibres from the "nerve-fibre layer" collect posteriorly, and leave the eyeball as shown in fig. 4; passing individually through the pigment and sclero-choroid layer which is not broken here, and then backwards, becoming lost among the connective tissue fibres, which form a sheath for them, continuous with the sclero-choroid itself, which "tails off" here posteriorly. Undoubtedly these fibrils represent a degenerate optic nerve, but it is impossible to actually prove their nervous character, as I have no fresh material to work on. Indeed, they might readily be accepted as fine connective tissue fibres with elongate nuclei, lying lengthwise between the fibres, there being no difference whatever in appearance, as indicated by fig. 4. In one other section there was a distinct but narrow cleft in the pigment wall near the same region, but nothing could be found passing through. Nor in the material at hand have I been able to detect any differentiation in the cells of the retinal layers. One is led to believe that a thin more or less definite layer of cells, exterior to the "nerve-fibre" layer, represents the ganglion cells, but no difference in structure can be seen from cells with slightly more deeply staining nuclei, which are scattered all through the nuclear layers.

In the other two stages of degeneration the cavity of the ball is occupied by a mass of cells, generally much crowded together, without any trace of special arrangement.

In Stage II (to which belongs the eye in which the indication of a pupil was seen, as described above) a choroid fissure was present at the outer and ventral side, which opened into a semicircular, very narrow slit-like cavity, bounded posteriorly by a hyaloid membrane. It was almost filled by connective tissue fibres, which have entered from the sclero-choroid by the choroid split (cf. Myxine [**6**, Taf. iv, fig. 40], Typhlichthys [**1**, Taf. xiv, fig. 49, and pp. 575-6], and Rhineura [**4**, pl. xxxiv, fig. 4, and p. 538]. The anterior boundary of this vitreous chamber, such as it is, is formed in

Notoryctes, as in Typhlichthys [**1**, Taf. xiv, fig. 46], by the rudimentary epithelium of the pars iridis retinalis. No nerve fibres could be detected in this stage within the pigment epithelium, though the "tailing off" of the sclero-choroid to form a sheath was well marked, and the elongate nuclei between the fibres outside the eye (? connective tissue or nerve-fibres) were very regularly arranged in this specimen. Just within the pigment wall, on the outer side of this one eyeball, is a definite, very narrow layer of cubical cells, with deeply staining nuclei, extending from the hinder edge of the vitreous split, posteriorly to the proximal end of the eye. I am unable to suggest any homology for them, unless they be concerned with the pigment epithelium, which is specially thick just here in this specimen.

In this eye also the walls of a small capillary blood-vessel could be seen running straight inwards and across the eye from the choroid split, and losing itself in the retina, somewhat similar to the eyes of Proteus [**6**, p. 86] and Typhlichthys [**1**, p. 576]. No blood was present in it.

Stage III.—A choroid fissure filled with connective tissue was present in some cases, but no vitreous chamber (cf. Amblyopsis [**1**, Taf. xiii]) nor differentiation of cells of any kind within the eyeball, and no nerve fibrils. This represents the lowest stage of degeneration, to which the eyeball has reached in any of these blind vertebrate eyes.

No Müller's fibres nor their nuclei, rods, nor cones have been seen under any conditions in Notoryctes. There seems to be a marked tendency in all the preparations of Notoryctes eyes for the retinal cells under the influence of reagents to separate away from the enclosing pigment (figs. 1 and 4).

Of optic nerve-fibres the most careful examination has failed to show a trace other than the fibrous appearance in one eye noted above, and seen in fig. 4 (*o. n.f.*); though in one specimen, in which the whole conical capsule was mounted intact and unstained, there is an undoubted though very short and faint double line of pigment, ·41 mm. long,

which must certainly indicate the position of the sheath for the nerve. I have examined both brain and eye very minutely, both by means of dissecting lens and microscopic sections through the brain and eye and through the whole head, and have found no further trace of it. In one specimen the dissecting lens showed a connective-tissue connection of the conical eye capsule with the brain, but this is seen to be merely superficial on close examination, and is probably the remnant of the sheath of the optic nerve still connected with the membrane of the brain.

This may be compared with the pineal eye, in which although clear remnants of the eye may still persist in many cases, the nerve is completely wanting. In Amblyopsis [**1**, p. 568], although the optic nerve can be traced to the brain in the young, it is not so in the older form. In Typhlichthys [**1**, p. 574] the nerve is not so distinct in the eye, but can always be traced to the brain (cf. Myxine also [**6**, Taf. iv, fig. 40]). In Siphonops [**6**, p. 114] as in Notoryctes it or its relic can only be seen for a short way from the eye, no connection being found with the brain.

It will be seen that the eye of Notoryctes in all its present stages is much more degenerate than is that of Talpa or Scalops, its analogous forms in other parts of the world. In each of these the vitreous humour, lens, retina, and optic nerve are comparatively well developed, the retina in Scalops being simply over-crowded, while the optic nerve is normal in the adult.

Summary of Structure and Comparisons.

A. Structure.

1. The eye has retired far beneath the skin which passes over it unaltered but for the presence of sense organs (? tactile).

2. A conjunctival sac is present, and the lachrymal glands

are extremely well developed—both being concerned with some function not connected with the power of vision.

3. Eye muscles—abnormal in position, structure, and very variable in development. Their usual nerve-supply is absent, its place being taken by a branch of the ophthalmic nerve.

4. Sclerotic and cornea not distinguishable from one another, nor from the choroid.

5. Lens absent always.

6. Vitreous body is practically absent, even in the least degenerate forms.

7. Pigment layer of retina very thick distally, and thinner proximally.

8. Pupil absent, iris being only represented by a few elongate nuclei in some cases. These also are absent in other eyes observed.

9. Rods and cones are absent, simplified nuclear and molecular layers being present rarely—otherwise the retina is represented by an undifferentiated mass of cells.

10. Optic nerve-fibres. Probable remnants are found only in one case within the eye. They cannot be traced towards the brain except rarely, and for a short distance by its connective-tissue sheath.

B. Comparisons.

To summarise the most important of these.

1. Aquatic Forms.—Of these Troglichthys and Amblyopsis are the most nearly comparable in structure with that of Notoryctes (higher stages). Typhlichthys and Typhlomolge have reached a similar condition in many points, but the muscles in each are more degenerate, and there is no conjunctival sac or lachrymal gland, and in Typhlichthys the nerve can always be traced to the brain.

The eye of Proteus is, in all points, more highly developed than that of Notoryctes, the retina only being similar to that of the higher stages of this form.

2. Burrowing Forms.—In Siphonops we find most of the parts such as the muscles, iris, and lens well developed,

as also the gland and duct, which leads either into a conjunctival sac, or direct into the nasal cavity. The optic nerve, on the other hand, is about in the same stage of degeneration as in Notoryctes, there being no connection with the brain.

In Typhlops all parts are comparatively well developed, the large glands, with their blind ducts towards the skin, being, however, very like those of Notoryctes, though the destinations of the internal ducts are different.

In Rhineura the most degenerate of previously-described eyes in burrowing animals the muscles and conjunctival sac are absent, though the gland is well developed. The lens is found but rarely, and a single cartilaginous bar is present in the sclero-choroid.

In the presence of a choroid split, and the general structure of the retina, this eye resembles the higher stage of that of Notoryctes, and, in the absence of an iris epithelium, the lowest stage.

Scalops and Talpa are so much less degenerate than Notoryctes that a comparison is needless.

It is, therefore, not possible to compare the eye of Notoryctes with that of any other animal in toto, but, omitting the lachrymal glands and ducts, which are well developed in all burrowing animals, and the muscles connected with the fibrous capsule, its higher stages are almost identical with that found in Troglichthys, which is regarded by Eigenmann as the most degenerate of vertebrate eyes. Thus it will be seen that the more degenerate condition found at present in Notoryctes is without a parallel among the Vertebrata, consisting simply of a fibrous sclero-choroid containing a hollow pigment ball filled with a mass of cells, devoid of all arrangement, and without any nerve or blood supply.

The presence of such abnormally well-developed lachrymal glands and of the ducts in all burrowing animals—except Scalops and Talpa, where they are as yet unaffected—is most interesting. The blind outer duct present in Typhlops, and

sometimes in Notoryctes, remains in the less degenerate forms as an indication of the path along which the conjunctival sac was drawn inwards by the retreating eye. This duct doubtless, as now in Talpa and Scalops, formed for some time a last direct communication with the exterior. This later on was lost, since evidently it was not of much use to the animal, the sole remaining escape for the secretion of the gland then being through the nasal duct into the nose. The action of the muscular layer round the conjunctival sac, as also that of the muscle bands which are attached to the conical capsule, would be, by their contraction, to increase the pressure on the conjunctival sac, directly and indirectly. Their common innervation with the gland alveoli also suggests that possibly efferent motor fibres may be associated with the efferent secretory fibres of the latter. The gland must have now a considerable functional value, since, with increasing degeneration of the eye and closing of the direct passage to the exterior, the gland has increased in size.

Presumably the present function of the secretion is—(1) to keep the snout and nasal cavity moist, and (2) chiefly, to hinder the entrance or accumulation of particles of sand in the nasal cavity when burrowing, as this animal does so rapidly in the fine sand in which it lives.

Indeed, this cavity is often so full of coagulated secretion that at first, in sections, no cavity at all can be found.

The least degenerate of the eyes which I have been able to examine is that in fig. 1, in which the gland is small, the blind duct of the skin much longer, larger, and more definite, while the appearance of the nerve-fibres is absent in every other case—and there is no doubt whatever that, as the eye becomes more degenerate, so the gland increases in size and importance.

Conclusions.

Eigenmann [1, p. 546] remarks:—"It must be apparent that an experiment on a vast scale has been conducted by nature, leaving us but to read the results. Moreover, the

experiment is one of evolution without the assistance or intervention of natural selection." This latter statement may or may not be true in the case of the blind fishes with which he is dealing. To explain their degeneration he invokes the aid solely of "disuse" of the eyes in "animals already predisposed to shun the light, or creep under rocks or into crevices" [**4**, p. 535].

But there can be no question of use or want of use in the eyes of Notoryctes, which "is, in reality, a more surface animal than the European mole," as already pointed out by Professor Spencer in the report of the Horn Expedition [**9**, p. 51].

Here several factors have come into play.

First, as the result of natural selection, degeneration has taken place because the presence on the surface of the head of such a sensitive structure would be deleterious to the animal. "The fine grains of sand through which it burrows would have been a fruitful source of irritation, resulting constantly in the production of inflammation," and, as Professor Spencer continues, "more than counter-balancing the advantage to be gained from the possession of an eye when it did come to the surface" [**9**, p. 51].

Second, when the eye had receded beyond the reach of irritation this factor would no longer operate. Then the fact of disuse may have operated to intensify the degeneration already well advanced, at all events in the accessory parts, such as the eyelids and character of the muscles.

Thirdly, the great degeneration of the eyeball itself with its nervous structures has undoubtedly gone on side by side with the great development of the gland structures both in the eye region and in the nasal region [see **12**] itself an adaptation to the burrowing habit, and so probably controlled by direct natural selection. Possibly the used organ has developed at the expense of the food and room of the disused organ by the law of compensation and economy of growth. It cannot be due to merely disuse or diminished use from want of light, since disused organs that are not concerned in

the struggle for room or food may maintain themselves for a long time.

Eigenmann [**1**, pp. 600, 601] doubts the efficacy of the struggle for room and food in the degeneration of the eyes of the Amblyopsidæ, since the position and room formerly occupied by the eye is now filled with fat. But in Notoryctes we found very little fat, and that only in one specimen, while on the other hand the more degenerate the eyeball itself the more highly developed the gland arrangements were found to be.

As to the object of the burrowing of the animal we must conclude that it is mainly for food, as suggested in the Horn Expedition Report, and as a means of escape from its enemies.

The loss of the eye as a means of knowledge of the external world is compensated for by the great sensibility of the animal to sound, and probably from the presence of the supposed tactile sense-organs to touch.

Its relation in point of degeneracy to Scalops and Talpa is interesting, since we may regard it in two ways. Either the eye of the Marsupial has had a longer time since it took to burrowing life in which to become reduced than has that of the insectivorous European Talpa or American Scalops, or, more probably, the sand in which Notoryctes lives is, and has been, more deleterious to the eye than the earth in which Scalops and Talpa burrow, and so degeneration has gone on more quickly in Notoryctes.

Professor Spencer suggests that this view is strengthened by the evidence as to the recent past of Central Australia. There we find deep gorges and broad river valleys comparatively intact, though now dry except on rare occasions, showing that this region was favoured with a more liberal rainfall at no very great distance of time. Then when the conditions became dryer the decomposition of the rocks and the wearing of their débris produced a finer and finer sand. Probably, up to this time, Notoryctes was a burrower with its eyes in a condition comparable to those of Talpa, and as the sand be-

came finer and the temperature higher, its eye would still further degenerate very rapidly with the increased liability to irritation. This also confirms the previously expressed opinion with regard to the cause of the degeneration of the eye of the Notoryctes.

In the eye of Notoryctes, then, we have an example of a very specialised sense-organ, degenerating in virtue of its environment, losing its original sensory function, and assuming an importance of quite a mechanical nature, also rendered necessary by reason of the habitat, and, further, this transference of function is even now in an incomplete and transitional stage, though it has in some animals reached a point of reduction not known in any other Vertebrate eye.

I understand that the brain of the Notoryctes is being investigated, and it will be of great interest to note whether the sensory nuclei in the optic centres have kept pace with the degeneration of their peripheral end-organ.

Biological Laboratory,
Melbourne University;
February 28th, 1906.

Bibliography.

1. Eigenmann, C. H.—"The Eyes of the Blind Vertebrates of North America," I. Amblyopsidæ, 'Archiv für Entwickelungsmechanik der Organismen,' Band viii, Heft 4, 1899.
2. Eigenmann, C. H.—"The Eyes of Typhlomolge rathbuni, Stejneger," 'Contributions from the Zoological Laboratory of the Indiana University,' No. 29, 'Trans. Amer. Mic. Soc.,' vol. xxi.
3. Eigenmann, C. H.—"Typhlotriton spelæus," 'Biological Bulletin,' vol. ii, No. 1, 1900.
4. Eigenmann, C. H.—"The Eyes of Rhineura floridana," 'Proc. Wash. Acad. Sci.,' vol. iv, Sept., 1902.
5. Eigenmann, C. H.—"History of the Eye of the Blind Fish Amblyopsis," 'Mark Anniversary Volume,' Art. ix, 1903.
6. Kohl, C.—"Rudimentäre Wirbelthieraugen," 'Bibliotheca Zoologica,' Bd. v, Heft 13, 1892.

6[1]. Kohl, C.—"Rudimentäre Wirbelthieraugen," 'Bibliotheca Zoologica,' Bd. v, Heft 14, und W. Nachtrag, 1893, 1895.

7. Klein.—'Quart. Journ. Mic. Soc.,' vol. xxi.

8. Slonaker, J. R.—"The Eye of the Common American Mole (Scalops aquaticus machrinus)," 'Journ. of Comparative Neurology,' vol. xii, 1902, p. 335.

9. Spencer, W. B.—"Report of Horn Scientific Expedition to Central Australia," Pt. ii, 'Zoology.'

10. Stirling, E. C.—'Trans. Roy. Soc. S. Australia,' 1888.

11. Stirling, E. C.—'Trans. Roy. Soc. S. Australia,' 1891.

12. Sweet, G.—'Proc. Roy. Soc. Victoria,' vol. xvii (N.S.), Pt. 1.

EXPLANATION OF PLATE 31,

Illustrating Miss Georgina Sweet's "Contributions to our Knowledge of the Anatomy of Notoryctes typhlops, Stirling. Part III.—The Eye."

Reference Letters.

c.n. Nodule of cartilage. *c.s.* Conjunctival sac. *e.d.* Blind external duct. *f.c.* Conical fibrous capsule. *l.g.* Lachrymal glands. *l.g.d.* Lachrymal duct. *m.* Muscle bands. *m.l.* Internal molecular layer. *o.n.f.* Optic nerve fibrils. *p.* Pigment layer. *p.c.e.* Fibrous capsule of eye = sclero-choroid. *r.r'.* Cells representing retina. *s.a.* Small artery. *v.* Veinlet.

All figures were drawn with the aid of the camera lucida, and Zeiss lenses were used.

Fig. 1.—Longitudinal section of eye of Notoryctes typhlops, showing long tube (*f.c.*) containing two muscle bands (*m.*) at this level, lachrymal glands (*l.g.*) and ducts (*l.g.d.*), with the precornеal space (*c.s.*); the eye itself with pigmented wall (*p.*), and remains of retina (*r.*), and surrounded by its own capsule (*p.c.e.*); two small arteries (*s.a.*) and veinlet (*v.*); also nodule of cartilage (*c.n.*) posterior to the eye. Zeiss A*, oc. 2.

Fig. 2.—Longitudinal section of anterior portion of conical tube enlarged, showing same parts as Fig. 1, and, in addition, the longest of the external

ducts (*e.d.*) running out towards the skin and ending blindly beneath it. Zeiss A, oc. 4.

Fig. 3.—Transverse section taken across the tube through the anterior part of the eye, showing parts as above, and, in particular, the close relationship of the precorneal space to the sclero-choroid capsule. Zeiss A, oc. 4.

Fig. 4.—Longitudinal section of small portion of outer tube, showing same parts as in Fig. 1, but more enlarged. This figure is compiled from two consecutive thin sections, and shows more clearly than does Fig. 1 the fibres (*o.n.f.*) running from front to back of eye and through the pigment wall. Zeiss A, oc. 4.

Structure and Origin of Canker of the Apple Tree.

By

James E. Blomfield, M.A., M.D.(Oxon.).

With Plate 32.

The object of the present communication is to give an account of the structure and origin of the tumours produced on the apple by the woolly aphis, Schizoneura lanigera.

These tumours are familiar objects in many orchards, and are well known to gardeners who call the disease "canker."

My reason for investigating these tumours and their mode of origin arose from the circumstance that in examining a transverse section stained differentially in carmine and methyl green there appeared to be a transition of the wood cells into the tumour cells suggestive of a malignant process. The literature of vegetable pathology, as far as I could gain access to it, gave me no help in discovering the meaning of this appearance. Prillieux had described the structure of the tumour in 'Bull. de la Soc. Bot. de France,' 1875. His researches are quoted by Küster in his 'Pathologische Pflanzen Anatomie,' but this authority notes that the subject of wood-galls requires more exact investigation. This book, published in 1903, gives exhaustive references to previous observations in vegetable pathology, and I think that I am justified in concluding that there is no satisfactory account of how these tumours originate.

The insect which produces this disease is an aphis of a purplish-red colour. Crushed between the fingers it leaves a blood-red stain, whence it derives its German name of Blood-louse, but its chief characteristic which makes its presence easily noted is a fluffy covering of a white or greyish colour, sticky and resinous in consistence which exudes from tubercles on the back of the insect, and which, from the fact that it is insoluble in water, serves to protect the insect from wet and damp, and to make difficult its eradication from an orchard when once it is seriously invaded by the aphis. The material is soluble in alcohol, and I imagine, though I can speak from no practical experience, that the reason that insecticides are so useless is owing to the fact that this resinous material is not removed before they are applied.

The general structure of the Schizoneura is the same as that of other aphides except that the cornicles are atrophied. The rostrum in the immature forms is as long as, or longer than, the body. The rostrum or haustellum is as in other Hemiptera an extension of the labium. It consists of three joints which are grooved on their upper surface to receive the setæ or lancing organs which prick the juicy parts of plants to cause a flow of sap on which the insects feed. The setæ are three in number, and represent the mandibles and maxillæ of other insects. During the act of sucking the rostrum is closely applied to the plant surface and secured there by coarse hairs at its tip, the setæ are run along their groove into the soft plant tissue, which they lance and stab to ensure a flow of sap which can be sucked up partly by capillary attraction and partly by a pumping action on the part of the insect.

The question whether salivary glands are present in all aphides appears to be yet unsolved. They were found by Buckston ('Brit. Aphides,' Ray Society) in some specimens, the name of which he does not give, but in connection with the present subject the matter would seem to have some importance because it is to the secretion of some such gland that we must look as a cause for the peculiar action of the

gall-producing Aphides, Lachnus, and Schizoneura. The common aphides of the ivy and the rose prick and suck the soft young stems, but there is no specific reaction on the part of the plant such as we see in the case under consideration.

The natural history of the Schizoneura is as follows. During the winter months the mature insects find shelter in the cavities and crannies of the nodosities, and in the early days of spring their presence is noticeable from the patches of white fluff. These increase in size owing to growth in number of the insects, and as the summer advances masses of sticky fluff envelope the branches in which are found crowds of aphides. Some of these become "nurses" and produce living young parthenogenetically. A new generation is said to be produced every fourteen days, and as the young twigs of the tree grow new colonies are founded. The place where the colony is started on the twig of the year does not appear to be a matter of chance but rather of selection. At the time that the twigs are invaded the young leaves are well formed, the distal end of the stalk is green, but towards the parent tree the green passes into a reddish-brown colour indicating the formation of a periderm, which, in the case of the apple, is derived from the epidermis. The place of selection is not the green portion of the twig at the extremity, but nearer to the old wood at a point where the wood has definitely formed and the formation of the periderm commenced. Some of the "nurses" descend to the roots as in the Phylloxera and establish colonies there producing deformities similar to those on the stem.

If a tumour is selected for study on which the aphides are actively feeding, and after fixing, hardening, etc., sections are prepared and stained in a manner to differentiate the tissue, such as by iron hæmatoxylin method and fuchsin, the appearance represented by fig. 3 will be seen.

On the outside is a layer of cells two or three in thickness, which is the periderm, beneath this is the cortex with strands of sclerenchyma. If we follow the cortex over the tumour we shall see that it has undergone slight alteration only, a

stretching and thinning from the growth that is taking place beneath it. The sclerenchyma bundles are, however, less numerous and less defined. Beneath the cortex is a layer consisting of bast and cambium, the separation of which is marked in all parts except over the tumour, where no distinction can be made. In the centre of the section is the pith, surrounded on all sides by the wood, the continuity of whose ring is interrupted by the tumour which dips into the wood ring in a wedge-like manner. As a rule it does not reach the pith unless the section has passed near a leaf-shoot, which may happen, because a favourite place for the young colony is just above a leaf-shoot which serves to shelter and protect it from wind and rain, both of which are very disastrous for the propagation of the species.

In such a section as I have described all lignified cells are stained pink with the fuchsin, and it is easy to distinguish the soft cellular cells of the tumour from the lignified cells of the wood. If examination is now made with a higher power it will be found that no hard and fast line separates the tumour from the wood, but that the tumour cells seem to arise by alteration of the wood parenchyma cells. Among the tumour cells, however, will be discovered pink-stained, large, irregular cells, which evidently are altered, pitted, and scalariform wood-vessels, enlarged in size, and irregular in shape. These must have arisen from altered cambium cells. The general arrangement of the cells of the tumour is in a radial direction. Large oblong nuclei can be seen in each cell, and in some cases there are several in each cell, a fact which was pointed out by Prillieux. The soft walls of the cells are composed of cellulose, as shown by Schultz reagent. There is hardly any starch present, as revealed by iodine, but there is sugar in relatively large quantities. If a section is made of a fresh tumour, and tested with Fehling's solution, this fact comes out plainly by the reduction that takes place in the tumour. A rough quantitative estimation showed 1 per cent. of sugar in the tumour, while small fragments from the same stem hardly yielded any reaction at all. A

small quantity of coagulated plasma may be found in each cell (fig. 4).

If the cambial region of the tumour is examined with a high power it is evident that this tissue is in a state of great activity. In appropriate sections the tracks left by the setæ of the insect may be traced through the periderm and cortex till they terminate in the cambium, and it is around these terminations that the greatest activity is taking place. The distinction between bast and cambium is made out with difficulty, as both kinds of cells are enlarged, and contain large, well-defined nuclei. In places the division of the nuclei exceeds in rapidity that of the cells, with the result that a multinucleated mass is produced. This cell division no doubt takes place by mitosis, evidence of which I obtained, but the material is difficult to cut with sufficient accuracy for a study of this process. After the cells are produced by the cambium further division is undergone, or multiplication of nuclei may take place without corresponding cell division. The protoplasm exhibits vacuoles, which increase in size till the whole cell consists of a wall of cellulose, a small quantity of plasma, with a nucleus and a large quantity of cell sap, consisting chiefly of sugar.

That these changes are produced by the aphides is shown by the fact that, if they are swept away by wind or rain, the cambium resumes its normal activity, and gives rise to cells, which pursue their destiny of lignification in a normal manner, enclosing a portion of the tumour, which itself undergoes lignification, but, from the displacement, increase in number (hyperplasia) and in size (hypertrophy), the elements are abnormally arranged, and produce a condition which is known as wound wood (fig. 6).

If the aphides linger on their tumour and the weather is dry the soft parenchymatous tissue may split and allow a genus of destructive fungi such as Nectria to enter, producing necrosis and ulceration of the tissue. This the plant tries to counteract by its powers of healing, and new cambial tissue is produced, to be quickly utilised by the aphides for

their nourishment, till large gnarled nodosities are produced consisting of dead, necrosed wood, hypertrophied tumours, and wound wood, which may attain the size of a man's fist. It is not necessary that the tumour be split for Nectria to gain entrance. In comparatively young tumours the fungus and its necrosing action may be seen, though there is no breach of the surface except the punctures made by the insect, and it is, no doubt, by these that the germ gains entrance.

To shortly resume the origin of these tumours, we have seen that they are produced by the pricks of the aphides. That during this process some influence is brought to bear on the active cambial cells which leads to their enlargement and increase. That the cells are arrested in their normal development and destiny, and that as long as this influence lasts they serve the purposes of their parasitic victors, that, when these retire, they are able again to pursue their development and destiny, but in such a way that the traces of their experiences are not obliterated.

I have sketched out this view in the diagrams (fig. 7).

What is the agent of this influence which the Schizoneura is able to exercise on the cambium of the young twig? As I noted above, mechanical irritation we must dismiss as a cause, and we can only fall back on the hypothesis of a ferment, such as Beyernick suggested under the term growth enzyme. This may come from the salivary glands of the Aphis.

I have tried to test this question by acting on a suggestion made by Prof. Farmer of inserting a glycerine and water extract of the insects by means of capillary tubes as near the cambium as possible in such a way that the liquid would constantly bathe the cells. No success has followed these attempts. The slight reaction visible at the point of insertion did not amount to more than that produced by a fine wire inserted in a similar way.

EXPLANATION OF PLATE 32,

Illustrating Dr. James E. Blomfield's paper on "Structure and Origin of Canker of the Apple Tree."

Fig. 1 is a photograph of branchlet of an apple representing the growth of three years. Each year's growth, numbered successively 1, 2, and 3, is infected by Schizoneura, and shows the characteristic boil-like swelling.

Fig. 2 is a photograph of two sections, one through a normal stem, and the other through a canker. At * there is a recent swelling caused by Schizoneura.

Fig. 3 is a drawing of a slightly magnified section through a young tumour. A is periderm commencing in the epidermis. B is the cortex. C is a sclerenchyma strand. D is the bast. E is the cambium. F is the wood. G is the tumour with a few large vessels cut across.

Fig. 4 is a portion of a longitudinal section of tumour, × 700, showing the pointed cells (W) which would have been wood-cells, and the square-shaped cells (M) which represent modified medullary ray cells.

Fig. 5 represents a portion of the tumour in the region of the cambium. A are cortical cells. B are modified bast cells. C, modified cambial cells. D, modified wood cells. E, the track of a seta with proliferating cells in its neighbourhood, × 700.

Fig. 6 shows a tumour passing into a condition of wound wood. The enlarged wood cells are twisted, dislocated, and separated by collections of cells, which represent the hypertrophied medullary rays.

Fig. 7 represents in a diagrammatic manner the changes undergone by a cambium cell (A) in becoming wood, and by an affected cambial cell.

The series *a* to *f* represents the normal course; *a'* to *f'* the course under the influence of the Schizoneura, with its cessation after a time

Review of Dr. Richard Goldschmidt's Monograph of Amphioxides.[1]

By

A. Willey,

Hon.M.A.Cantab., D.Sc.Lond., F.R.S.

Among the many acquisitions of the German Expedition to the Deep Sea in the S.S. "Valdivia" (1898—1899), which was organised under the direction of Professor Carl Chun, not the least valuable was the relatively large series (27 specimens) of pelagic Acraniata belonging to the genus Amphioxides, Gill. This material was entrusted to the skilled hands of Dr. Richard Goldschmidt, who may be congratulated on his important monograph, which takes its place in a section of zoological literature associated with the honoured names of Johannes Müller, A. von Kölliker, de Quatrefages, Kowalevsky, Hatschek, Huxley, Ray Lankester, van Wijhe.

Dr. Goldschmidt's memoir will almost certainly tend to enhance the morphological importance of Amphioxus (if it be permitted still to employ this name in a cursory, non-italicized sense), based as it is upon such careful observation and logical deduction. It is, however, frequently necessary to discount the force of logic when dealing with discussions of the kind before us.

The type species of Amphioxides was originally described from a single specimen taken in the Pacific Ocean, under the name Branchiostoma pelagicum by Dr. Günther (1889) in his report on the Pelagic Fishes collected during the "Challenger" Expedition. Further examples from the Indian Ocean have been recorded by C. Forster Cooper

[1] Richard Goldschmidt, "Amphioxides," 'Wiss. Ergebn. der deutschen Tiefsee-Expedition,' Bd. xii, 1905, pp. 92, ten plates and ten text-figures.

(1903),[1] W. M. Tattersall (1903),[2] and G. H. Parker (1904).[3] Two other species have been added by Dr. Goldschmidt, A. valdiviæ and A. stenurus. All three species were taken during the "Valdivia" Expedition at considerable depths in the vertical tow-net in the high seas, often several hundred miles from the nearest coast. Specimens were also obtained in the Atlantic Ocean;[4] the distribution of the genus is therefore circumequatorial, but there is no correlation between the specific forms and their geographical range. In the Bay of Bengal, 300 miles east of Ceylon, twelve examples were captured simultaneously at one station between a depth of 2500 metres and the surface, of which nine belonged to A. valdiviæ, three to A. pelagicus. Off the west coast of Africa A. valdiviæ was taken south of Teneriffe and A. pelagicus in the Gulf of Guinea. Lastly, all three species have been taken in the neighbourhood of the Seychelles.

No sexually mature individual has yet been seen. Dr. Goldschmidt has found the immature gonads developing only on the right side, lying in the gonocœl which is shut off from the ventral ends of the myotomes. No specimens in the "Valdivia" collection exceed 10 mm. in length. At this size the gonads were observed to be as far developed as in a Branchiostoma lanceolatum of 28 mm.

[1] C. F. Cooper, "Cephalochorda," 'Fauna and Geography of the Maldive and Laccadive Archipelagoes' (J. Stanley Gardiner), vol. i, part 4, 1903, p. 352.

[2] W. M. Tattersall, "Report on the Cephalochorda collected by Professor Herdman at Ceylon in 1902," 'Ceylon Pearl Oyster Fisheries,' part 1, 1903, p. 214.

[3] G. H. Parker, "Maldive Cephalochordates," 'Bull. Mus. Harvard,' vol. xlvi, 1904.

[4] It is interesting to note that no examples were procured during the "Plankton" Expedition. Hensen (Einige Ergebnisse der Plankton Exped., 1892, p. 24—25) says that they frequently obtained young Amphioxus lanceolatus up to some centimetres in length, as many as two to ten individuals in one catch of the Plankton Net in the North Atlantic. He notes that it is remarkable that they should remain so long at the surface over great depths, because Amphioxus is a coastal and littoral form, only the larvæ being pelagic in the coastal zone. These observations are important as indicating that the prolongation of the pelagic life does not involve a persistence of the larval asymmetry in A. lanceolatus.

Amphioxides is remarkable for the possession of many characters which are proper to the larva of the European species of Amphioxus, e. g. the absence of a closed atrial chamber, a sinistral slit-like mouth, an unpaired series of gill-clefts which lie in the mid-ventral line, an anterior dextral endostyle, a club-shaped gland, and a sinistral præoral pit. On account of these and some other characters the genus is made the type of a new family, Amphioxididæ Goldschmidt, in contrast with the first family, Branchiostomidæ Bonaparte, 1846.

The characters mentioned above are regarded by Dr. Goldschmidt as being essentially primitive, emphatically not as indications of a persistent larval organisation. In other words, in his opinion Amphioxides is the most primitive Acraniate, and stands more or less in the direct line of Vertebrate descent.

One of the finest additions to our knowledge of the anatomy of Amphioxus which has been made in recent years is Professor J. W. van Wijhe's discovery of the sinistral innervation of the mouth. Anyone who has handled Amphioxus will probably subscribe to this statement. The conclusion drawn by van Wijhe from this discovery and accepted by Goldschmidt, namely, that the mouth of Amphioxus is from the beginning to the end an organ of the left side, may seem to be clearly indicated, and is held by Dr. Goldschmidt to be a fact of primary phylogenetic significance in Amphioxides where the sinistral position of the mouth is said to be dependent upon the structure of the pharynx. It may be mentioned here that my own theory is still what it was fifteen years ago so far as its essential point is concerned, that the mouth, or situs oris, of the lancelet has migrated from a dorsal position such as it holds in the Ascidian larva.

The pharynx of Amphioxides is characterised by the presence in its floor of a median series of gill-perforations to the number of thirty-four, opening directly to the exterior on the ventral side of the body between the metapleural folds. Above the gill-arches the wall of the pharynx projects

inwards as a ridge on each side, delimiting a dorsal pars nutritoria from a ventral pars respiratoria. The gill-openings are simple clefts destitute of tongue-bars, but the gill-arches which bound them are considerably folded, and exhibit a bilaterally symmetrical structure being incompletely divided by a deep median groove into right and left halves. The gill-arches are somital, the gill-slits intersomital, in their topographical relation to the myotomes.

The primitive condition of the pharynx of Amphioxides

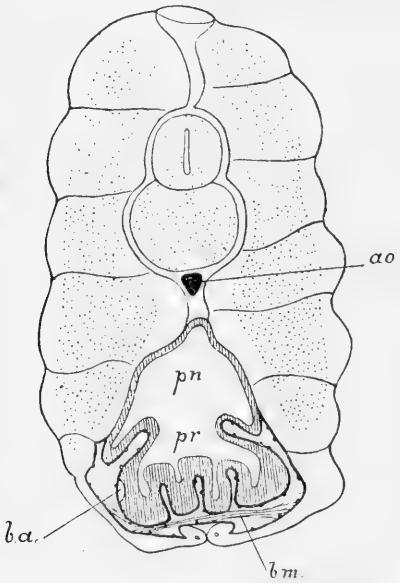


FIG. 1.—A. pelagicus. Section through the middle of the branchial region. After Goldschmidt. *ao.* Aorta. *b.a.* Branchial artery. *b.m.* Branchial muscle. *p.n.* Pars nutritoria of pharynx. *p.r.* Pars respiratoria.

would therefore consist in the median ventral series of unpaired gill-slits, the partial separation of the ventral pars respiratoria from the dorsal pars nutritoria, and, consequent thereupon, the perforation of a sinistral mouth into the dorsal division, and the development of a ciliated glandular organ, the endostyle, opposite to the mouth, also leading up to the dorsal division. The club-shaped gland is

regarded as an accessory organ to the endostyle; it is well developed in Amphioxides, opening into the pharynx behind the posterior end of the endostyle into the dorsal nutritive portion. Goldschmidt has not found an external orifice of the gland such as was first observed by Hatschek (1881), and subsequently confirmed by Lankester and Willey (1890), and Willey (1891) in the larva of Branchiostoma lanceolatum.

Now there is a reflection which must occur to the minds of those who may be conversant with the living larvæ of Amphioxus, and with the extreme contractility of their tissues, which may raise a doubt concerning the fundamental importance of the lateral ridges of the pharynx of Amphioxides as interpreted by Dr. Goldschmidt. There is not a shadow of doubt that the gill-slits and gill-arches of Amphioxides would wear a very different appearance from that which they present in Dr. Goldschmidt's excellent figures if they were seen fully expanded with the body in a state of turgidity, and it seems not unlikely that under those conditions the projecting ridges[1] of the pharynx would vanish and the folds of the gill-arches straighten out.

Again, it follows, from the interpretation of facts which has been outlined above, that the anterior dextral position of the endostyle, with its unequal limbs, is also a primitive feature; but Goldschmidt has not observed in Amphioxides those paired ciliated peripharyngeal bands which proceed upwards and backwards from the anterior ends of the endostyle in the larva of Amphioxus, and offer such a striking analogy with the similar organs of the Tunicata. These bands, while distinctly pointing to an affinity with the Ascidians, also indicate that the endostyle, although asym-

[1] These ridges, the so-called limiting folds or plicæ limitantes, are described as being lined by cylindrical flagellate cells, the long flagellum of which is connected through the cell-body with the nucleus by a clear and deeply staining rod. It seems highly probable, after all, that they are homologous with the peripharyngeal bands of Amphioxus. Forster Cooper also figures them in larvæ of Amphioxides taken at the Maldive Islands.

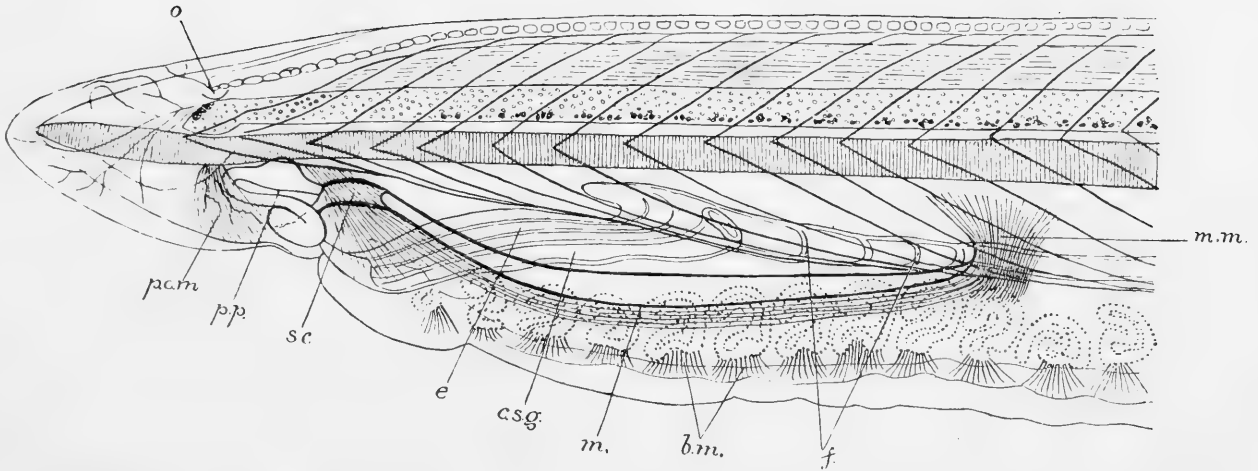


Fig. 2.—A. valdiviæ. Anterior region of the body from the left side. After Goldschmidt, somewhat simplified. *b.m.* Branchial muscles. *c.s.g.* Club-shaped gland. *e.* Endostyle. *f.* Semilunar folds or visceral septa. *m.* Lower lip of mouth. *m.m.* Muscle of lower lip (occlusor oris). *o.* Olfactory pit. *p.c.m.* Constrictor muscle of præoral pit. *p.p.* Præoral pit. *s.c.* Sulcus communicans between præoral pit and mouth.

metrical in the larva of Amphioxus, was originally symmetrical.

The gill-slits of Amphioxides are arranged eumetamerically, that is to say, there is, in general, an exact correspondence between branchiomerism and myomerism in the branchial region. An individual having twenty-nine gill-slits, for example, will have thirty gill-arches, of which the first arch corresponds with the first myotome, while the thirtieth arch lies in the thirtieth segment. This applies to two of the species, A. pelagicus and A. stenurus. In A. valdiviæ repeated counting showed that there were more gill-slits than myotomes in the branchial region, so that in a specimen with twenty-seven gill-arches the last lay in the twenty-third segment, or, more correctly, under the twenty-third myotome. Such a specimen is interpreted as having four supernumerary gill-arches; the highest number observed was seven. The supernumerary arches are considered to indicate the occurrence of prosomital gill-slits in A. valdiviæ. This unexpected interpretation appears simpler in the writing than in the illustration (fig. 2). Those who can appreciate it will be able to make the best use of it. For my part, the lack of coincidence between gill-slits and myotomes in A. valdiviæ appears as an indication of the independence of branchiomerism and myomerism in the Acraniata, however closely they may be correlated.

In A. valdiviæ the ventral halves of the anterior myomeres are bent backwards at a very acute angle in correlation with the great length of the mouth, 1—1·4 mm. (fig. 2). This again strikes me as being a slight myomeric disturbance independent of the gill-slits.

Above the gill-arches and between every two gill-slits the pars nutritoria of the pharynx is fused with the body-wall, the lines of fusion coinciding with the dissepiments of the myotomes. Between two lines of fusion the body-cavity extends upwards as a pouch to the base of the notochord. Seen from the side, the lines of fusion appear as semilunar folds (fig. 2). This is the inter-segmental concrescence of the

gut with the myosepta, in other words, a partial segmentation of the ventral mesoderm.

The metapleural folds closely resemble the corresponding structures in the larva of B. lanceolatum; the right fold is larger than the left (especially in A. valdiviæ), and reaches farther forwards. Goldschmidt finds, in general accord with previous observations on Branchiostoma by MacBride (1898) and van Wijhe (1902), that in Amphioxides the pterygocœl or metapleural lymph-space communicates in front by a fine opening with the general body-cavity, on the left side behind the mouth, on the right side in front of the mouth. Posteriorly the metapleural folds terminate freely, directly behind the last gill-slit, and are thus independent of the median ventral fin as in Branchiostoma. Goldschmidt says that they cannot serve as equilibrating organs in Amphioxides on account of their ventral position and asymmetry; he thinks they are gill-covers and can become turgid by fluid-pressure, thus approximating together and closing under the gill-clefts, synchronously with the respiratory movements. This view, however, is not confirmed by observations on the living larvæ of Branchiostoma. Another suggestion made by Dr. Goldschmidt may conveniently be mentioned in this place. He says (p. 32) that in life the lateral folds of the pharynx (plicæ limitantes) may be capable of being pressed together and so prevent food from falling into the pars respiratoria. I think this is highly improbable, and our author seems to overlook the circumstance that the ingestion of food and the respiratory current in Acraniata are alike effected by ciliary action.

From the arrangement of the branchial musculature which is described in detail, Goldschmidt deduces a mechanism of breathing by expansion and reduction of the body-cavity, analogous to lung-breathing. As I have stated above, however, the respiration of Branchiostoma, like that of Ascidians, is primarily promoted by ciliary currents, not by muscular contraction. The branchial muscles serve only for the protection and regulation of the branchial apparatus.

Their contraction occurs under stimulus, but, far from inducing respiratory currents, it temporarily inhibits them. In point of fact rhythmic muscular respiratory movements have not been observed in Acraniata.

The gill-arches of Amphioxides (fig. 3) possess the essential qualifications of a true vertebrate gill-arch, namely, the endodermal pharyngeal epithelium; the ectodermal portion of the body-wall; the branchial cœlom; the branchial muscles, which are true visceral muscles derived from the wall of the cœlom. They appear, in the preserved material, to pro-

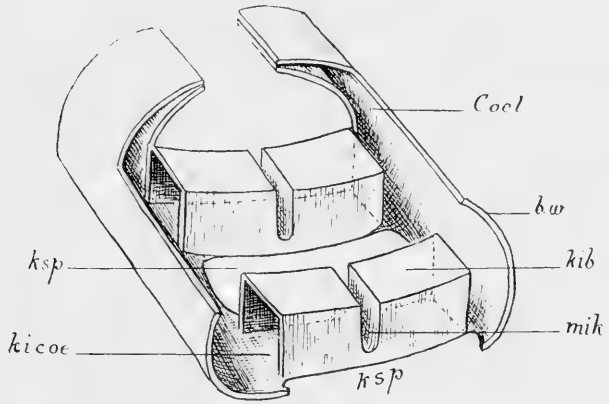


FIG. 3.—Diagram representing the structure of the branchial apparatus of Amphioxides. The ventral half of the body with the pars respiratoria has been exposed by a frontal incision. From Goldschmidt. *ksp.* Gill-slit. *kib.* Gill-arch. *mik.* Median furrow of gill-arch. *ki.coe.* Cœlom of gill-arch. *Coel.* General cœlom. *b.w.* Body-wall.

ject into the pharynx like hollow sacs between the gill-slits, each arch being apparently divided incompletely into right and left compartments by a median groove, and the suggestion is made that this bilateral disposition may be a stage towards the duplication of the slits. The respiratory epithelium is described as a many-layered ciliated epithelium; a similar appearance may be noted in other species, but it has been shown, in the first place by Langerhans, that

the branchial epithelium consists of very high filiform columnar cells in which the nuclei occur at different levels; it is probable that the appearance of stratification is still further increased by the compression of the epithelium as a consequence of contraction.

What may be described as a sensational conclusion is that which proceeds from the author's comparison of Amphioxides with Branchiostoma, i.e. that the so-called secondary gill-slits which suffer a retardation of development in the larva of Branchiostoma are heterogeneous formations, not homologous with the primary series. Of course this conclusion is not rendered in an arbitrary manner, but is led up to by a number of arguments based upon the grand assumption that Amphioxides alone is primitive, and that the larval development of Branchiostoma points no farther back, but is a mere recapitulation of the characters of the original pelagic Acraniate as represented by the former genus.

Dr. Goldschmidt adopts the method of assuming that his form is primitive, and then explaining the facts on that assumption; and he claims to explain all the facts, whereas other theories only explain some of them. But Amphioxides may be as highly adapted to a pelagic life as Branchiostoma to a benthonic life. I am aware that this is an easy objection, but it is none the less true. In most or many other sharply defined orders, those forms in which an entire organic system is functionally deranged or obsolete, are not usually the most primitive. A simple example is the eyeless condition of many cave animals, deep-sea animals, and othe cryptozoic forms; another is the limbless condition of some Teleostean Fishes, Batrachia, and Reptiles. They may be primitive in other respects, but not in respect of their lack of parts. Perhaps a closer analogy is afforded by the pelagic Tunicata of the class Copelata which, like the Acraniata, comprises two families, Kowalevskidæ and Appendicularidæ, the former characterised by the absence of an endostyle, but it is not suggested that the Kowalevskidæ

are the more primitive on that account.[1] In the same way it seems to me to be improbable that Amphioxides is primitive in respect of the absence of an atrial chamber, of antimeres to the gill-slits, and of the hepatic cæcum.

Another unexpected conclusion to which Dr. Goldschmidt has been led by his researches is the virtual denial of the homology of the hepatic cæcum of the other Acraniates with the liver of Craniota, but he does not refer to recent work on this organ.[2]

Turning now to the cavities in the rostrum of Amphioxides we find a remarkably clear account of their relations to each other and of their connections with the cavities (myocœl) of the first pair of myotomes. The special character of these myotomes, as compared with those which follow, has been pointed out by Hatschek (1881), and more recently by MacBride (1897), who compared them with the collar cavities of Enteropneusta.

The diagram (fig. 4) and the transverse section (fig. 5) illustrate the arrangement, which is sufficiently elucidated in the explanation of the figures. Perhaps the most important fact to note is that the ventral rostral cavity is that which represents the right head-cavity of the embryo; it is aptly described as the ontogenetic partner of the præoral pit, for which it provides a splanchnocœl and a visceral musculature.

From the mode of branching of the cephalic nerves, their relation to the ventral rostral cavity, and the alleged prosomital gill-clefts of A. valdiviæ, Dr. Goldschmidt deduces a scheme of the segmentation of the head, for the particulars of which the reader should consult the original monograph. I regret that I cannot follow it myself, chiefly because I cannot believe that there is such a fundamental difference between closely-allied species as would follow if it were actually true that A. valdiviæ is the possessor of pro-

[1] Cf. H. Lohmann, 'Die Appendicularien der Plankton-Expedition,' Kiel and Leipzig, 1896.

[2] Guido Schneider, "Einiges über Resorption und Excretion bei Amphioxus lanceolatus, Yarrell," 'Anat. Anz.,' xvi, 1899, pp. 601—605.

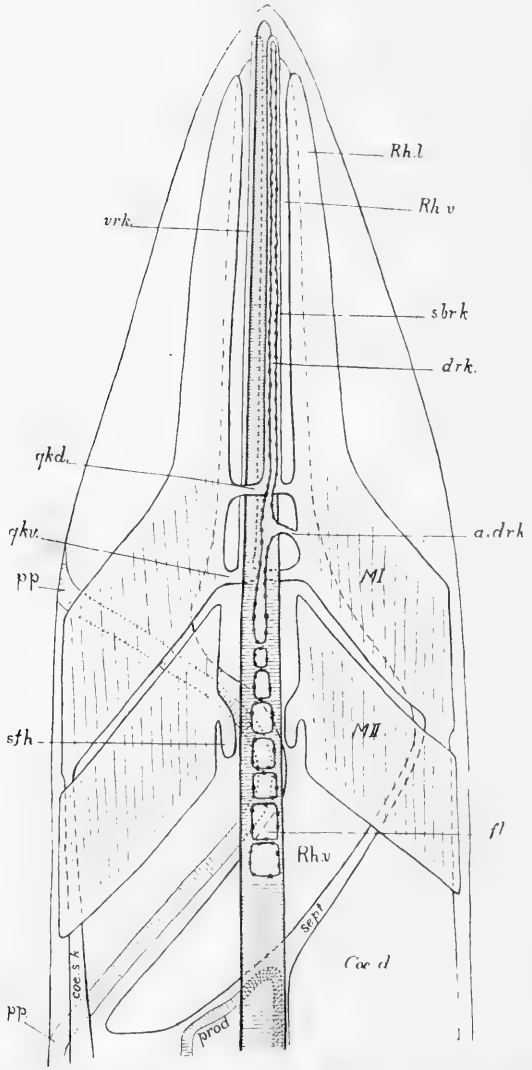


FIG. 4.—A. valdiviæ. Diagram of anterior end from above to show the relations of the rostral cavities. After Goldschmidt, somewhat simplified. *Rh.l.* Lateral rostral cavity proceeding from the first myocœl. *Rh.v.* Ventral rostral cavity. *vrk.* Ventral rostral canal proceeding below the notochord from the ventral canalis communicans (*qkv*) between the first myotomes. *sbrk.* Subdorsal rostral canal proceeding above the notochord from the dorsal canalis communicans (*qkd*). *drk.* Dorsal rostral canal continued

somital gill-clefts which are lacking in the other two species.

In his description of Hatschek's nephridium Dr. Goldschmidt records the important discovery of the presence of solenocytes in this small tube, which occurs sinistrally under the notochord, and opens into the præoral portion of the gut. In a specimen of 8 mm. it attains the considerable length of half a millimetre. It is closely applied to the anterior end of the aorta, so that only a thin membrane separates the two structures. The part of the tube in the vicinity of the orifice into

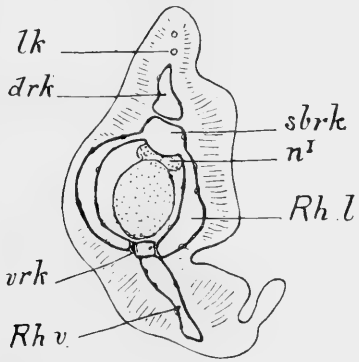


FIG. 5.—A. valdiviæ. Section through rostrum. After Goldschmidt. *lk.* Lymph-canals which arise from the dorsal rostral canal (*drk*). *sbrk.* Subdorsal rostral canal at its point of origin from the dorsal canalis communicans between the lateral rostral cavities, *Rh.l.* (Anterior ends of cavities of first myotomes.) *vrk.* Ventral rostral canal. *Rh.v.* Ventral rostral cavity.

the præoral gut consists of high cubical cells. Dorsally this epithelium ceases, and gives place to isolated large round

---

forwards from the fin-chambers (*fl.*), and communicating by a canal on the right side only (*a.drk*) with the cavity of the first myotome. *M.I* and *M.II.* First and second myotomes. *sept.* Septum between the ventral rostral cavity and the splanchnocœl. *Coe.d.* Anterior end of the splanchnocœl on the right side communicating with the first myocœl. *coe.s.k.* Anterior cœlomic canal placing the splanchnocœl in communication with the first myocœl on the left side. *prod.* Præoral termination of the gut. *p.p.* Anterior and posterior borders of the præoral pit. *sfh.* Sclerotome diverticulum of the second myocœl.

cells which lie upon the membrana limitans of the aorta. These latter cells are identified as solenocytes, since each of them gives off peripherally a long and delicate tubule, which passes straight across the lumen of the nephridium to the opposite wall, where it penetrates between the cells of the cubical epithelium. The solenocytes occur along the entire dorsal wall of the organ, and all the tubules converge towards the orifice, from which numerous fine long flagella depend into the præoral gut; these are the flagella of the solenocytes. Goldschmidt therefore defines Hatschek's nephridium as a portion of the cœlom constricted from the left head-cavity (which gives rise to the præoral pit or præoral organ), and effecting a communication with the præoral gut by a pronephric

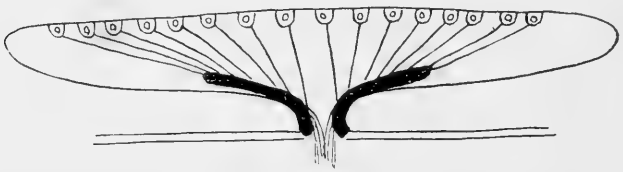


Fig. 6.—Diagram of Hatschek's nephridium, showing solenocytes and the orifice into the præoral gut. After Goldschmidt.

canal. It thus appears that Hatschek's nephridium has a structure analogous to that of Boveri's tubules as corrected by Goodrich.

Dr. Goldschmidt has found no other excretory tubes in Amphioxides, but describes some structures which he calls "Schwammkörper" occurring segmentally on the left side at the dorsal recess of the body-cavity in the region of transition between pharynx and intestine; they appear as a feltwork or mass of spongy tissue with nuclei and solenocyte tubules.[1]

Noteworthy features in the vascular system of Amphioxides are the absence of a portal system, the presence of an unpaired aorta, and especially the fact that the branchial artery, which is a direct continuation of the sub-intestinal

[1] Possibly they have some relation to Lankester's brown funnels (see also Burchardt, 'Jena Zeitschr.,' 1900).

vein, is displaced to the right side. In front of the pharynx the branchial artery bends up more dorsally and ends blindly, co-extensive with the aorta and the præoral gut.

One word concerning the classification adopted by Dr. Goldschmidt. He only recognises two genera of Branchiostomidæ, namely, Branchiostoma, Costa, 1834, and Epigonichthys, Peters, 1876. Opinion may be reserved regarding the necessity of abolishing certain other generic terms which have been introduced, but the resuscitation of Epigonichthys is clearly correct.

A great deal more information is contained in Dr. Goldschmidt's monograph than what I have outlined above. In order to render the presentation of the portion of his theoretical excursions which I have selected for criticism more complete, it should be added that he traces the origin of what he considers to be the primitive condition of the pharynx as determined by the ventral series of gill-slits, to the secondary extension of the segmental musculature towards the ventral side; this circumstance (and here he is in agreement with Boveri) would also account for the existence and peculiar method of development of the atrial chamber of Amphioxus. It may be admitted that there is very likely a good deal of truth in this correlation when regarded from the point of view of the mechanical conditions of development, without prejudicing supposed morphological or phylogenetic relations one way or the other.

By the courtesy of the Cambridge University Press[1] I am able to reproduce a set of diagrams which may serve a useful purpose as indicating different points of view, and thus helping to clear the issues.

In a recent article Professor van Wijhe[2] states that I have

[1] 'Zoological Results' (A. Willey), part vi, 1902, p. 728. The matter is introduced into that portion of my "Contribution to the Natural History of the Pearly Nautilus," which is devoted to "Personal Narrative." I take this opportunity of noting an unfortunate misprint on page 800 of that publication, where the word "Branchial" should have been "Brachial."

[2] J. W. van Wijhe, "Die Homologisirung des Mundes des Amphioxus und die primitive Leibesgliederung der Wirbelthiere," 'Petrus Camper,' April, 1906, p. 17 of reprint.

confused topographical with morphological conceptions in regard to the organ of fixation in the larva of Ciona intes-

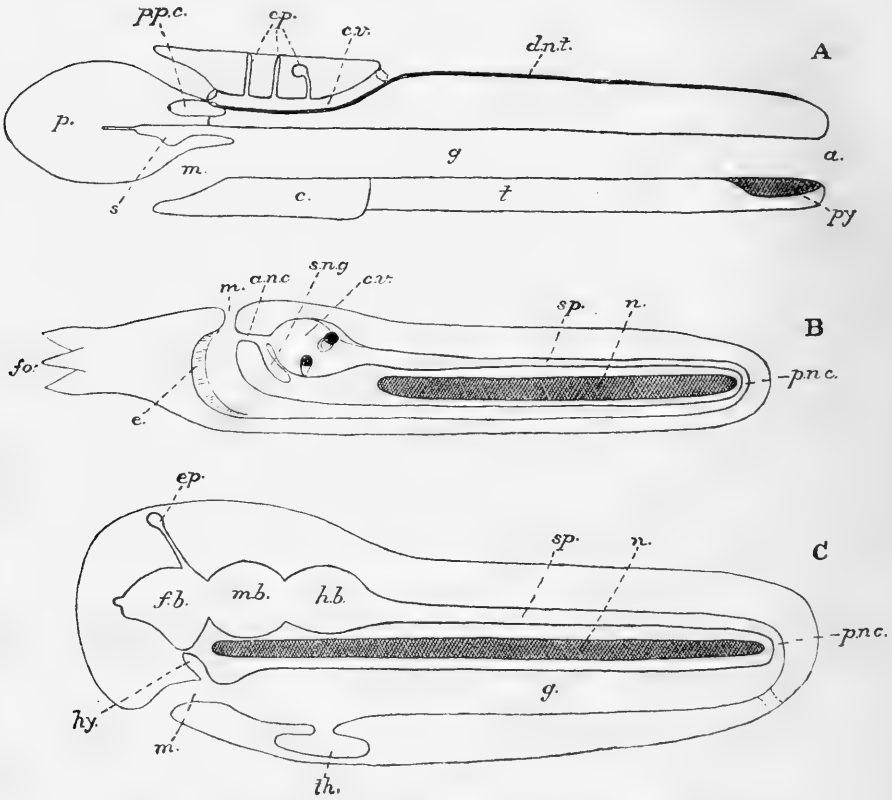


FIG. 7.—Diagrams of an Enteropneust (A), an Ascidian larva (B), and a Craniate embryo (C). After Willey ('Zoological Results,' part vi, 1902), by permission of the Cambridge University Press.

A.—*p.* Proboscis. *p.p.c.* Proboscis pore-canal opening externally close to the anterior neuropore. *c.v.* Collar nerve-tube. *ep.* Epiphysial roots. *s.* Stomochord. *m.* Mouth. *c.* Collar region. *g.* Gut. *t.* Trunk region. *d.n.t.* Dorsal nerve tract. *a.* Anus. *py.* Pygochord.

B.—*f.o.* Organ of fixation. *e.* Endostyle. *m.* Mouth. *a.n.c.* Anterior neurenteric canal. *s.n.g.* Subneural gland. *c.v.* Cerebral vesicle. *sp.* Medullary tube. *p.n.c.* Posterior neurenteric canal. *n.* Notochord.

C.—*ep.* Epiphysis cerebri or pineal organ. *f.b.*, *m.b.*, and *h.b.* Fore-, mid-, and hind-brain. *hy.* Hypophysis cerebri. *th.* Thyroid gland. Other letters as above.

tinalis which I have likened to a præoral lobe. I may be allowed to remark that whether or not there has been any objective confusion, there has at least been no unconscious mental confusion on my part on this particular point.

The præoral lobe or proboscis should, I suggest, be regarded as an axial organ, forming part of the normal body-length, neither dorsal nor ventral. The functional situs oris is determined by special factors (such as its relation to the anterior neurenteric canal in the Tunicate larva) and should be considered on a basis of its own. The mouth may be dorsal, ventral or lateral in actual position. That the præoral lobe is essentially axial is indicated by the manner and order of its development in the embryos of Acraniates and Enteropneusta, and also in the regeneration of the proboscis of the latter (see Dawydoff, "Ueber die Regeneration der Eichel bei den Enteropneusten," 'Zool. Anz.,' xxv, 1902, pp. 551–6).

In conclusion, as to the relation of the Acrania to the Ascidians, Dr. Goldschmidt is of the opinion that the developmental tendency leads from Amphioxides to Amphioxus, and beyond this in a straight line to the Ascidians, whose organisation appears to him to have arisen by degeneration from the Acrania. In opposition to this theory I submit that the Ascidians have degenerated from an extinct cœlomate perennichordate type, but not from a cephalochordate type.

When, however, Dr. Goldschmidt asserts that the capacity which resides in the pharynx of Acrania, as illustrated in the particular instance of Amphioxides, of forming a gill-slit between the segments over a great region of the body, indicates the original existence of very numerous primitive gill-slits, and supports the theory of the primary polytremism of Vertebrates, I am glad to say that I agree with him heartily.[1]

September, 1906.

[1] Cf. A. Willey, "Enteropneusta from the South Pacific," 'Zoological Results' (Cambridge University Press), part iii, 1899; and R. C. Punnett, "The Enteropneusta," 'Fauna and Geography of the Maldive and Laccadive Archipelagoes,' vol. ii, part 2, 1903, see p. 669.

The Modification of the Sexual Characters of the Hermit Crab caused by the Parasite Peltogaster (castration parasitaire of Giard).[1]

By

F. A. Potts, B.A.,
Trinity Hall, Cambridge.

With Plates 33 and 34.

I. Introduction.

The discovery of the fact that an alteration in the sexual characters, both primary and secondary, of an organism may be brought about by parasitic infection, and the demonstration of the widespread occurrence of this phenomenon in the animal kingdom, we owe to Professor Alfred Giard. Admirable summaries of the work done in this department up till the date of writing, are to be found in two papers of Giard's cited below (**1** and **3**). Most of the cases are drawn from the observations of Giard himself among the Crustacea, which group affords particularly favourable opportunities for study, on

[1] Mr. Sedgwick has pointed out to me that the term "castration parasitaire," or at least its literal rendering in English, is somewhat misleading, and should not be applied to the phenomena which form the subject of this paper. Castration means destruction of the gonad, and though the phenomenon here described may eventually culminate in this, yet the changes principally here discussed occur before the complete destruction of the gonad, which is consequent on the parasitism, and, so far from being the cause of the other modifications, is probably merely an effect of the same order as the rest.

account of the well-marked secondary sexual differences and the frequent ecdyses, allowing the modifying influence of the parasite on the secondary sexual characters to be clearly demonstrated. But that in cases of this phenomenon the parasite and the host may belong to any phylum is shown by such varied instances as the brittle-star Amphiura parasitised by Orthonectids and a copepod crustacean, the Hymenopteran insect Andrena infected by the parasitic strepsipteran Stylops, the mollusc Lymnæa by Trematodes, and the crab Inachus by the gregarine Aggregata.[1]

In his conclusions Giard recognises a tendency of the secondary sexual characters of either sex to become modified toward the type of the other under the influence of parasitism, while at the same time those originally possessed may tend to be effaced. Thus in the Oxyrhynchid crab Stenorhynchus phalangium, attacked by Sacculina fraissei, the male almost entirely loses its copulatory styles and assumes, in greater or less degree, the abdominal ovigerous appendages proper to the female; in the female these last-mentioned characters become less accentuated, and the abdomen gradually approximates to the appendageless condition of the male, Giard regarding this as a true modification toward the male type. In other cases, as, for instance, Palæmon infested by Bopyrid Isopods, there is a different type of effect, the parasite merely preventing the full development of the secondary sexual characters of the male, causing them to remain in the less advanced female condition. In the primary sexual characters Giard observes a reduction in the size of the gonads and suppression of sexual activity, and the altered condition of the gonads he regards as leading in turn to the above-mentioned modification of the secondary sexual characters. This sterility of an infected animal is considered to be merely temporary, though satisfactory cases of the regeneration of the gonad and the resumption of the sexual functions, after the death of the parasite, are not quoted.

[1] This case does not occur in Giard's original list, but is the subject of a recent paper by Smith. See Bibliography (7).

Lately, also, the study of the effects of the parasitism of various members of the Rhizocephala on crustacean hosts has been continued by Mr. G. W. Smith, of New College, Oxford, and I am permitted to make a preliminary reference to his results, which will shortly appear in a work on the Rhizocephala in the Naples series of monographs. Two most important points are clearly indicated which place our knowledge of this phenomenon on an entirely fresh footing. The capability of assuming the secondary sexual characters of the other sex is shown to be in the cases studied really confined to the male, and, as proved by experiment and observation, modified males (and, apparently, males only) may, on recovery from parasitism by Sacculina, regenerate a completely hermaphrodite gonad. This examination of parasitic effects in the hermit crab was undertaken at the suggestion of Mr. Smith, to whom I am greatly indebted for much kind help, with the object of ascertaining whether evidence supporting and extending these remarkable results could be derived from the study of other cases.

Before commencing the description of the effect which the parasite Peltogaster produces upon its host it will be well to mention a few facts with regard to its life history. It belongs to the Rhizocephala, and after completing its free-swimming existence as a Cypris larva passes through a completely internal stage in the body cavity of the hermit crab, like that described by Delage and confirmed by Smith in Sacculina. The Peltogaster internus, which is constituted of a ramifying root system and a small central tumour, which later forms the external body of the adult, was first discovered by Pekarsky (**6**), and has also been found on some few occasions both by Smith and myself. With regard to the duration of existence of the internus stage we are still ignorant, owing to the absence of success attending attempts to raise Peltogaster larvæ and artificially infect hosts, and also to the comparative rarity in nature of crabs containing it. When, however, the Peltogaster is ready for emergence to the exterior it need not wait for the assisting agency of a moult, but may, as we have

actually witnessed, dissolve its way through the soft skin of the abdomen. In the external stage the Peltogaster is a sufficiently striking object with its sac-like body of a clear red colour and the great mass of dark green roots shining through the semi-transparent skin of the host. Practically always it is found in the same position of fixation between the second and third abdominal segments on the left-hand side of the body, occupying in a noteworthy way the space where, in the normal female, the embryos are borne. Once become external the adult size is quickly attained, and this latter, then, is no indication of the age of the parasite, but only of the food-supplying capabilities of the host. The same crab frequently supports two Peltogasters, and less often examples of the Cryptoniscid Liriope pygmæa, are found fixed on or near the Peltogaster itself. But neither the size of the Peltogaster nor the presence of one, or a plurality of parasites appear to be correlated with the degree of modification, due to the parasite, which I go on to describe. It may, however, be mentioned here that the character of the root system of the parasite seems to change with the duration of infection, being more diffuse and extensive in the earlier stages, and more condensed, compact, and dark coloured as time goes on.

II. General Effect of the Parasitism of Peltogaster on Eupagurus.

Parasitic castration has been studied by Giard amongst his numerous other cases, also in the genus Eupagurus (**2**). At Wimereux the occasioning parasites are the Bopyrid Phryxus and the Cirripede Peltogaster (P. paguri), and these are found on Eupagurus bernhardi. At Naples one of the most frequently occurring species of hermit crab is E. meticulosus,[1] very common at a depth of 10 metres, and 20—30

[1] A small percentage of the crabs examined, infected and uninfected, appear referable to the species E. angulatus, which is distinguished from E. meticulosus by certain characters of the chela. Yet intermediate specimens are found, and the effect of the parasite is the same as in E.

per cent. are infested with a species of Peltogaster (P. curvatus, Kossmann). The same parasite is also found on E. prideauxi, associated with E. meticulosus on the same ground and in almost equal numbers, but infected specimens are very rare, and in nearly a thousand crabs of this species examined I could only find three with Peltogaster.

Some hundreds of infected crabs of the former species were examined with respect to the internal and external sexual characters. It is quite easy to keep the animal in captivity, and to observe that this differs from other cases of parasitic influence in Crustacea in that the parasite does not completely check the general growth of the host after the moult, at which it becomes external, and that under aquarium conditions moults are fairly frequent. No exact comparison of the frequency of moulting in infected and uninfected crabs are made, but experience seemed to show that normal crabs did not moult so frequently in captivity as infected. That castration increases the rate of growth of animals is by no means a new idea, and is put into practice in the rearing of stock and poultry, and since E. meticulosus, at Naples, reproduces the whole year round, it would appear most likely that a relief from the reproductive functions would increase the somatic growth.

It is also interesting with regard to the general effect of the parasitism to notice that infected crabs seem fully as active and healthy as normal specimens. Though the internal organs (for instance, the heart in its abdominal extension) may be entirely enveloped by the enormous root system of the Peltogaster, yet, with the exception of the gonads, they do not appear to be affected by the parasitism. But even in the period of the internus stage one finds the gonad reduced in size, and the brown mature ova of the female giving place to white ova with little yolk. In the male, however, sperm production still occurs at this stage, and even for a time after the commencement of the externus stage. The reduction in

meticulosus. Probably then E. angulatus is only a variety of the last named species.

size of the gonad is first effected at the expense of the glandular portion—the testis of an infected specimen consists typically of a coiled duct with reproductive cells only toward the termination,—and it is noticeable that the secretory function of the duct is not checked, but even accelerated up till an advanced stage of infection. The duct is at first filled with irregular spermatophores, which may contain a reduced number of spermatozoa, but at later stages, where the testis is still clearly recognisable, the ducts present a swollen appearance, and are filled with irregular and unequal masses of chitin derived from the cells producing the dense cap-part of the spermatophore (pl. 34, fig. 5, *ch.*). It was often observable that the duct on the same side as the Peltogaster presents this phenomenon in a greater degree. Perhaps the excessive production of chitin in the male ducts and the increased capacity for moulting may be closely related.

With regard to the way in which the reduction of the gonad is effected it should be said that though the Peltogaster roots often completely enclose the gonads, in no stage of infection was it shown by dissection or serial sections that they penetrated the bounding wall. That the parasite derives its nourishment from the general body fluid rather than the gonads, and hence that the effect on the latter is indirect and effected through the general metabolism, seems conclusively shown by the fact that though the parasite in the internal stage proceeds to envelop first the gonad of the side where it is destined to become external—and so must exercise a more extended action on that gonad,—yet in the occasional cases observed in which the aperture of one side had disappeared, indicating entire and long-standing abortion of the corresponding gland and duct, this had taken place on the side opposite to the Peltogaster. This may be contrasted with the case of Andrena parasitised by Stylops, worked out by Perez and quoted by Giard, where the testis of the side on which the parasite is situated, becomes non-functional, but that of the other side continues to produce sperm.

We will now go on to consider more specially the effect on the sexual characters.

III. Effect on the Sexual Characters.

a. The Secondary Sexual Characters.

The external characters by which the sexes may be distinguished in E. meticulosus are the following:

(*a*) The Genital Apertures.—In the female these are situated on the internal corner of the basal joint of the third pair of walking legs, and are noticeable oval apertures.

In the male, on the basal joint of the fifth thoracic leg, there is a papilla directed somewhat posteriorly bearing the male aperture on its apex, bordered with setaceous hairs.

(*b*) The Abdominal Appendages.—In both male and female there are appendages present on segments 2, 3, 4, 5, 6, a character which distinguishes this species from E. bernhardus, longicarpus, and others in which the appendage of segment 2 is absent in the male, and from E. prideauxi, in which the appendages of segments 2, 3, 4, 5 are all absent in the male. Those of the last segment are paired and form with the uropod the organ by which the crab maintains its hold on the columella of the shell it inhabits. The fifth segment is also furnished similarly in both sexes, bearing a single appendage consisting of a basal joint and two rami, of which the external member is long, and flattened and furnished with an edging of stiff hairs; the internal is pointed and rudimentary. The remaining three segments also bear a single appendage, but while in the female these are of markedly biramous character (pl. 33, fig. 6), in the male they are practically uniramous (pl. 33, fig. 1), and similar to that of the 5th segment.

The relative development of the rami varies slightly in the female. In the second segment the external ramus is but slightly the larger, while in the third and fourth respectively the development of the internal ramus is progressively less.

Also in young (though even then sexually mature) hermit crabs of 1·0—1·3 cm. of carapace length, the internal ramus is less developed than in the adult, and everything points to the conclusion that the biramous character of the abdominal appendage has been acquired to provide a more secure anchorage for the developing embryos. For this purpose the basal joint and the internal ramus, both in the middle and apical part, are provided with long, radiating bunches of hairs. Of the bunch on the basal joint only is there any representative in the male. But though the external ramus is well provided with hairs it is noticeable that eggs are never attached to them, so that this ramus is left quite free to subserve its proper function of aërating the interior of the shell, for which it is admirably adapted by its oar-like shape and border of stiff hairs.

In the male the external ramus is developed as in the female and is used in the same manner; but in correlation with its non-participation in the nursing functions, the internal ramus is rudimentary in the first four appendages.

Account of Modification in the Male.

The effect of the parasitism of Peltogaster on the secondary sexual characters of the male E. meticulosus is to stimulate the development of the rudimentary internal ramus of the first three abdominal appendages. The extreme members of the modified series reach a stage indistinguishable from the corresponding normal female appendage. The modification is rendered complete by the assumption of the typical gibbous form and the bunches of egg-bearing hairs of the internal ramus of the female.

Various stages of the complete series which exists between totally modified and unmodified crabs are here figured (pl. 33, figs. 1—5). An examination of these shows that, as in the development of the female, so in the modification of the male, it is in the first appendage that the internal ramus earliest responds to the stimulus (fig. 3). And though in some cases

the internal ramus attains the same degree of alteration in the first and second appendages, in the third it remains for the most part in a condition of suppressed development (figs. 2, 3, 4), though in the most marked examples of modification this appendage exhibits the effect in the same degree as the two first (fig. 5). The retardation of modification proceeding backwards in the series is apparently in correspondence with what takes place in the normal development of the female, which reaches its extreme in the fourth abdominal appendage, where the internal ramus remains throughout life in a rudimentary state, and where also no alteration occurs in connection with parasite castration. These facts are apparently to be explained by the supposition that the abdominal appendages have been successively pressed into the service of bearing the embryos, and the modification entailed by this is more complete in those longest so employed.

On account of the completeness of the modified series it is impossible to do more than roughly classify the infected males with respect to the degree of modification. It may be said, however, that at least a quarter of those examined were quite unmodified, that in considerably more than half the modification range from a condition of bare perceptibility to one in which the internal ramus, though distinctly small, showed the typical female form, and the remainder alone could be spoken of as completely modified.

Amongst the infected crabs there is a considerable proportion which it is impossible to assign to their sex by merely external examination, for they are entirely without genital apertures, which have to be taken as the external criterion of sex. In these then the modification has been of so long standing duration that the gonad or at least the duct has become completely extirpated, and succeeding moults have closed the genital apertures. As referred to above, a midway stage to this condition is to be noticed in certain crabs in which one only of the apertures is closed, this in almost all the cases of both sexes examined being the one opposite the Peltogaster.

By dissection of these crabs of problematical sex it was in rare cases possible to discover a diminished gonad, and on such occasions this was more often an ovary than a testis, though instances of both were found. The appendages of these animals vary from slightly modified to completely female type, but the former part of the series is represented by only a small number of individuals. Probably then the slightly modified individuals and a small percentage of the fully modified belong to the male sex, while the rest which form a majority are really females.

In Carcinus mœnas infected by Sacculina, as described by Giard, the old males are entirely unmodified, and the varying amounts of modification in different individuals are hence connected with the age at which they became infected. Though the solution of the question is to a certain extent interfered with in Eupagurus meticulosus by the continued growth after the external appearance of the parasite, yet the observation that young males of 1·0—1·3 cms. carapace length were perhaps more often than not unmodified, while the largest infected males found of 1·6—2·0 cms. carapace length were equally likely to be modified or unmodified shows that in this case the male sexual characters are not more susceptible to alteration of type at an earlier age. It has also been pointed out above that the degree of modification is independent of the presence of a larger or smaller parasite, of one or a greater number.

Apparent modification in the female.

Although the majority of the infected females resemble the young rather than the adult normal female with regard to the development of the internal ramus, yet cases in which any real approach to the uniramous condition is found are extremely rare, and I have been only able to procure three specimens which were, in Giard's sense, clearly modified toward the male type. All three were of small size; in no case were any of the first three appendages of pure male

type, but in the second and third appendages the development of the internal ramus was quite slight, while in the first it had attained about a half of its usual size.

The consideration of some facts dealing with the later metamorphoses of the hermit crabs may help us to decide whether these cases fall under the heading of true modification or retardation of development.

The life-history of Eupagurus meticulosus is not known in detail, but that of the American species, E. longicarpus, has been the subject of careful research by Thompson (**8**). This species as noted above differs from E. meticulosus in the loss of the first abdominal appendage by the male.

After escaping from maternal gestation the larva leads a pelagic life, the last stage of which is known as the Glaucothoë. In this the animal has an appearance and anatomy nearly similar to the adult, but the abdomen is symmetrical in form, and it also differs in the possession of a pair of appendages for each segment. These are of male type, though the rami are but slightly developed.

In the stage, after the moult from the Glaucothoë, during which the animal seeks a shell, the abdomen becomes asymmetrical, the appendages of the right side are totally lost, and the first appendage of the left becomes rudimentary, while throughout the remaining appendages the external ramus increases in size. At this time there is no differentiation of sex, both male and female possessing appendages with a rudimentary internal ramus. And it is not till thirty days after the glaucothoë stage that the rudiment on the second segment even begins to develop into a typical female appendage. Ten days after that the alteration to the female type begins in those of the third and fourth segments, but the complete development requires several further moults. Quoting Giard's case of the parasitism of Peltogaster on Eupagurus bernhardus, where the form of the appendages of the female is said to approximate to the male type, Thompson says, "The parasite presumably might attach

itself any time within the first fifty days of adolescent life, and yet be able to check the complete development of the female type of appendage." But the parasite does not exert its retarding stimulus directly after fixation, and we are without data which would give the period after which it commences to do so. To support this explanation of the apparent modification of the female characters, it is probably necessary to postulate a larval infection. During the last moults of larval life the Peltogaster would remain in the internus stage and in the time of the first adolescent moults, whether or no its stage of development was sufficiently advanced for an external appearance, the checking effect on the further development of the female appendage could be brought about.

Assuming then that the course of development runs fairly similarly in Eupagurus longicarpus and meticulosus, it is perfectly open to consider the exceptional cases mentioned above as due to unusually early, probably larval, infection and consequent midway arrest of the assumption of the female characters. That this is the true explanation of the phenomenon is indicated by the unusually small size of the infected individuals and by the fact that the development of the first appendage was more distinctly female than the other two. It will be later seen from other evidence that the parasitism does not cause alteration in the once assumed female characters.

Effect on the Secondary Sexual Characters of Eupagurus prideauxi.

Before proceeding to describe the effect of parasitism on the primary sexual characters of Eupagurus meticulosus it will be well to mention the conclusions gleaned from the small amount of infected material of E. prideauxi I have been able to collect. This species differs from E. meticulosus in the complete absence of the abdominal appendages of the second,

third, fourth, and fifth segments in the male. In the female appendages are found on the second, third, and fourth segments, of the usual biramous type. One of three individuals with Peltogaster was a female; in it I was unable to detect any alteration from the female type. Of the two males one was unaltered; the other was provided with three pairs of abdominal biramous appendages exactly as in the female. But these appendages, though of an exact female form, did not possess the long hairs which serve as egg attachments in the female, and were only about one third the size of those of a slightly smaller normal female with which it was compared. It may be mentioned that the modified male was much smaller than the unmodified, but the examination of a more considerable number of cases is needed before the connection of modification with infection at an early stage is asserted.

Evidence concerning the Permanence of the Modification caused by Parasitism.

(1) From moulting.

(*a*) A large number of infected crabs, particularly unmodified males, were kept in aquaria and fed generously. In periods varying from a fortnight to two months the crabs moulted, and in no cases, either of infected females or males of any degree of modification, was there any alteration of the relative proportions of the two rami in the new appendage.

(*b*) Primarily, for another purpose, to be treated of below, operations were made on a large number of crabs, the external part of the Peltogaster being removed by scissors. They were then kept as far as possible in natural surroundings and well fed, and after a month moults became of frequent occurrence. On comparing the old and new appendages no difference in the relative proportion of the rami, concurrent with the removal of the occasion of modification, could be distinguished. It is interesting to compare the

stability of the effect produced here with the case recorded by Giard of Phryxus paguri and Eupagurus bernhardi. The parasite is fixed on the abdomen of the hermit crab, and it is comparatively easy to remove it from its host without injury to the latter. Giard did this in the case of a modified male crab, and when, after being kept for a month, it moulted, he thought he could observe an incipient return to the unmodified condition of the appendages, the gonads being still of small size. It may, perhaps, be affirmed that the two cases are fundamentally different, and that Phryxus is purely an ectoparasite, its effect naturally passing away after removal, while it is impossible to entirely nullify the effect of the Peltogaster by operation, the roots continuing to live and play the same part as the complete parasite, but the same results have been obtained with Eupagurus meticulosus even in cases where the partial recovery of the gonads showed that the parasitic stimulus had finally ceased to act.

(2) From regeneration.

As in the American species on which T. H. Morgan experimented, the abdominal appendages of Eupagurus meticulosus are easily capable of regeneration. Various abdominal appendages were cut off from crabs, in a large number of which the Peltogaster was at the same time removed. In all cases at the next moult the appendages were found to be regenerating. When the appendage was cut off proximal to the bifurcation the rami regenerated but feebly, but if the cut was made at a level slightly distal to the bifurcation an almost complete regeneration took place. Unfortunately the number of operations of the latter class were but few, and in those of the former it was difficult to see whether the degree of modification in the old and new appendages exactly corresponds. But the point which comes out clearly as a result of these experiments is that females and modified males (pl. 34, fig. 1), from which the Peltogaster has been removed, regenerate appendages in which both rami are of approximately equal size, while unmodified and slightly modified

males (pl. 34, fig. 2) regenerate appendages in which they are manifestly unequal, and in which at first, indeed, only the external ramus may be developed.

In those on which the Peltogaster was allowed to remain the interesting fact was observed that a female with an abdominal appendage removed regenerates one of purely female type, and this, together with the evidence derived from moulting, seems entirely to prove that the Peltogaster, at least in the later stages of parasitism, exerts no influence at all on the secondary sexual characters of the female, or on the processes which regulate their reformation. So also with regard to the male it appears that the modifying cause operates at an early stage in the history of infection, and that the degree of modification is not subsequently increased. I am inclined to attribute the great variability in degree of modification in infected male crabs to constitutional differences in the crabs themselves, not to date of infection, for the same range of variation is found in crabs of all sizes, nor to the comparative vigour of the Peltogaster, for quite large Peltogasters may be present on unmodified, and small Peltogasters on modified, hosts.

The permanence of modification in recovering crabs may be compared with a kindred phenomenon in Mammalia. In deer the females may assume the horns characteristic of the male, as a consequence of atrophy or disease of the genitalia. But cases are known of female deer with horns which, on dissection, showed normal ovaries. Herbst (**4**) refers the growth of the horns to a period when the ovaries were in a diseased condition, but while these later recovered their normal state, the modification of the secondary sexual characters remained.

Effect of the Parasite on the Cellular Character of the Gonad.

A large number of testes of infected males have been examined by sections, showing a very curious cytological change of a widespread nature. This is the appearance of small but distinct ova-like cells in the glandular part of the testes (pl. 34, figs. 4, 5, ov_1, ov_2). They may be followed in their development from spermatocytes by the enlargement of the nucleus, the appearance of a single large and distinct nucleolus, and the loosening of the chromatic network, together with the aggregation of cytoplasm round the nucleus. This phenomenon may occur while sperm production, though in diminished activity, is still proceeding in other parts of the testes (pl. 34, fig. 5). A very large number of infected males with external Peltogasters exhibit it, and it seems likely that all of them, when a certain period of infection was reached, would be capable of producing ova in their testes. As to the period of appearance it is impossible to be sure; but in the only male I found, with an internal stage of Peltogaster, spermatogenesis was but slightly checked, and there was no sign of the appearance of female cells. But though in the majority of infected crabs with small just external Peltogasters the testis was of normal character, in one or two I found signs of incipient change on sectioning. Probably there is much individual variation in this particular, though the variability of the organism is much more clearly demonstrated in the reaction of the secondary sexual characters to the parasitic stimulus.

It must not be forgotten that the occurrence of ova is a phenomenon of normal occurrence in the testes of various animals, such as, for instance, Orchestia and Homarus among Crustacea, in Phalangids, and in the toad. A number of normal testes of Eupagurus were examined, but without finding that it could be added to the above list.

With the object of ascertaining whether this apparently

hermaphrodite state of the gonad of the infected male is merely transient, or persists on the removal of the parasite and the recovery of the host, from a large number of infected crabs of both sexes, the external part of the Peltogaster was removed, cut off with scissors as close to the crab's abdomen as possible. As mentioned above, the operated animals, which were fed regularly, soon became quite healthy and moulted very frequently. They were kept for various periods, varying from two to five months, but up till the time of writing no crabs had so far recovered as to completely regenerate the gonad. It is difficult in most cases to prove that the gonads increase in size at all, but in one of the crabs of problematical sex (in which the gonad, if present, is of very small size), when two months had elapsed after removal of its Peltogaster, a fairly large sized ovary with eggs approaching maturity was found. From this fact and others it appears certain that a slow regeneration of the gonad does take place. How slow this regeneration is may be judged from the evidence of a crab once infected, but which was found naturally freed from its Peltogaster. Though, in the few other crabs of the kind which were found, the experience appeared to have been recently undergone, this one, a slightly modified male, preserved no trace of the Peltogaster externally, indicating that several moults must have occurred, enabling it to lose the scar of the Peltogaster. On dissection, the roots, though degenerate, were found, but the gonad of the host, in spite of the presumably extended period since the loss of the parasite, was still very small, though showing some signs of regeneration.

With regard to the question whether the egg cells, which appear during the period of activity of the parasite, are resorbed when the influence is removed, or whether, with recovery of the gonad, they grow and become functional ova, there is some little evidence to be cited from a histological study of the testes of operated crabs. In nearly all those, sectioned ova were still present, and in one case at least seemed to have attained a greater size than had been ob-

served in infected testes (pl. 34, fig. 6, ov_2). Such evidence, as far as it goes, seems to show that, as in the secondary sexual characters, so in the primary, the effect induced by the parasite is a permanent one, at least for a considerable time, and does not pass away immediately with removal of the primary cause.

Eupagurus prideauxi.—In one (the unmodified) infected specimen of this species testes of medium size were found on dissection. The ducts exhibited the same phenomenon of excessive chitin secretion; but while spermatogenesis had entirely ceased, there was no appearance of egg cells in the glandular part. The study of more material might well, however, establish the existence of the phenomenon described above also in Eupagurus prideauxi.

In concluding this account of the hermaphroditism of Eupagurus, due to the action of the parasite Peltogaster, a case of an apparently naturally occurring hermaphrodite must be mentioned. This animal possessed typical female appendages, but perfectly formed male apertures. On dissection, the gonad of the right side appeared completely and normally male, though of somewhat reduced size; but on the left side, though testis and duct were present, there were large masses of mature brown ova. It is just possible that this specimen was one which had recovered from parasitism of Peltogaster, for we have seen above that the attachment scar of the parasite may disappear with lapse of time, and if complete absorption of the roots also supervenes no further evidence of former infection remains. But this isolated case cannot be cited as serious evidence of the capability of an infected male to regenerate a complete hermaphrodite gonad. I may, however, indicate the fact that the animal was predominantly male, possessing male ducts and apertures, and gonads principally male; and since we have seen that the effect of parasitism is to occasion the appearance of ova in the testis, and alter the male secondary sexual characters toward the female type, so we may imagine that some other unknown cause, acting on a primarily male

organism at some unknown possibly embryonic period of development, may produce a hermaphrodite of the character described above.

It will be interesting here to briefly compare the phenomenon of hermaphroditism in the gonad of Inachus scorpio caused by Sacculina neglecta, as described by Smith. Here the testis of infected males, before completely atrophying, showed no appearance of ova. In those, however, from which the Sacculina was removed, and which regenerated their testes, the externally modified and slightly modified males show a completely male gonad; the perfectly modified, on the other hand, possess gonads with mature products of both kinds. In the males of Inachus scorpio, then, the capability of assuming the internally hermaphroditic state would appear to be more restricted than in the crustacean here considered. I am unable to say, however, whether the male infected hermit crabs are able to regenerate a completely hermaphrodite gland in place of the testis on removal of the parasite, as happens in Inachus.

Many examples of infected and recovering ovaries were likewise examined for evidence of hermaphroditism. But though the cellular elements are so reduced in size from lack of nourishment that many appear exactly similar to spermatocytes, none of the stages of spermatogenesis were ever seen, and in recovering ovaries the cells throughout increased in size and recovered their ova-like appearance.

Correlation of the Effects on the Primary and Secondary Sexual Characters.

It was expected at first that some correspondence between the degree of modification exhibited in the primary and secondary sexual characters respectively might be traced, but this does not appear to exist, and the development of egg cells appears to take place in the same degree in entirely unmodified animals as in those in which some

degree of modification has been developed. It has been noticed that the effect on the primary characters as one which very often supervenes, and may in fact be general for the whole male sex; while on the other hand individual variation plays a great part in the effect produced on the male secondary sexual characters, which possibly in about a quarter may be entirely null. The correlation which exists between the primary and secondary characters must be of a loose nature, and this fact and more particularly the observation of cases in which modification of the appendages had begun before the corresponding changes in the gonad, appears to conclusively show that the modifying cause, acting on the secondary sexual characters, is not to be sought specifically in the altered character of a secretion of the gonad, but rather in a more general rearrangement of the metabolism, occasioned by the parasite, which affects both primary and secondary characters.

Summary.

(1) The infection of the hermit crab Eupagurus meticulosus by the cirripede Peltogaster curvatus has the effect of diminishing immediately the size of the gonads and suppressing their functions. This is probably effected through interference with the general nutrition of the host, and not through direct action on the gonads.

(2) At an early stage of the external parasitism ova make their appearance in the glandular part of the testis. Experiments planned to discover the fate of these ova on recovery of the host from the parasite were not very successful owing to the slow regeneration of the gonad, but it seems probable that they persist and grow. No corresponding changes could be traced in the ovary.

(3) The male secondary sexual characters are stimulated to development towards the female type under influence of the parasitism. There is a complete series between un-

modified crabs and crabs which have almost entirely assumed the female characters.

(4) The apparent cases of modification in the female toward the male type are probably due to unusually early infection, a retarding influence being exerted by the parasite on the incompletely developed secondary sexual characters causing them to remain in the less advanced male stage.

(5) In Eupagurus prideauxi a similar development of the female secondary sexual characters in the male was observed.

(6) Observations on the moulting and regeneration of the abdominal appendages show that the final degree of modification is attained early after the commencement of the external stage of Peltogaster, and is never afterwards altered either by continued action of the parasite or by its extirpation. The great range of degree in modification is the expression of individual variability in the hermit crabs.

(7) The looseness of the correlation between the changes in the primary and secondary sexual characters shows that the latter are not directly consequent upon the former, but rather that both are attributable to some change in the general metabolism.

Note.—In "Expéd. Sci. Travailleur et du Talisman, Crustacea Decapoda, pt. i, Brachyura et Anomura, 1900, p. 228," A. Milne-Edwards and E. L. Bouvier assign the crabs here called Eupagurus angulatus to Herbst's earlier species E. excavatus, and that known as E. meticulosus to E. excavatus var. meticulosus.

September, 1906. F. A. P.

Bibliography.

1. Giard, A.—"La castration parasitaire," 'Bull. Soc. Dep. du Nord,' ser. 2, 10 Année, 1887.

2. Giard, A.—"Sur la castration parasitaire chez l'Eupagurus Bernhardus et chez la Gebia stellata," 'C. R. Acad. Paris,' t. civ, p. 113.

3. Giard, A.—"La castration parasitaire (nouvelles recherches)," 'Bull. Soc. Dep. du Nord,' (3), i, 1888, p. 12.

4. Herbst, C.—'Formative Reize,' 1901.

5. Perez.—"Des Effets du parasitisme des Stylops sur les Apiaires du Genre Andrena" (Act. Linn. Soc. Bordeaux, t. 40).

6. Schimkewitsch, W.—"Ueber die Untersuchungen von J. O. Pekarsky; ueber die Entwicklung von Peltogaster paguri," 'Trav. Soc. Natural Petersbourg,' vol. xxviii, p. 218, 1898.

7. Smith, Geoffrey.—"Note on a Gregarine which may cause the Parasitic Castration of its Host," 'Mitt. Zool. Stat. Neapel,' 17 Band, 3 Heft, 1905.

8. Thompson.—"Metamorphosis of Hermit Crab," 'Mem. Boston Soc. Nat. Hist.,' 1903-4.

EXPLANATION OF PLATES 33 and 34,

Illustrating Mr. F. A. Potts paper on "The Modification of the Sexual Characters of the Hermit Crab caused by the Parasite Peltogaster (castration parasitaire of Giard)."

In all cases the first three abdominal appendages only are figured; they are denoted respectively *a*, *b*, and *c*.

PLATE 33.

Fig. 1.—Normal male: abdominal appendages.

Fig. 2.—Infected male: abdominal appendages, Stage 1.

Fig. 3.—Infected male: abdominal appendages, Stage 2.

Fig. 4.—Infected male: abdominal appendages, Stage 3.

Fig. 5.—Infected male: abdominal appendages, Stage 4 (appendages 1 and 3 only are figured).

Fig. 6.—Normal female: abdominal appendages (appendages 2 and 3 only are figured). *e.r.* External ramus. *i.r.* Internal ramus.

PLATE 34.

FIG. 1.—Regenerated appendages (1st and 2nd abdominal) of modified male. The animal, which had only undergone one moult since operation, regenerated appendages in a modified sense.

FIG. 2.—Regenerated appendage (1st abdominal) of unmodified male. (One moult after operation.)

FIG. 3.—Transverse section through normal testis. Tubule with duct stained with Delafield's hæmatoxylin and eosin. *spc.* Spermatocyte. Duct filled with spermatozoa (*spz.*). Objective 7*, ocular 2 of Koristka.

FIG. 4.—Preparation of infected testis with Schneider's aceto-carmine. Small and irregular spermatophores (*sp.ph.*) with much reduced numbers of spermatozoa. *sp.ph.c.* Chitinous cap-part of sphermatophores. *spc.* Spermatocytes, and ov_1, ov_2, two stages in the development of ova. Obj. 7*, oc. 5, Koristka.

FIG. 5.—Transverse section through infected testis tubule. Stained with borax carmine. *ch.* Irregular masses of chitin in the duct. Other lettering as before. Obj. 7*, oc. 2, Koristka.

FIG. 6.—Transverse section through recovering testis tubule. Reduced number of egg-cells (possibly some resorbed); but ov_2 here represents a more advanced state of development than in infected testes. Cytoplasm shows reticular structure. Higher magnification than last. Stained with Mayer's carmalum. Obj. 7*, oc. 6, comp. Koristka.

(The figures are drawn with Zeiss's camera lucida.)

On the Medusa of Microhydra ryderi and on the Known Forms of Medusæ inhabiting Fresh Water.

By

Edward Potts,

Of Philadelphia, U.S.A.

With Plates 35 and 36.

The three species of freshwater jellyfish as yet discovered were announced during the years 1880, 1893, and 1897 respectively. As the first of these dates was twenty-six years ago, and as the paper reporting the North American form was unaccompanied by illustrative figures, it has been suggested to the writer that a general review of the subject, so far as known, would be useful at this time, and the kind assurance was given that if it included "good drawings and a full account of the polyp and medusa" of Microhydra ryderi, it would be gladly accepted and published by the 'Quarterly Journal of Microscopical Science.'

To make such a re-presentation of value I propose first to offer copies of the published figures of the earlier forms, viz.: Limnocodium sowerbii, Allman and Lankester; found in the Victoria Regia tanks of Regent's Park Gardens, London, England (Pl. 35, figs. 5, 6, 7, 8), and Limnocnida tanganyicæ, Dr. R. Böhm, Lake Tanganyika, Central Africa (Pl. 35, fig. 12). Following these I give figures showing the lateral and polar aspects of the medusa of Microhydra ryderi, Potts (Pl. 36, figs. 13 and 14). Both

of the figures of this medusa show, as described in the 'American Naturalist,' the eight tentacles, one at the termination of each radial canal, and the others alternating with them; the latter figure also shows at the centre, as in an optical section, the quadrate character of the mouth parts of the manubrium. The polyp of this species was found in two localities in the neighbourhood of Philadelphia, Pennsylvania, U.S.A., in January, 1885, and studied more or less continuously for twelve years before the medusæ were detected in August, 1897. The figures of the medusa here given (figs. 13 and 14) were drawn by Dr. J. Percy Moore, of the Biological Department of the University of Pennsylvania, from medusæ placed by myself in a very dilute aqueous solution of formalin some time in August or September, 1897; and, although possibly somewhat shrunken, they correspond very closely with my remembrance of them when fresh, and with my description published in the 'American Naturalist' of December in that year. These medusæ have as yet only been seen as they were developed from the hydroids in comparatively small culture-jars at my home, where they were quickly lost sight of amongst water-plants kept floating at the surface. Under these circumstances none probably reached an age of more than two or three days, and can only be safely compared with figs. 5 and 6, Pl. 35, supposed to be mature, by experts familiar with similar types of marine medusa. During several weeks of the year 1897, and occasionally during the two following years, Dr. Davenport, Prof. Cheyney, with myself and some others, were cognisant of the budding and liberation of several medusæ.

I have felt an especial pleasure in finding, as represented in figs. 15 and 16, Pl. 36, two rough drawings (the latter by camera lucida) among my papers dated September 5th, 1897. They represent sketches made, during the afternoon and evening, of the same medusoid bud at an early stage of its development; and, under date of the 7th of the same month in my letter press-book, I found copies of the following letter addressed to Dr. C. B. Davenport, referring to the same

observations. "Most important was my success in removing a swelling bud to the stage of my microscope, where its connection with the stem of one of a colony of two hydroids was unquestionable. The pup, of course, grew faster than his daddy, who curled his head to one side in evident disgust, the pedicle of the medusa-bud assuming the position of the main stem. I made a rude camera lucida sketch at the time." (The freehand drawing (fig. 15) had been made earlier in the day.)

"On the following morning I was pleased to see that development had gone on unchecked by its removal. When examined that evening the tentacles had been projected to nearly their full length, the expansion and opening of the disc was nearly complete, and pulsations had evidently continued for some time. This morn (September 7th) the jelly-fish was swimming about the little dish.

"Another important point. At the time of my last evening examination, the cellular structure of the nearest hydroid stem, as well as that of the pedicle of the medusa, had entirely disappeared, leaving the struggling creature attached only by a diaphanous cuticle almost impossible to define, within which were a very few spherical floating cells and numbers of detached thread-cells. This disintegration of tissues serves to account for my previous failures to discern the supporting stems, and to explain the long detention of the medusa when there seemed to be no attachment.

"I do not mean that the Microhydra had entirely disappeared. The stem bearing the other head had, in fact, elongated, and the nearer stem seems to be re-adjusting its conditions and reforming a capitulum."

It must not be at all understood that this was the only instance of the liberation of a medusa observed; we saw several of them, but none that so entirely eliminated the personal equation of a poor memory.

Before introducing a number of drawings illustrative of the structure of the hydroid form of Microhydra ryderi, I beg

leave to reproduce for comparison figs. 3 and 4, and 9, 10 and 11, Pl. 35, given by Alfred Gibbs Bourne and F. A. Parsons in their descriptions of a certain hydroid found in the Regent's Park Gardens (but in other tanks than those in which the Limnocodium had been discovered), and assumed to be the hydroid condition of that medusa. I have not been informed that such a derivation had been actually traced.[1]

The remaining figures of Plate 36 (one or two exceptions noted) have been very accurately copied by F. von Iterson, artist, from excellent drawings made some years ago by my friend, Dr. John A. Ryder, of the University of Pennsylvania, who was enthusiastic in the study of the polyp with which his name is associated, but who died before the medusa had been seen, much to my sorrow and to the loss of science. They are—

Fig. 17 (Pl. 36).—A bifid or bi-capitate representation of M. ryderi from life, of which one branch (*a*) taken by itself may be considered as showing the appearance of the single form of the hydroid, and (*b*) as the compound form, where two are found branching near the base: *cpm.*, the capitulum, more or less spherical, terminating the distal end of each mature polyp, and charged upon the surface with varying numbers of *n.*—nematocytes or thread-cells; *p.d.* is the pedal disc or foot, and *l.* a fully-formed asexual larva about to be liberated from one of the polyps. × 64.

Fig. 18.—Another bifid form which Dr. Ryder describes as "a freehand sketch of M. ryderi on the side of the culture-jar with the capitulum of one (much elongated branch ring-like mouth depressed, stem of glass-like transparence, with the exception of cell-ends and boundaries shimmering in the centre." Incomplete "basal branch (*b*) very granular and relatively very opaque."—J. A. R., October 12th, 1891.

Fig. 19.—Of this Ryder says, "Oral end of adult polyp,

[1] This derivation, however, had been traced in 1890 by Fowler. The chief figure of the Limnocodium polyp, showing a terminal "medusa" in section, is reproduced here in Pl. 35, fig. 1.—E. Ray Lankester.

showing manner of eversion of hypostomal organ, in longitudinal section."—J. A. R., × 365.

Fig. 20.—"Oblique section of pedal disc of adult, showing cuticular sheath."—J. A. R., × 365.

Fig. 21.—Original diagram by the writer to show the locality of the larval bud (*l.b.*), its gradual growth, and the manner of separation of the fully formed larva. A course of 24 hours or less.

Fig. 22.—Highly magnified section (× 244), showing budding larva (*l.b.*).

Fig. 23.—Another bicapitate hydroid, showing larval bud in situ, lasso threads highly stimulated. × 67.

Fig. 24.—Young larva forming capitulum; thread-cells accumulating at distal end. × 112.

Fig. 25.—Single hydroid adherent to glass of jar; base and adjacent parts showing adherent threads of Nostoc and adventitious particles. × 30.

Fig. 26.—Drawing by J. P. Moore from cross section of mature hydroid by Harold Wingate, showing structure, enteric folds, etc. Magnified 480 diameters.

In the latter part of January, 1885, while examining under the microscope the surface of some small fragments of gneiss and mica-schist, chipped from larger rocks in the bed of Tacony Creek, a small mill-stream near Philadelphia, Penn., an affluent of the Delaware River, I first observed the primitive freshwater hydroid, since classified as M. ryderi. The stones had been brought into my house during the previous summer to enable me to study the winter condition of a newly discovered bryozoan, Paludicella erecta, mihi (since named Pottsiella erecta by Prof. Kraepelin), that grew abundantly upon them.

The polyp, as seen, was about one half a millimetre in length by one tenth of a millimetre in thickness, nearly cylindrical, sometimes simple, in other instances divided near the base of support into two nearly equal, divergent branches.

Body soft (hydra-like), with but slight power of extension or retraction; without tentacles or cilia, but terminated by conspicuous symmetrical capitula varying from time to time from a generally hemispherical shape, and bearing upon or near their surfaces fifty or more nematocysts, each with slightly protruding palpocils. At the extremity of each capitulum an oval aperture, expansible and contractile, was with difficulty determinable, but was finally abundantly proven (fig. 19, Pl. 36).

The creature was fixed in position, and apparently without power of locomotion when once attached—standing or swaying upon its proximal extremity, which latter was specialised, as in the common Hydra, into a pedal disc or foot (fig. 20). Besides the nematocysts collected within the limits of the capitulum, others were rather sparsely distributed throughout all parts of the ectoderm.

The animal was often entirely quiescent; at other times its sole movement consisted in a very deliberate writhing or swaying of the whole body, or a curving of the capitulum abruptly against the body as a bent finger curves upon itself.

During the fifteen weeks or thereabouts that these creatures were under close daily and nightly observation, at least fifteen individuals, old and young, were seen; and as the positions of the mature forms seemed unchangeable, and the larvæ had but slight—or even accidental—powers of locomotion, there was little difficulty in identifying them from day to day. It must be observed, however, that on account of the scarcity of specimens, and the apparent impossibility of removing any from its place of attachment (on rough stones) without fatal result, I was unable to place them in good positions for examination by transmitted light, and that, standing as they generally did, upon opaque surfaces, and in positions directed more or less toward the observer, their internal structure, and the changes occurring therein, could not be seen with entire success in the living animal. For instance, notwithstanding the most careful watching during

this long period, I had entirely failed to see the act of feeding until the last days of the last surviving mature individual.

I copy from my note-book, under date of May 11th, 1885:—"On this date, for the first time, I saw M. ryderi capture and swallow a small rotifer. A hydroid standing upon the clear surface of my glass dish had developed a lateral branch, which had only recently completed its capitulum. While watching the branch this evening, one of the turtle-back rotifers, that have been so plentiful, came swimming by and, fortunately for me, touched some of the palpocils, when it immediately lost all power of motion, and adhered, or was held, to the surface of the capitulum. This extremity was then deliberately curved around in a direction toward the rotifer, pressing and holding it against the adjacent body of the hydroid, when, by some method not clearly discovered, its head was directed toward and absorbed into the expanded oral aperture of the latter. Its downward progress was then relatively quite rapid, so that it was little more than five minutes from the moment of capture before the caudal appendage of the victim had passed into the orifice, and the whole creature could then be followed through the channel of nearly transparent cells to its resting place about the centre of the polyp. The so-called gizzard-motion of the rotifer still suggested life; but Microhydra's digestion must have been quite energetic, for about an hour later the empty test had already been ejected.

"About the same time I witnessed the act of ejection of the test of a similar rotifer from the main extremity of this hydroid, and the next evening I counted six of such 'relics of mortality' surrounding this form of voracious simplicity."

Two modes of reproduction or development were noticed.[1] The most important of them, perhaps unique in its character, was seen in the formation of asexual larvæ non-ciliated and inert (represented in diagram fig. 21), which, after one or two weeks, became fixed upon their pedal discs, developed capitulæ, and appeared essentially mature (fig. 24).

[1] This was twelve years before the medusa was discovered

One of the first discovered polyps produced larvæ February 3rd, March 2nd, 11th, and 15th. Another produced larvæ on February 5th, 16th, and 28th, and March 3rd. They were all thrown off from the same neighbourhood in the parents, and the visible process was accomplished in a few hours. In every case the act was preceded by an increased opacity of the lower half of the polyp, as though it became filled with granules, an effect, perhaps, of the rapid segmentation of nuclei in the endodermal cells (see fig. 22). The external stages in their formation, often completed within a very few hours, are rather suggested than illustrated in diagram fig. 21 mentioned above. It will be noticed that the product, whatever it may be called, is not a self-sustaining duplicate of the parent hydroid, but an immature individual, needing entire development of its functional parts before it becomes capable of ministering to its own growth or of reproducing others.

When detached the larvæ rest wherever they may chance to fall, changing place only as a result of the slight writhing motion sometimes noticed, or of a protoplasmic, amœboid action within the surface cells. They are long, oval, cylindrical bodies, the central parts exhibiting infinite numbers of yolk-like granules from which nucleated endodermal cells are gradually developed, while the superfices, even before separation, show a continuous, clear, hyaline ectoderm. The external surface seems generally smooth, but a high magnifying power brings out, particularly near the extremities, a delicate, variable cumulation, the outer convexities of minute ectodermal cells. A few nematocysts can very early be detected, indifferently scattered around the margin. After a few days of quiescence, one extremity (Ryder says that last attached to the parent, the nearest to its proximal end), becomes attached to the supporting surface, and the other is gradually elevated into a more or less erect position when the thread cells are formed in numbers about the distal extremity (fig. 24), and maturity is reached with the fully-formed capitulum.

The other mode of reproduction or of propagation is by branching. This was seen in two instances, one of them from the same polyp that had been already known to have produced four larvæ, and the branch originated upon the same side and locality. The action was even more rapid in that the branch appeared and completed its capitulum within twenty-four hours. Its formation was not preceded by the turgidity and granular appearance mentioned in the case of the larval buds, but an uninterrupted sequence of clear endodermal cells was traced from the main stem into the branches. These branches were, possibly, later constricted off, as in the case of the common Hydra, to form independent hydroids, but I did not see it.

During the twelve years intervening between 1885 and 1897 collections had frequently been made from the Schuylkill canal, on the banks of the Schuylkill River, far above tide water, and from a water-shed quite separate from that supplying the locality previously mentioned. The special objects of search were here also stones bearing localised forms of Bryozoa, and, as before, I found M. ryderi amongst them. These stones were dredged up from a depth of six or seven feet at a place where a continuous rush of water kept them from being covered, and their encrusting fauna from being smothered by the mud that in other places embeds the canal.

Whether, under these circumstances, medusæ are commonly and normally developed, and their eggs hatch into hydroid polyps, or whether nature here provides more abundantly the asexual larval method of reproduction above described must, for the present, remain a problem.

In concluding, it is hardly necessary to invite the attention of scientists to the fact that we have, in these three fresh-water forms, an equal number of species, no one of which has been conclusively studied; that their appearance in three of the five grand divisions of the earth points very plainly to the probability that closer methods of research may very soon discover others in familiar but unsuspected places; that,

whatever we may believe as to the origin of Limnocodium sowerbii, it is hardly possible to doubt that Limnocnida tanganyicæ and M. ryderi are native to the districts in which they have been found, and that, unless or until the polyps of Bourne and Parsons have been seen to produce the Limnocodium, it is quite within the limit of possibility to suppose that they have had their origin in the Thames, or from any other source from which the tanks in which they were found may have been filled.

EXPLANATION OF PLATES 35 AND 36,

Illustrating Mr. Edward Potts' paper, "On the Medusa of Microhydra ryderi, and on the Known Forms of Medusæ inhabiting Fresh Water."

PLATE 35.

FIG. 1.—Diagrammatic section of a terminal medusa-bud of the hydroid colony of Limnocodium sowerbii. After Fowler, 'Quart. Journ. Micr. Sci.,' vol. 30, 1890, Pl. 32, fig. 8.

FIG. 2.—Portion of hydroid colony of Limnocodium sowerbii. After Fowler, loc. cit., Pl. 32, fig. 2.

FIG. 3.—Hydroid form of Limnocodium sowerbii upon roots of pond-weed. After Bourne, 'Proc. Roy. Soc.,' xxxviii, 1884, p. 10.

FIG. 4.—Portion of one of the hydroids of Fig. 3 seen in optical section. After Bourne, loc. cit., p. 10.

FIG. 5.—Limnocodium sowerbii, as seen floating. After Lankester, 'Quart. Journ. Micr. Sci.,' vol. 20, 1880, p. 357.

FIG. 6.—Limnocodium. After Allman, 'Journ. Linn. Soc.,' "Zool.," xv, 1880, p. 134. × 8.

FIGS. 7 and 8.—Two views of an embryo medusa of Limnocodium sowerbii. After Lankester, 'Quart. Journ. Micr. Sci.,' vol. 21, 1881, Pl. 13, figs. 4 and 1.

FIGS. 9, 10, and 11.—Hydroid figured by Parsons, 'Journ. Quek. Micro. Club,' ser. 2, vol. ii, Pl. 6, p. 130.

Fig. 12.—Medusa of Limnocnida tanganyicæ. After Moore, 'The Tanganyika Problem,' 1903, p. 299.

PLATE 36.

Figs. 13 and 14.—Medusa of Microhydra ryderi, lateral and polar aspects. Drawings made by Dr. J. P. Moore. × 116.

Figs. 15 and 16.—Medusoid bud developing upon a hydroid colony of Microhydra ryderi. Two stages, drawn by the author, during the afternoon and evening of the same day.

Figs. 17—20.—Drawings of Microhydra ryderi, made by Dr. J. A. Ryder (see text, pp. 626–7).

Figs. 21—25.—Drawings of Microhydra ryderi, by the author (see text, p. 627).

Fig. 26.—Section across a mature hydroid of Microhydra ryderi. Drawn by Dr. J. P. Moore. × 480.

Note on the Foregoing by E. Ray Lankester.

Mr. Potts has been so very kind as to send to me, at my request, a specimen of the minute medusa liberated by Microhydra and preserved in formalin. It is one of two which remained in his possession. Whilst further study of Microhydra and its medusa is urgently called for, it is clear that further research is needed in order to settle the doubt entertained by Mr. Potts as to the actual genetic connection of Bourne's hydroid with the medusa Limnocodium. It is within the bounds of possibility that that hydroid is a native European hydroid, similar to Microhydra, and not connected with the Limnocodium life-cycle.

On the Freshwater Medusa liberated by Microhydra ryderi, Potts, and a Comparison with Limnocodium.

By

Edward T. Browne, B.A.,

Zoological Research Laboratory, University College, London.

With Plate 37.

Through the kindness of Professor Ray Lankester, I have had the pleasure of examining a specimen of the Medusa liberated from the freshwater Hydroid Microhydra ryderi.

The veteran American naturalist, Mr. Potts, of Philadelphia, recently sent to Professor Lankester a manuscript on "Known Forms of Medusæ Inhabiting Fresh Water," for publication in the 'Quarterly Journal,' and this communication of mine forms a kind of appendix to it; it should be regarded as such, since I have before me an advanced proof of Mr. Potts' paper. The title given by Mr. Potts to his communication did not adequately convey the importance of its contents, and has been modified accordingly by the editor. Mr. Potts has at last given us a description with excellent figures of the Hydroid phase of Microhydra and the first figure of the Medusa.

When Professor Lankester showed me the original drawings of Microhydra I noticed the remarkable resemblance between the hydroid phase and that of Limnocodium, but we were doubtful about the Medusa, as Mr. Potts had not given a detailed description of it. Since we were not sure about the presence of sense organs, Professor Lankester asked

Mr. Potts if he could spare a specimen for further examination. A specimen was very kindly sent over from America by Mr. Potts, and I sincerely thank Professor Ray Lankester for his generosity in handing it to me for examination.

The figures of Medusa illustrating Mr. Potts' paper were not drawn from a living Medusa, but from a specimen which had been in weak formalin for several years. To obtain a really satisfactory drawing of a Medusa it must be made whilst the animal is alive. In drawing a preserved specimen allowances have frequently to be made for contraction and distortion, and therein lies a source for error. After studying living Medusæ for a few years the allowance for defects can be fairly well estimated, though occasionally one may go badly astray.

The specimen which I examined was in formalin and in good condition, but the umbrella was badly crumpled on one side. It was a difficult object to examine owing to its minuteness, being less than half a millimetre in diameter, and not easy to fix in a definite position. The drawings which I have made were finished before I received the proofs of Mr. Potts' plates. I then noticed that my drawing of the Medusa did not quite agree with that made by Dr. Moore, so I again examined the specimen, but found that it was not necessary to make any alterations.

The Description of the Medusa of Microhydra (Pl. 37, fig. 1).

Umbrella.—The umbrella is campanulate, a little broader than high (0·4 mm. in width and 0·3 mm. in height), with thin walls. No nematocysts could be found on the exumbrella, though the ectoderm cells were plainly visible. The velum is broad, and here also the ectoderm cells with a rounded nucleus could be easily seen.

Stomach.—The stomach is large for the size of the Medusa, being about three quarters the length of the cavity of the umbrella. It appears to be more cylindrical than quadrangular in transverse section, and tapers slightly towards the mouth. In this specimen the mouth is fairly

well expanded, but there are indications of four small lips, which are simply infolds of the margin.

Canal system.—There are four radial canals, which are not at all conspicuous, and have the appearance of thin lines running from the base of the stomach to the margin of the umbrella. In fact the canals are rendered visible by the brownish colouration of their endoderm cells. A circular canal is probably present, but the thick layer of ectoderm round the margin of the umbrella prevented me from finding a definite canal. To demonstrate the existence of a circular canal would have necessitated the cutting of sections.

Absence of gonads.—The specimen does not show the slightest trace of gonads. The figures of the Medusa illustrating Potts' paper (Pl. 36, figs. 13 and 14) would lead one to believe that gonads were present along the whole length of the radial canals and that they also extended round the stomach. The Hydromedusæ either have their gonads upon the stomach and its lobes, or else upon the radial canals, but none are known to possess gonads in both positions. If a Medusa was found with gonads upon the stomach and radial canals it would have to be placed in a special order. These shaded thickenings are undoubtedly misleading, and I fail to see what they are intended to represent.

Tentacles (Pl. 37, fig. 2).—There are eight tentacles (four per-radial and four inter-radial) similar in size and shape. They are probably in a semi-contracted condition, which gives them a rather stunted appearance. The base of the tentacles is apparently attached for a very short distance, on its upper side, to the margin of the umbrella. The manner in which the tentacles curl at their base and hang down points to an attachment, though I could not clearly see it. There is no indication of a definite basal bulb at the base of the tentacles. Nematocysts are very scarce in the tentacles, and it was only after a long search that I found any, as they were not on the surface but underneath the ectoderm cells.

Around the margin of the umbrella there is a thick layer of ectoderm cells, similar in structure to those of the tentacles,

and between these cells there are a few nematocysts, like those found in the tentacles.

Absence of sense organs.—When I saw the original drawing of Plate 36, fig. 14, before I received the specimen, I thought that the little circles at the bases of the tentacles represented marginal sensory vesicles situated just above the root of the tentacle. They certainly have the appearance of sense organs with a single otolith. In diagrams, and often in good drawings, sense organs are drawn in a similar manner. I thoroughly searched the margin of the umbrella for sense organs, finally using an oil immersion lens, but failed to find any indications of a sense organ either at the base of the tentacles or in between the tentacles. The margin of the umbrella has a slight brownish colour, as if the Medusa had been killed with Flemming's solution. All the nuclei are of a faint brownish colour, easily seen with an oil immersion lens, and the cell walls are also well defined, so that if any sense organs had been present they ought to have been visible. The little circles in the figure are intended for optical sections of the bases of the tentacles. They represent the attachment of the tentacle to the margin of the umbrella, and have nothing whatever to do with sense organs.

A Comparison between Limnocodium and Microhydra.

When I first saw the drawings of Microhydra and compared them with the figures of Limnocodium, it seemed quite possible that Microhydra might turn out to be the well-known Limnocodium, but after examining the Medusa of Microhydra the idea of such a possibility soon vanished.

Hydroid.—In the Hydroid phase the resemblance between Limnocodium and Microhydra is very close, as will be seen on comparing Pl. 35, fig. 10, with Pl. 36, fig. 17. In both forms the hydranth has degenerated to its simplest condition—i.e. to merely a body without tentacles, and when the native place of Limnocodium has been discovered we may obtain a clue to the cause of degeneration.

In the case of Microhydra the Hydroid appears to attach itself to rocks and stones in swift-running streams.

Under such conditions long flexible tentacles, like those possessed by the common freshwater Hydra, would stream out with the current, and be of little use for the catching of food. One would rather expect to find in such a situation a Hydroid with very short and fairly stiff tentacles, like those of Coryne, which lives between tide-marks. But Microhydra has, perhaps, conquered its new habitat at the expense of its tentacles, as it may be reasonably assumed that this Hydroid is descended from one which formerly inhabited the sea.

There is, however, a difference between the Hydroid phase of Limnocodium and that of Microhydra. The Hydroid of Limnocodium secretes from its body a glutinous mucus, to which adhere particles of mud and other débris, so that a protecting case is formed round the body, leaving only the oral end free, and this end is capable of contracting within the tube.

The Hydroid of Microhydra, so far as I can judge from the figures and description, forms no protecting case to its body. Potts' figure 25 shows the Hydroid attached to the glass of the aquarium with the "base and adjacent parts showing adherent threads of Nostoc and adventitious particles." Any one who has kept a freshwater aquarium knows that the glass becomes thickly coated with unicellular Algæ. Mr. Potts figures this coating surrounding the base of the Hydroid, the body of the Hydroid being shown by him quite naked.

Medusa.—The comparison between the Medusa of Limnocodium and of Microhydra is not so simple as that of their Hydroids. In the first place a stage exactly similar to that of Microhydra has not been described and figured for Limnocodium.

Fowler has described the Medusa-bud on the Hydroid of Limnocodium at a very early stage, whilst still attached to the polyp (Pl. 35, fig. 1), but as his supply of material failed he was

not able to proceed any further. Next we have some very early free-floating stages described by Lankester (Pl. 35, figs. 7 and 8). Although there is still no absolute proof that the Medusa-buds found upon the Hydroid do develop into the Medusa known as Limnocodium, still the circumstantial evidence is very strong.

I have seen many species of marine Hydroids bud off Medusæ, but have never seen Medusæ liberated at such an early stage as those of Limnocodium, which look as if they were developing direct from eggs. On the other hand, the Medusa of Microhydra looks like a Medusa which has just been liberated from its Hydroid, and Mr. Potts states that probably none of the specimens seen were more than two or three days old. The Medusa of Microhydra on liberation is at a far more advanced stage than the earliest floating embryos of Limnocodium. (Compare Pl. 35, fig. 8, with Pl. 36, fig. 13).

The embryo of Limnocodium has already got one sense organ developed, so one would expect to find sense organs in the Medusa of Microhydra if it had any. If an adult Medusa has sense organs one always finds (I cannot remember an exception) a certain number (generally about four or eight) of sense organs present in the young Medusa when ready for liberation. To pin one's faith on the absence of sense organs in preserved specimens is not a safe proceeding, because sensory vesicles have at times a wonderful way of becoming invisible after preservation, especially when alcohol is used. Their disappearance is generally due to excessive shrinkage of the tissues when the specimens are too rapidly transferred from sea water to strong alcohol. The great advantage of dilute formalin is that it does not produce a shrinkage of the jelly, and that the sense organs can be more easily found.

The great difference between the Medusa of Microhydra and that of Limnocodium lies in the structure of the tentacles. A few years ago I found out that the shape and structure of the tentacles, and particularly the shape of the basal bulb, were an exceedingly useful and reliable aid in

the determination of species. The tentacles of Limnocodium are quite unlike those of Microhydra. I have a few specimens of Limnocodium which came from Regent's Park in my collection, and they show the tentacles in all stages of development. The tentacles even at their earliest stage, when as mere buds upon the margin of the umbrella, show a character which is not found in the tentacles of Microhydra, nor have I found or yet met with it in any other Medusa. The nematocysts are definitely arranged at the ends of little papillæ. At first there are one or two nematocysts in each papilla, but later on the number increases to about five or more. For the purpose of comparison I selected a very small tentacle of Limnocodium, a little over one millimetre in length, and made a drawing (Pl. 37, fig. 3) from the central portion of the tentacle to the same scale as the drawing of the tentacle of Microhydra (Pl. 37, fig. 2). It will at once be seen that there is a marked difference between the tentacles of these two Medusæ. The nematocysts have also a different shape (Pl. 37, figs. 4 and 5).

The Reproduction of Microhydra.

The hydroid has two methods of reproduction; one is asexual, the other is sexual. Mr. Potts considers the budding of new hydranths, which are not set free, to be a second asexual method. The Hydroid is at first a single polyp, later on from its base another polyp is developed, but as the second polyp is not detached a colony of two individuals is formed. The Hydroid phase of Limnocodium in the same manner is also colonial, but has from two to four polyps. This is not a case of reproduction, as there is no increase in the number of independent individuals, but simply one of branching to form a colony.

The asexual method of reproduction of Microhydra seems to me, from the appearance of the figures given by Potts (Pl. 36, figs. 17, 21, 24), to be reproduction by fission, which occurs in certain marine Hydroids.

Allman, in 1871, gave an account, with figures, of reproduction by spontaneous fission in a Hydroid which he named Schizocladium ramosum. Although Allman had worked for many years upon British Hydroids, yet he had never before met with a case of fission amongst them. Hincks, in 1872, found Campanularia neglecta reproducing by fission in a similar manner, and refused to accept Allman's new genus Schizocladium, "which seems to rest on a single character, the development of fission-frustules in a certain way—a character which, there is reason to believe, may have a wide range amongst the Hydroida." Hincks suggests that Allman's Schizocladium is probably an Obelia. Allman found the colony in Loch Long (Firth of Clyde), and states that it bore a considerable resemblance to that of Obelia dichotoma. It was without gonosomes, and it was the absence of the gonosomes that led Allman to establish a new genus for a Hydroid that was reproducing by fission. Allman states that the frustule on liberation has a distinct endoderm and ectoderm, but no perisarc. It has no means of locomotion, and attaches itself by a mucous excretion from its surface to the glass of an aquarium. Soon after attachment the mucous excretion forms round the fission-frustule a very thin tube, which is the perisarc. Once attached a hydranth develops from it, then later on other hydranths are formed and a little colony arises by branching.

Fission is not merely the nipping off a small portion of the cœnosarc, as the fission-frustule contains all the elements necessary for the formation of a new colony. It does not usually take place at the same time as sexual reproduction. In Microhydra the method of fission is different from that in marine Hydroids, as there are no branches. An outgrowth takes place from the side of the hydranth (Pl. 36, fig. 21), and this is nipped off and develops into a hydranth.

It is very probable that the Hydroid of Limnocodium also reproduces asexually by fission. Parsons, who kept the Hydroids in an aquarium, states "that the polyps made their appearance on the side of a sponge which had been in contact

with a pipe (the hot-water pipe in the Victoria Regia tank). This fact leads me to the inference that the polyps were developed from germs contained in the water which I brought away with me, for I do not see how they could have got there while the sponge was alive; moreover, they were in different stages of development, the earliest stage seen by me being a little mound of fuscous coloured sarcode." Fowler has figured a section of a bud, which "may either remain attached to the parent, or may be nipped off and settle close by, its tissues in either case gradually undergoing the differentiations which characterise the adult."

The sexual method of reproduction of Microhydra is no doubt by means of Medusæ. Up to the present time only the earliest stage of the Medusa is known. The young Medusa has the appearance of an Anthomedusa, but it is impossible to assign it to a definite family until the later stages have been seen. It would be most interesting to know what became of the Medusa after leaving the Hydroid. The Medusa is set free in a stream or river, so that it must be carried along with the current in the direction of the sea.

The exact method of the sexual reproduction of Limnocodium still remains a mystery. During the period it lived in England only the male Medusa was found, and not the slightest evidence on the presence of the female sex was obtainable. The Medusa has not been seen in England since 1893, so it is evident that our stock has completely died out.

In 1901 Limnocodium suddenly appeared in the Victoria Regia tank at Lyons, and an account of it is given by Vaney and Conte. The Hydroid phase was searched for, but was not found, and the Medusæ were all males.

In 1905 Boecker recorded the appearance of Limnocodium in the Victoria Regia tank at Munich, and again only males were seen. The author does not mention the occurrence of the Hydroid phase.

References.

On Microhydra.

1885. Potts, E.—"Microhydra ryderi," Science Bulletin, p.v. (a supplement in 'Science,' vol. v, No. 123).

1897. Potts, E.—"A North American Freshwater Jelly Fish (Microhydra ryderi)," 'American Naturalist,' vol. xxxi, pp. 1032–1035.

On Limnocodium.

1890. Fowler, G. H.—"Notes on the Hydroid Phase of Limnocodium sowerbii," 'Quart. Journ. Micr. Sci.,' vol. xxx, pp. 507–513, pl. xxxii. (This paper contains the bibliography of Limnocodium up to 1885.)

1893. Lankester, E. R.—"Reappearance of the Freshwater Medusa (Limnocodium sowerbii)," 'Nature,' vol. xlix, p. 127. (Records the occurrence of Limnocodium at Sheffield, and gives an excellent summary of its life-history.)

1901. Vaney, C., and Conte, A.—"Sur le Limnocodium sowerbii," 'Zool. Anzeiger,' Bd. xxiv, pp. 533–534; two figures in text. (Records the occurrence of the Medusa at Lyons.)

1905. Boecker, E.—"Ueber das Vorkommen von Limnocodium im Münchner botanischen Garten," 'Biol. Centralbl,' Bd. xxv, pp. 605–606.

On Reproduction in Hydroids by Fission.

1871. Allman, G. J.—"On a Mode of Reproduction by Spontaneous Fission in the Hydroida," 'Quart. Journ. Micr. Sci.,' vol. xi, pp. 18–21, pl. ii.

1871. Allman, G. J. 'Monograph of the Gymnoblastic Hydroids,' p. 151, fig. 61.

1872. Hincks, T.—"Contributions to the History of the Hydroida," 'Ann. Mag. Nat. Hist.,' Series 4, vol. ix, p. 390.

1901. Billard, A.—"De la Scissiparité Chez les Hydroides," 'Comptes-rendus. Acad. Sci. Paris,' tom. 133, pp. 441–443.

EXPLANATION OF PLATE 37.

Illustrating Mr. E. T. Browne's paper "On the Freshwater Medusa liberated by Microhydra ryderi, Potts, and a Comparison with Limnocodium."

Fig. 1.—Lateral view of the medusa of Microhydra ryderi. × 150.

Fig. 2.—A tentacle of the medusa of Microhydra. Outer side. × 500.

Fig. 3.—The central portion of a very young tentacle of the medusa of Limnocodium. Drawn for comparison with fig. 2. × 500.

Fig. 4.—A group of three nematocysts in the tentacle of the medusa of Microhydra. × 1000.

Fig. 5.—Nematocysts from the tentacle of Limnocodium. × 1000.

Postscript.—The writing of this paper has led me to commence investigations on the methods of asexual reproduction amongst Hydroids, and the work is now being carried on in the Marine Laboratory at Plymouth. I have found Allman's "Schizocladium ramosum" and have observed the formation of fission-frustules, their liberation, and subsequent development. My observations completely confirm those made by Allman.

The frustule when nipped off consists of a thin, transparent layer of ectoderm with nematocysts, a thick layer of endoderm loaded with granules, and a hollow central cavity. The bud which is detached from the hydroid phase of Microhydra (Potts, Pl. 36, figs. 17 and 24) is exactly like the fission-frustule of Schizocladium, both in shape and structure.

Hincks' suggestion that Schizocladium is probably an Obelia has turned out to be correct. Some of the colonies have liberated Medusæ which belong to the genus Obelia.

I have also found a Clava-like hydroid detaching numerous fission-frustules from its hydrorhiza. These buds drop to the bottom of the aquarium and lightly attach themselves by one end to the glass. They are now a week old and have not yet begun to develop.

Nov. 4th, 1906.

INDEX TO VOL. 50,

NEW SERIES.

PRINTED BY ADLARD AND SON, LONDON AND DORKING.

THE MARINE BIOLOGICAL ASSOCIATION

OF THE

UNITED KINGDOM.

——:o:——

Patron—HIS MAJESTY THE KING.

President—Prof. E. RAY LANKESTER, LL.D., F.R.S.

——:o:——

THE ASSOCIATION WAS FOUNDED "TO ESTABLISH AND MAINTAIN LABORATORIES ON THE COAST OF THE UNITED KINGDOM, WHERE ACCURATE RESEARCHES MAY BE CARRIED ON, LEADING TO THE IMPROVEMENT OF ZOOLOGICAL AND BOTANICAL SCIENCE, AND TO AN INCREASE OF OUR KNOWLEDGE AS REGARDS THE FOOD, LIFE CONDITIONS, AND HABITS OF BRITISH FOOD-FISHES AND MOLLUSCS."

The Laboratory at Plymouth

was opened in 1888. Since that time investigations, practical and scientific, have been constantly pursued by naturalists appointed by the Association, as well as by those from England and abroad who have carried on independent researches.

Naturalists desiring to work at the Laboratory

should communicate with the Director, who will supply all information as to terms, etc.

Works published by the Association

include the following:—'A Treatise on the Common Sole,' J. T. Cunningham, M.A., 4to, 25/-. 'The Natural History of the Marketable Marine Fishes of the British Islands,' J. T. Cunningham, M.A., 7/6 net (published for the Association by Messrs. Macmillan & Co.).

The Journal of the Marine Biological Association

is issued half-yearly, price 3/6 each number.

In addition to these publications, the results of work done in the Laboratory are recorded in the 'Quarterly Journal of Microscopical Science,' and in other scientific journals, British and foreign.

Specimens of Marine Animals and Plants,

both living and preserved, according to the best methods, are supplied to the principal British Laboratories and Museums. Detailed price lists will be forwarded on application.

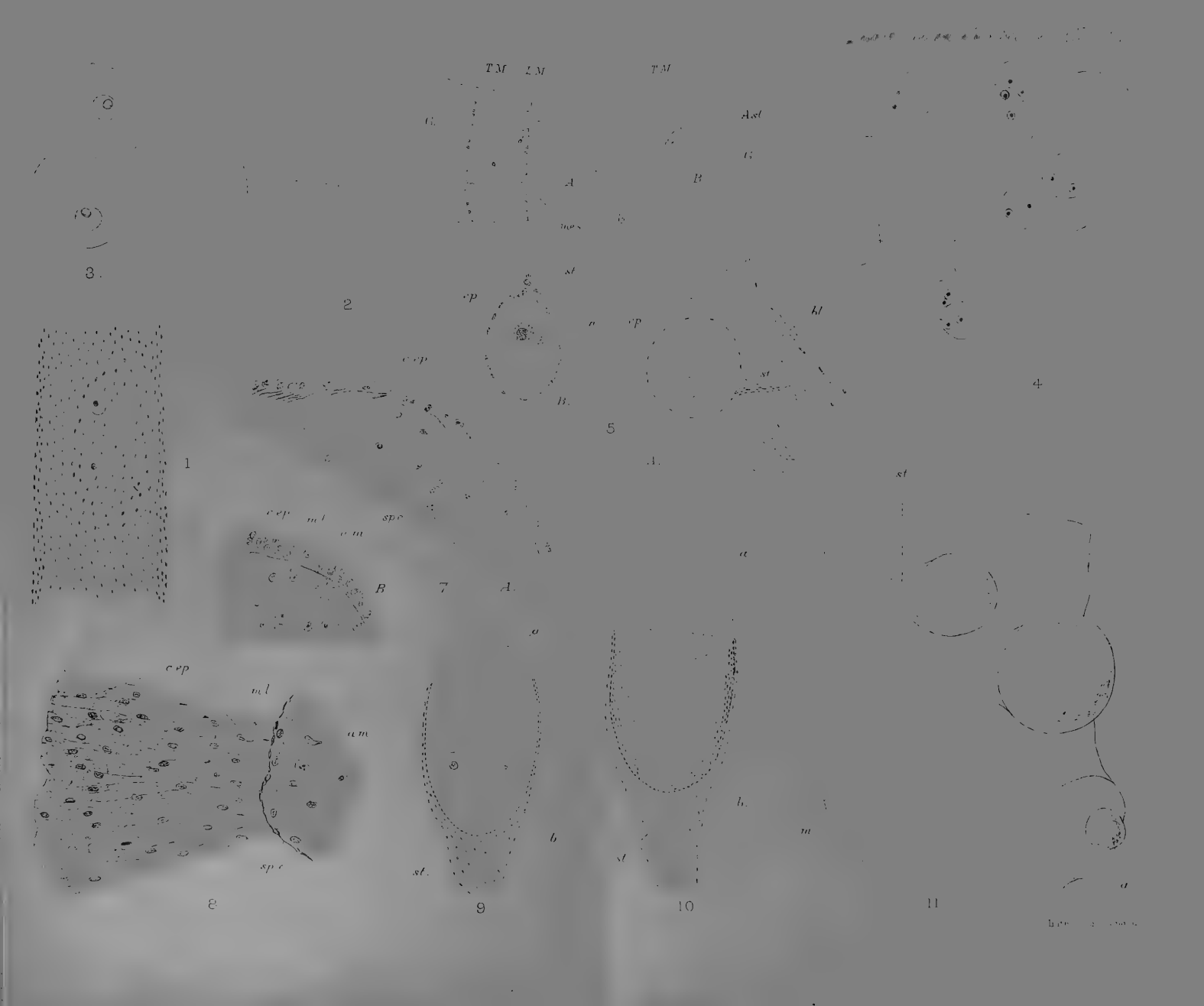
TERMS OF MEMBERSHIP.

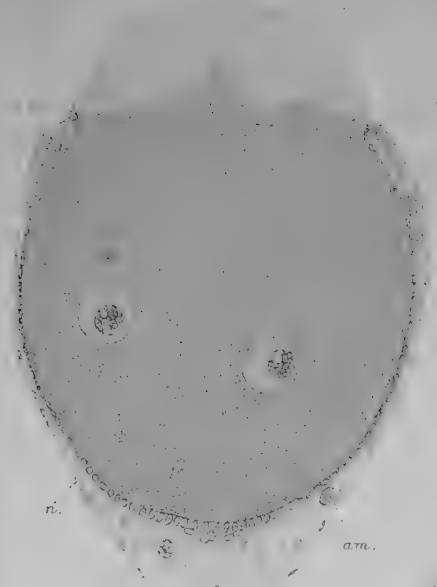
ANNUAL MEMBERS	£1	1	0	per annum.
LIFE MEMBERS	15	15	0	Composition Fee.
FOUNDERS	100	0	0	„ „
GOVERNORS (Life Members of Council)	500	0	0	

Members have the following rights and privileges:—They elect annually the Officers and Council; they receive the *Journal* free by post; they are admitted to view the Laboratory at any time, and may introduce friends with them; they have the first claim to rent a table in the Laboratory for research, with use of tanks, boats, etc.; and have access to the Library at Plymouth. Special privileges are granted to Governors, Founders, and Life Members.

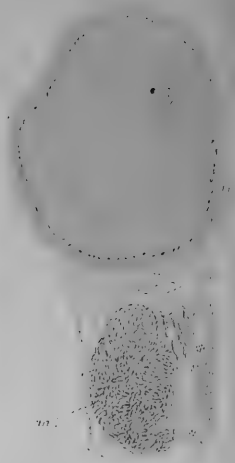
Persons desirous of becoming members, or of obtaining any information with regard to the Association, should communicate with—

The DIRECTOR,
The Laboratory,
Plymouth.

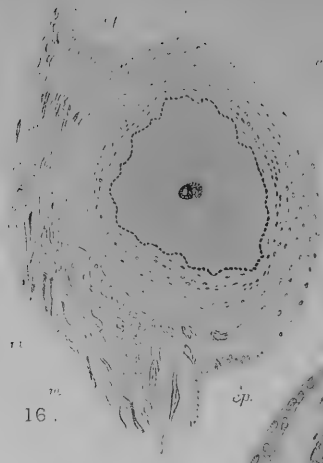

TM
LM
TM
Ast
A
B
ins
st
cp
cep
hl
3.
2
1
4
5
B.
A.
cep
mt
spe
cm
B
7
A.
a
cep
mt
cm
spe
8
st.
9
b
10
b.
m
st
11
a



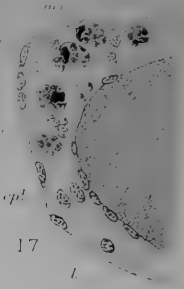
12.



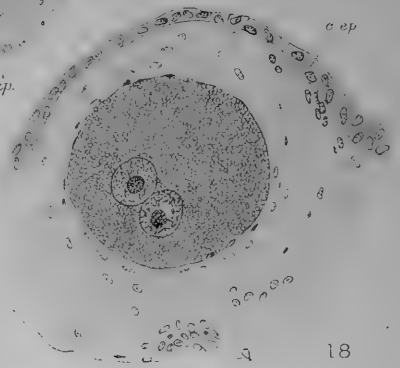
15.



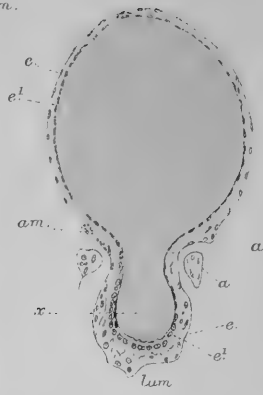
16.

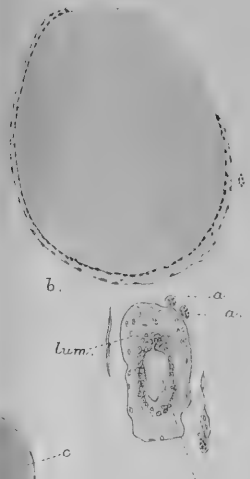


17



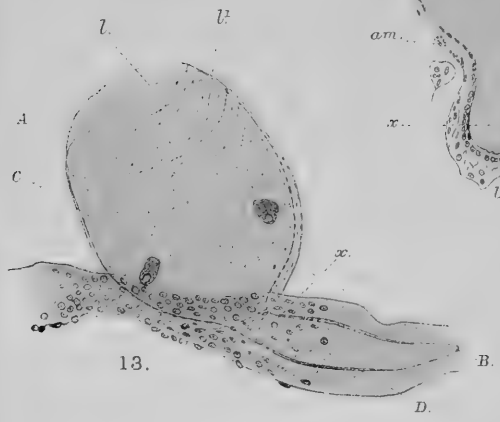
18



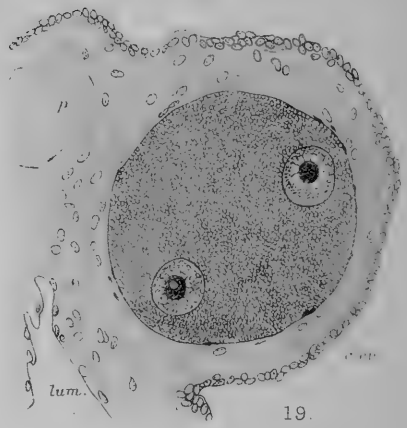


14.

20.

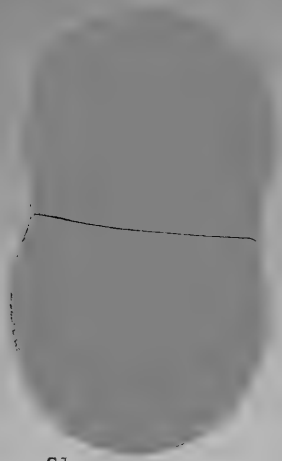


13.

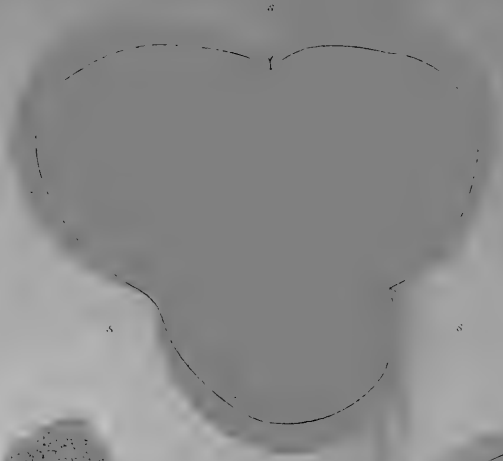


19.

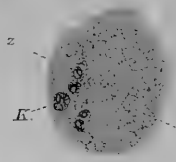
Huth, Lith^r London



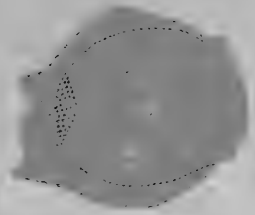
21



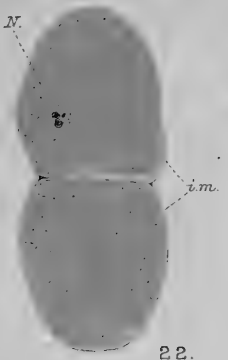
24.



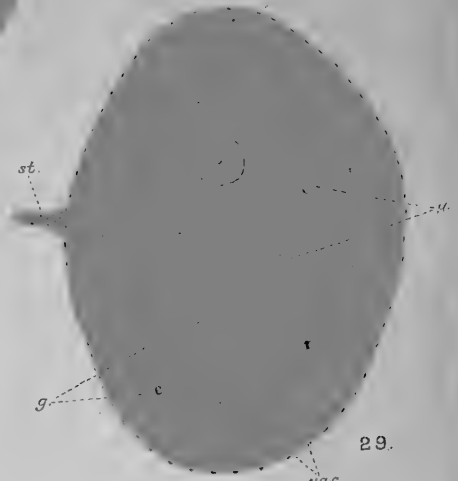
26.



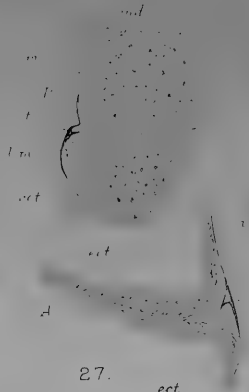
28.



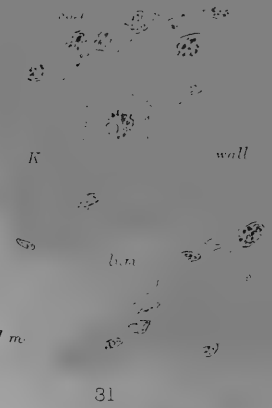
22.



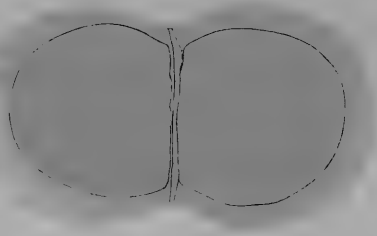
29.



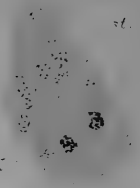
27.



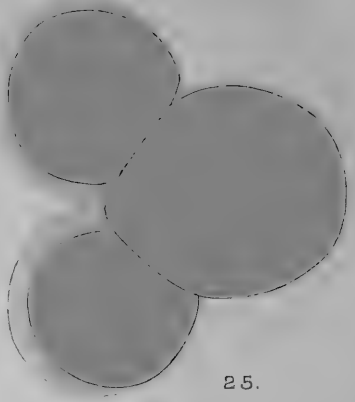
31



23.

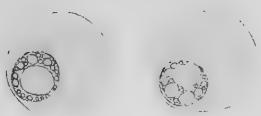


30.

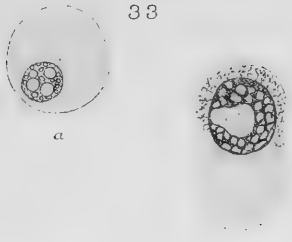


25.

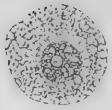
32



33

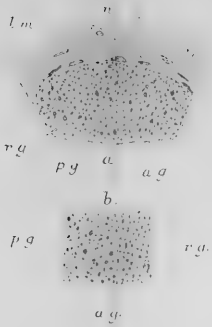


34.

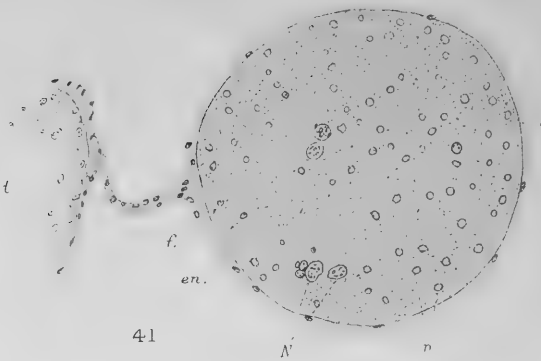


35.

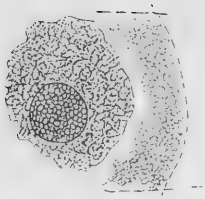
37.



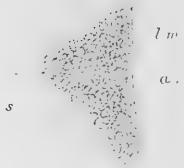
38.

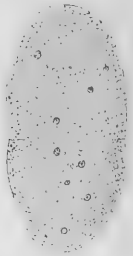


41

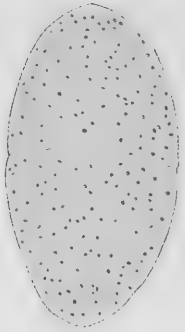


36.

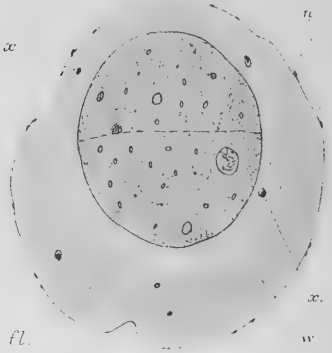




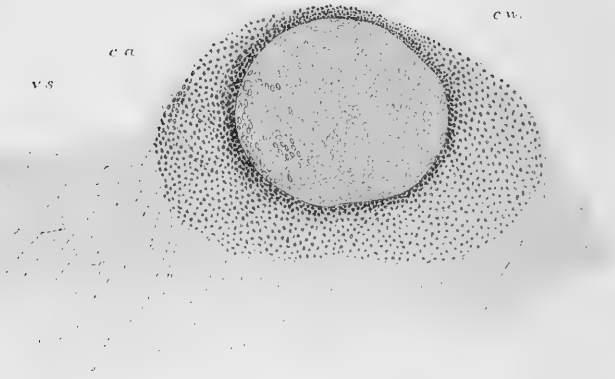
39.



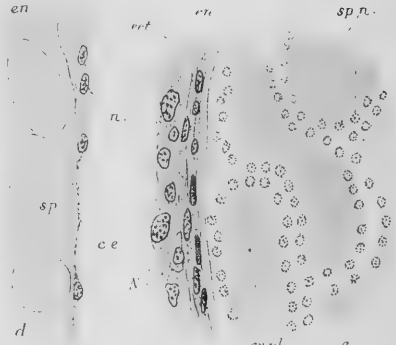
40.



42.

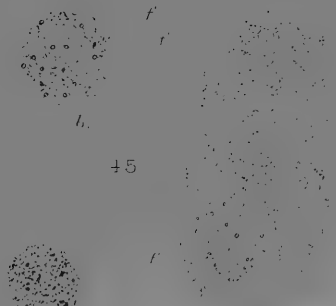


43.



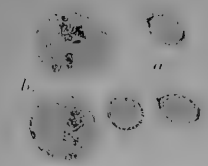
44

Huth, Lith. London

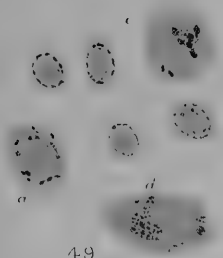


45

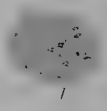
46

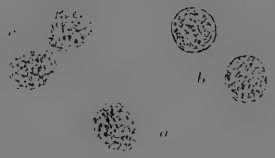


50.

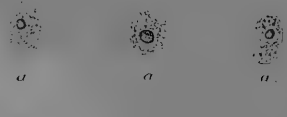


49

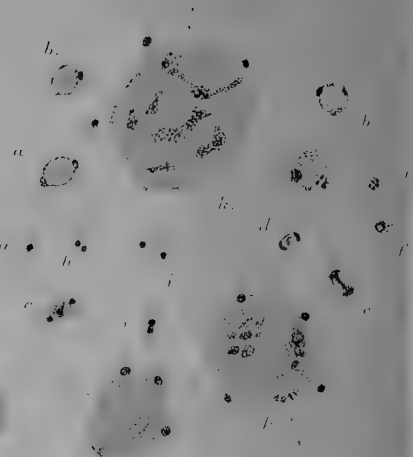




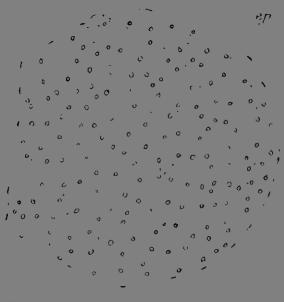
47

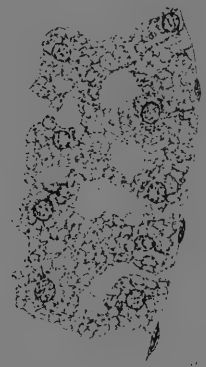


48

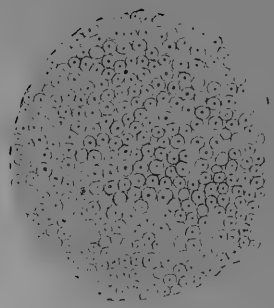


51



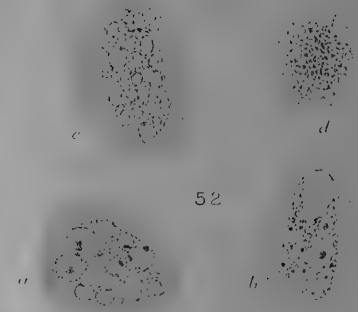


53



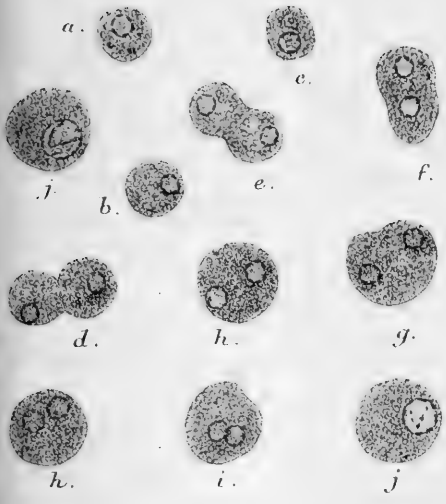
54.

55

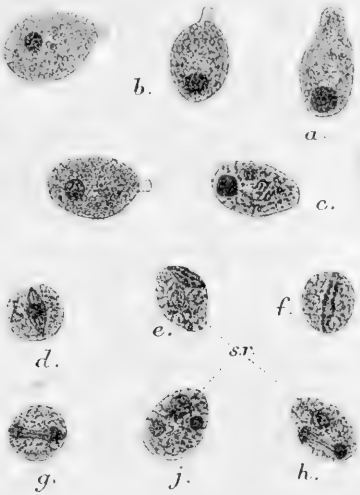


52

56

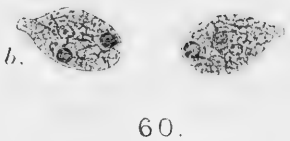


57.



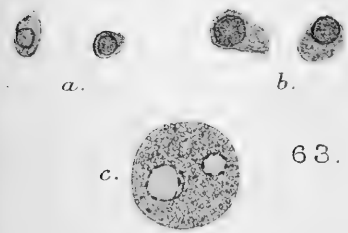
59.

58.



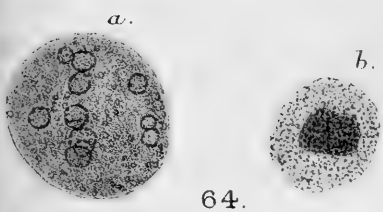
60.

62.



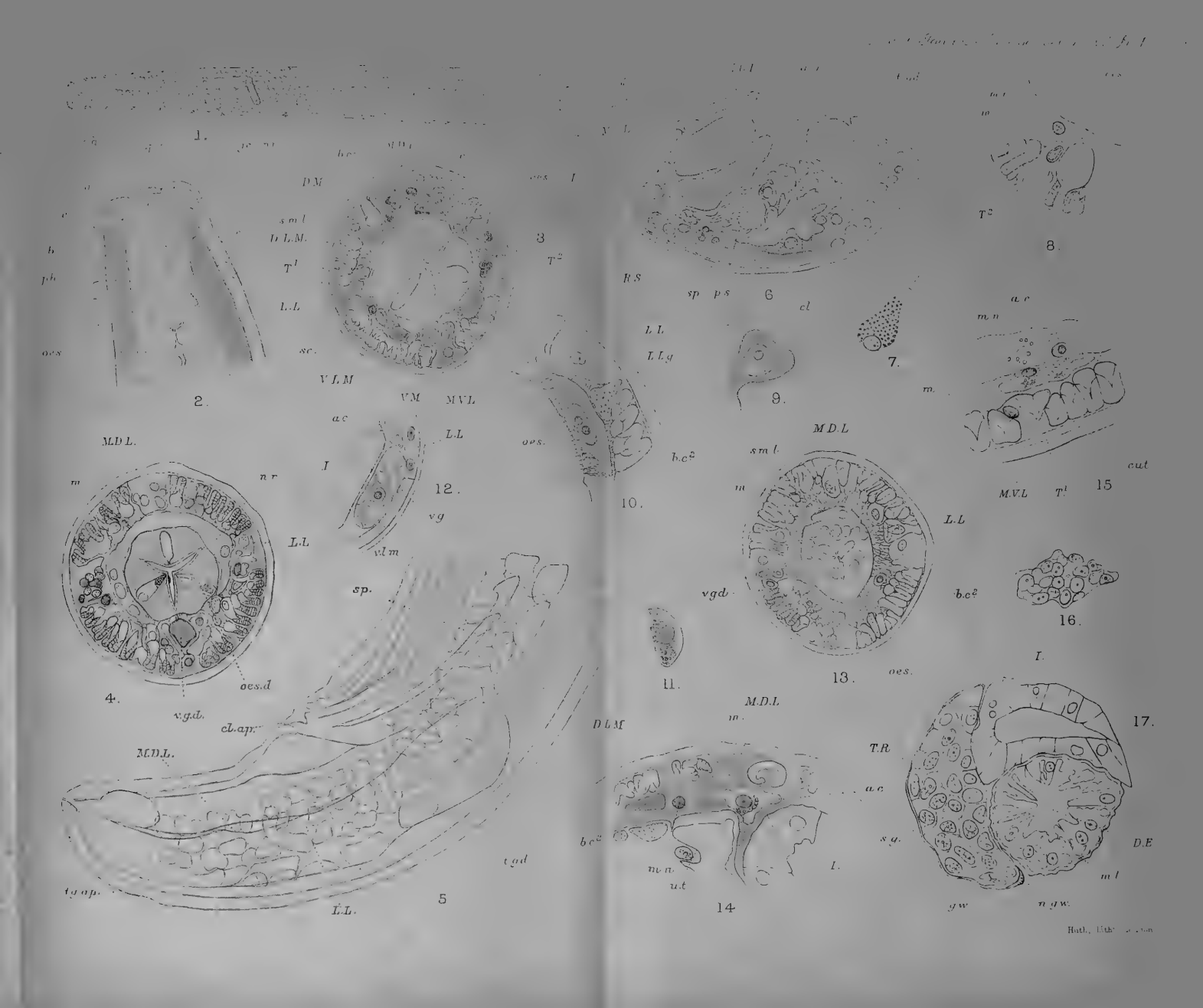
63.

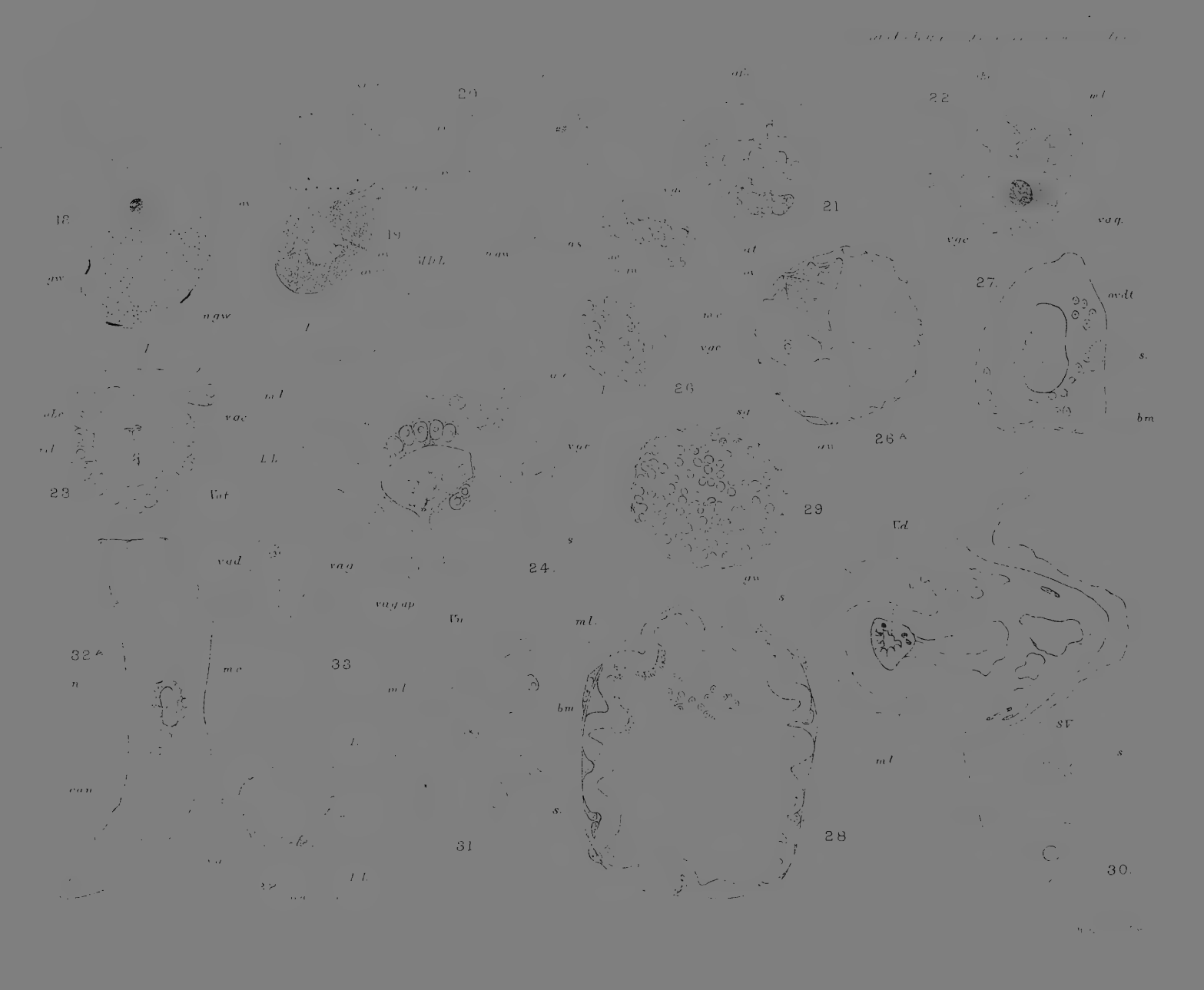
61.



64.

Huth, Lith^r London


1.
2.
3
4.
5
6
7.
8.
9.
10.
11.
12.
13.
14
15
16.
17.
DM
sml
DLM.
T1
LL
sc.
VLM
VM
MVL
ac
MDL.
m
nr
I
oes.d
v.g.d.
cl.ap.
tgap.
LL.
sp.
vlm
vg
oes
T2
RS
sp
ps
cl
LLg
bc2
vgd
MDL
oes.
MVL
T1
cut
DLM
TR
sg.
DE
ml
gw
ngw.
ut
Roth., Lith.



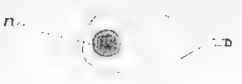


Fig. 34.

Fig. 35.

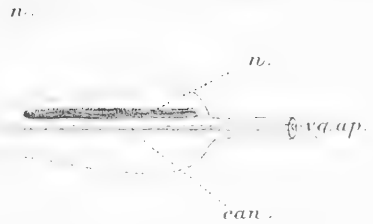


Fig. 36.

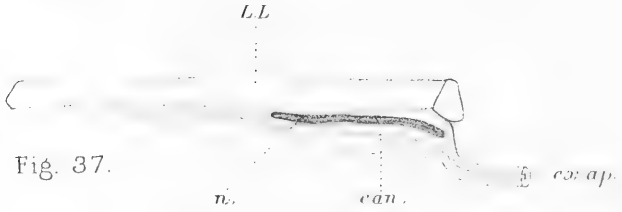


Fig. 37.

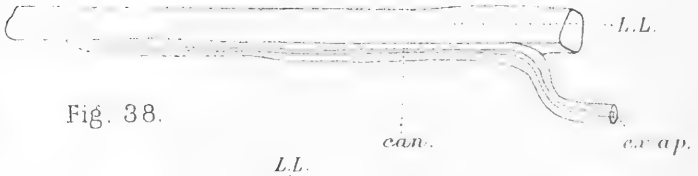


Fig. 38.

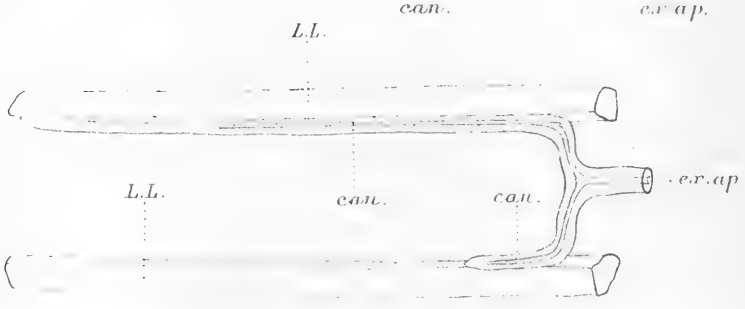
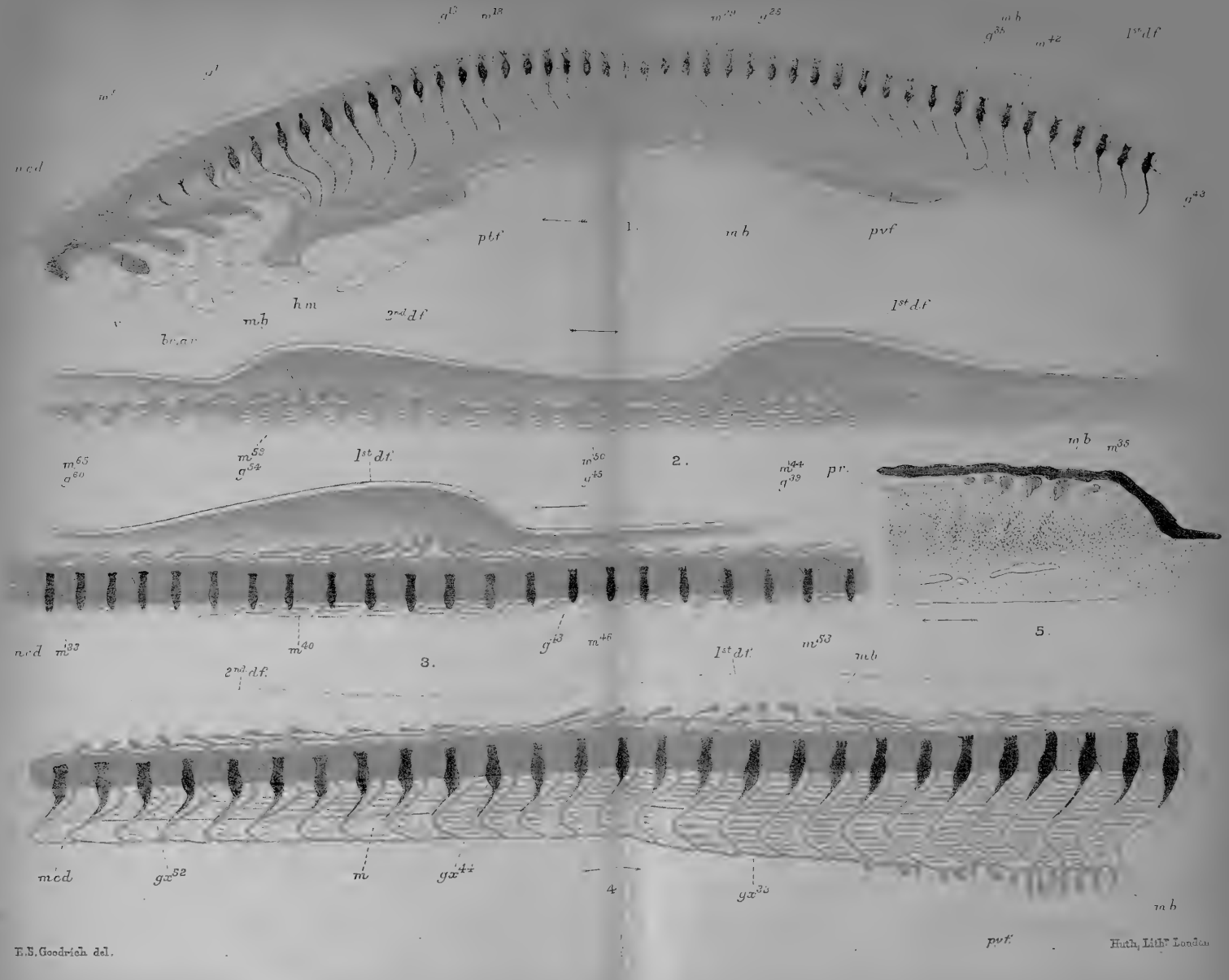


Fig. 39.

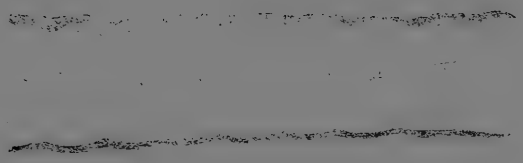
Fig. 40.

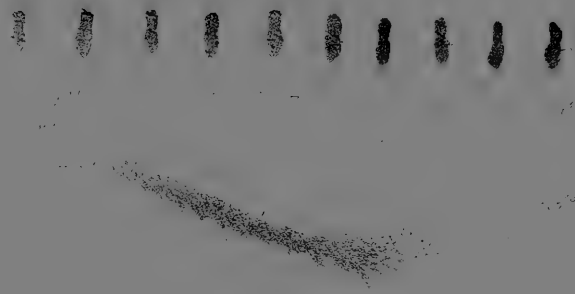
Huth, Lith^r London.

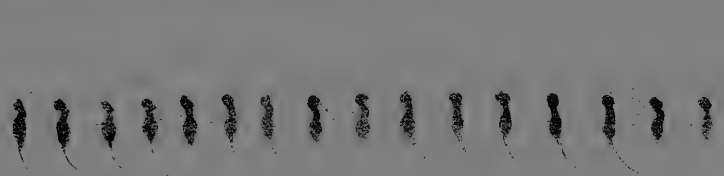


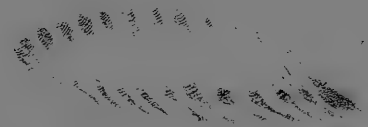
E.S. Goodrich del.

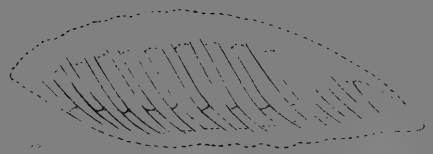
Huth, Lith. London

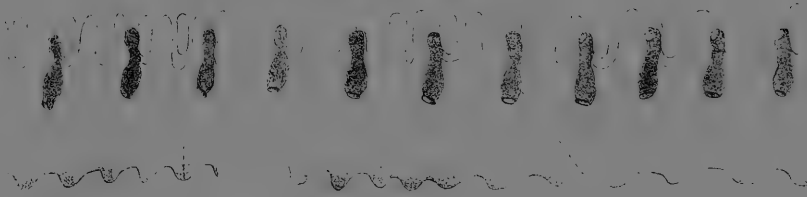


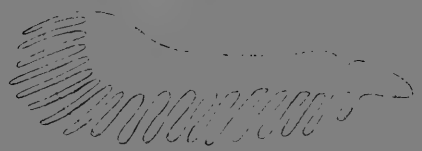


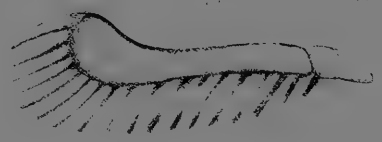


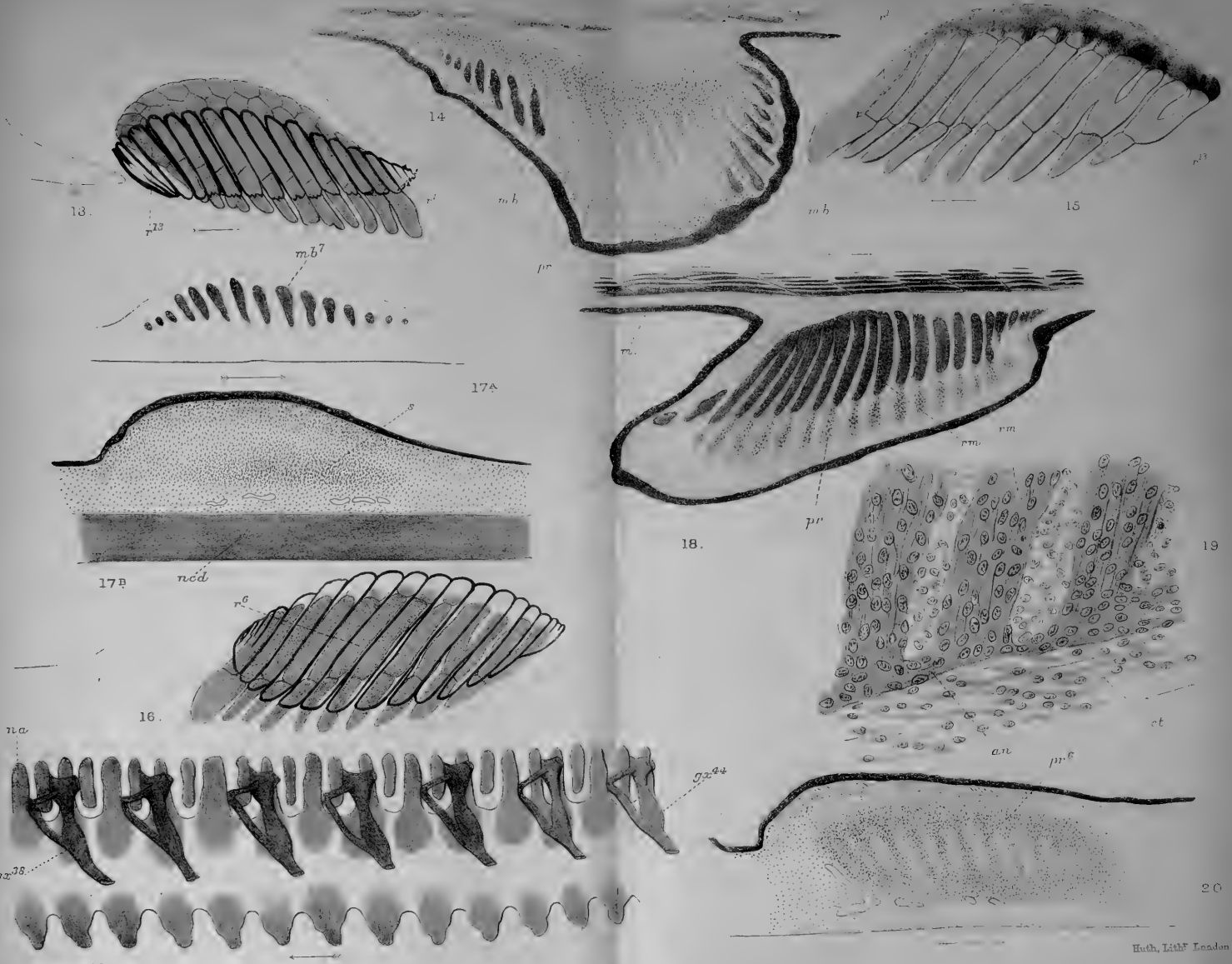


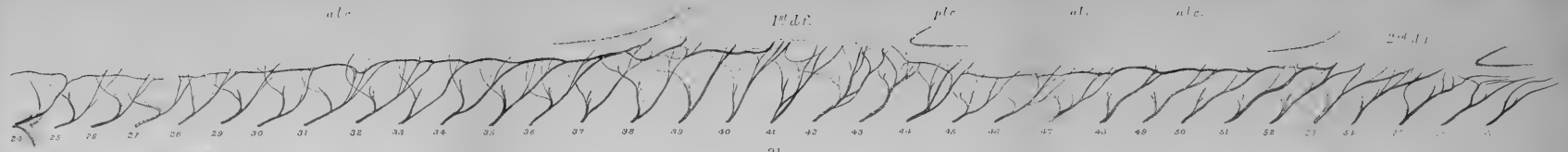




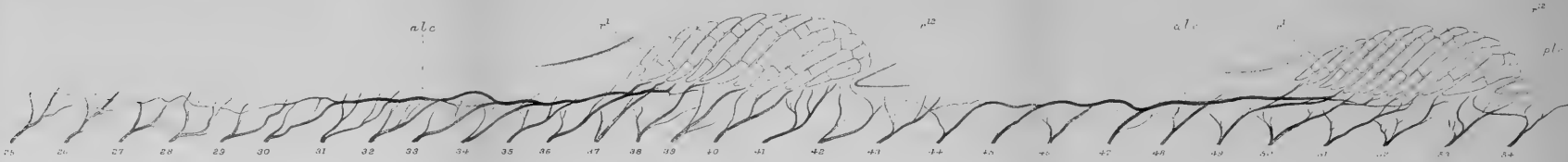




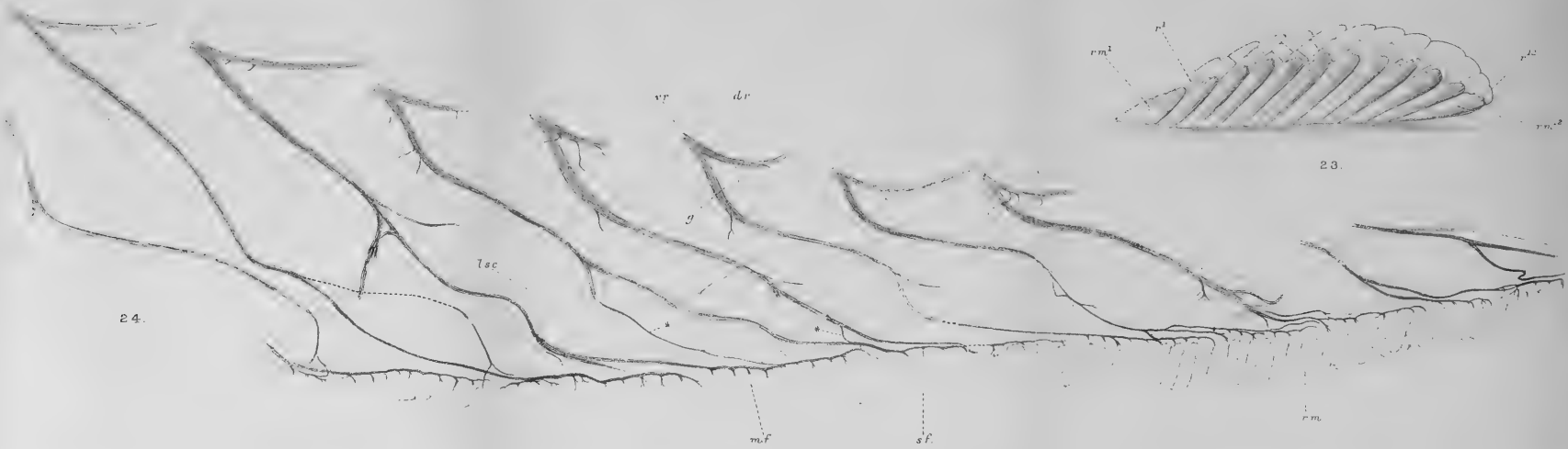

13.
14
15
16.
17A
17B
18.
19
20
mb
pr
rm
ncd
na
ar
ct
E.S. Goodrich, del.
Huth, Lith^r London


a l c
1st d r.
p l c
a l.
a l c.
2nd d r.

21.


a l c
r1
r12
a l c
r1
r12
p l c

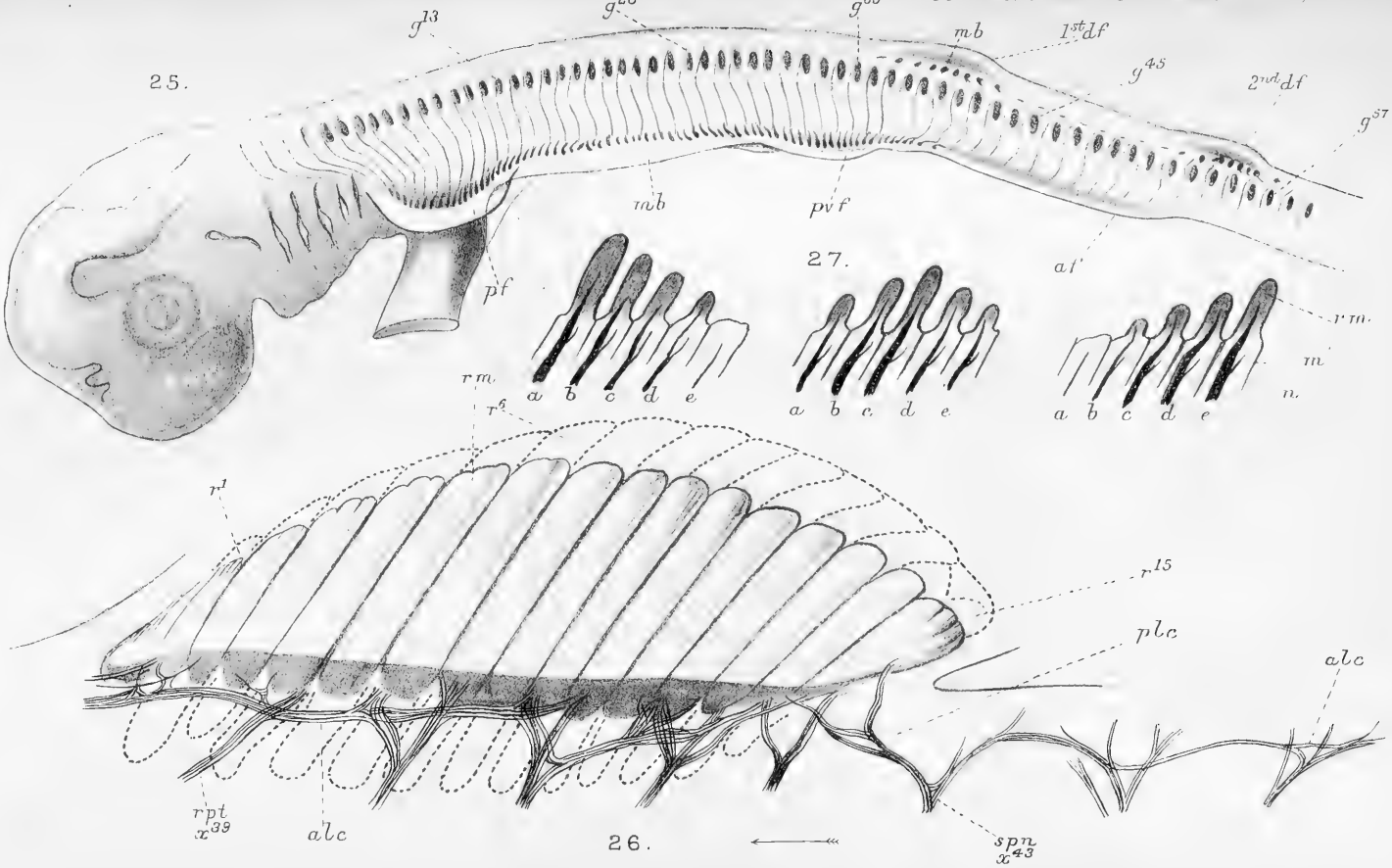
22.


v r
d r
l s c
g
m f
s f
r m

24.

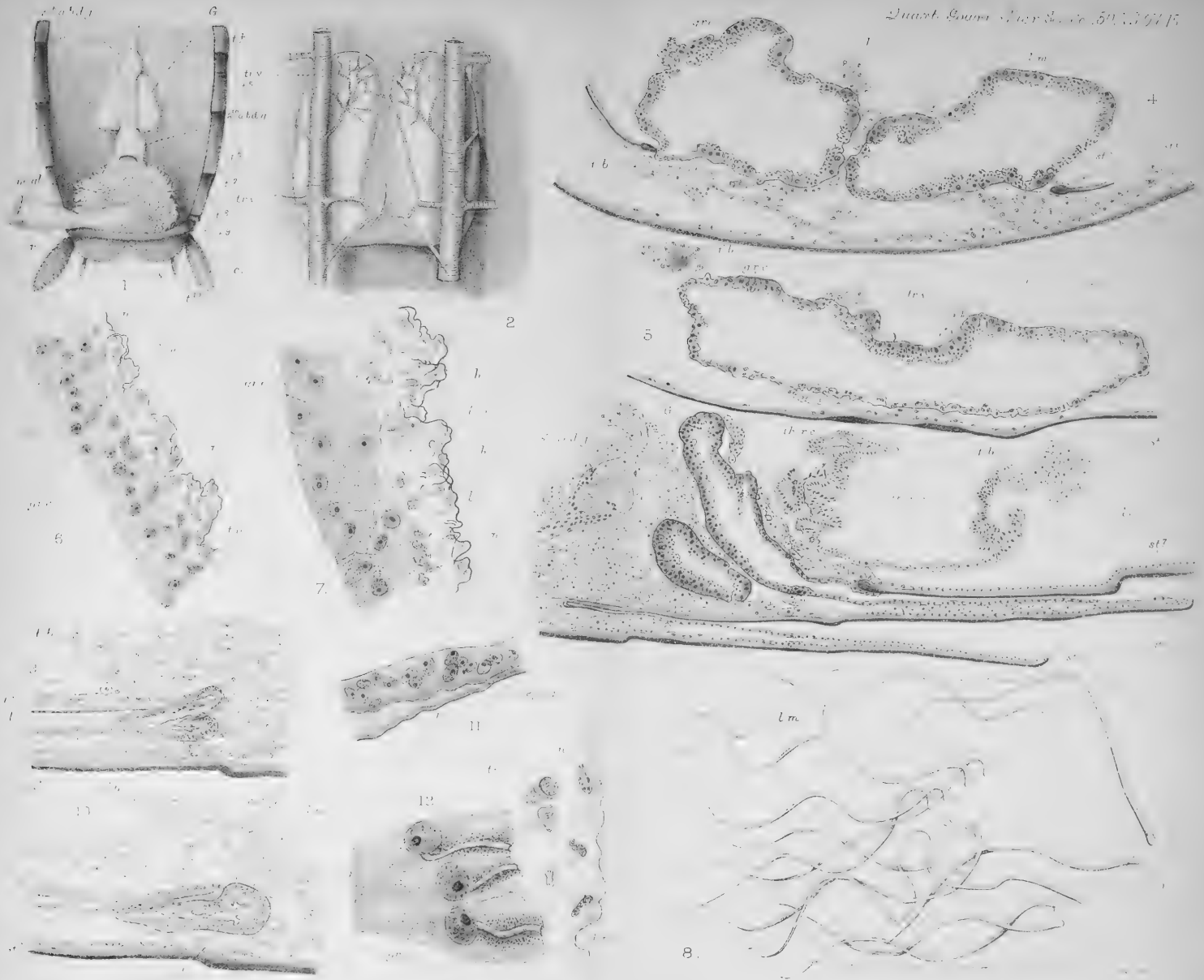
r1
r m1
r12
r m2

23.

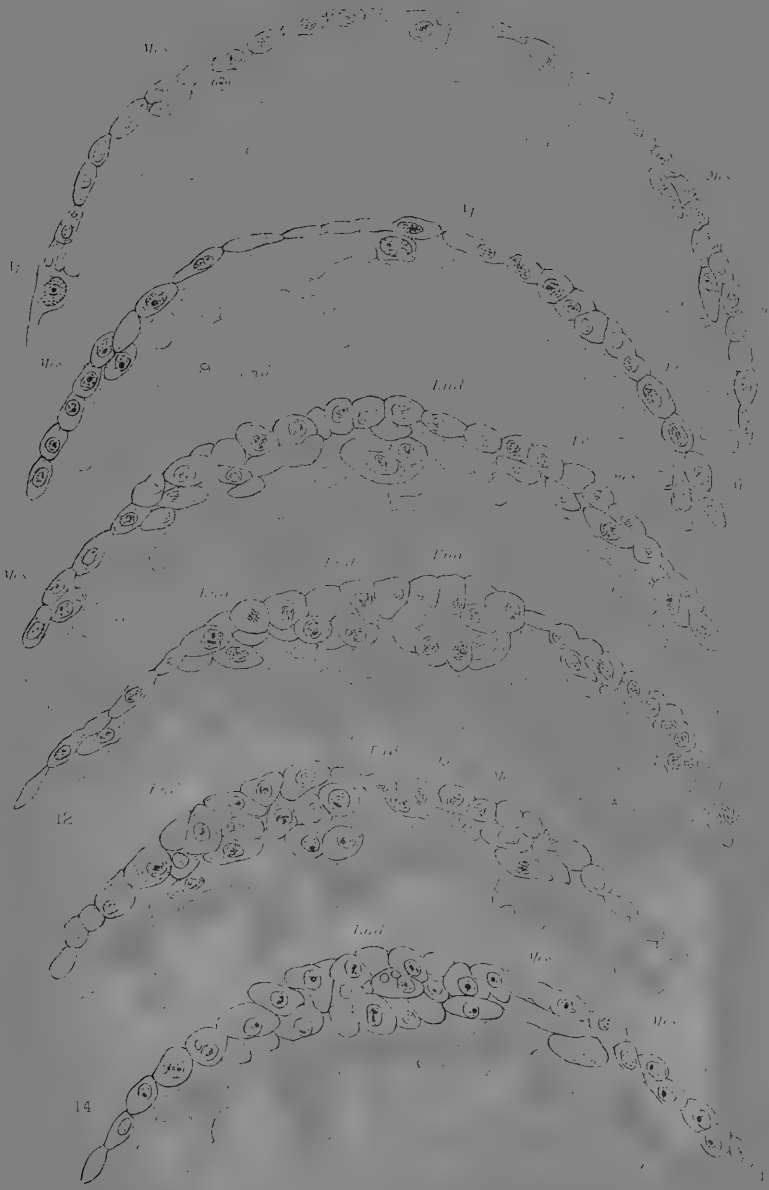


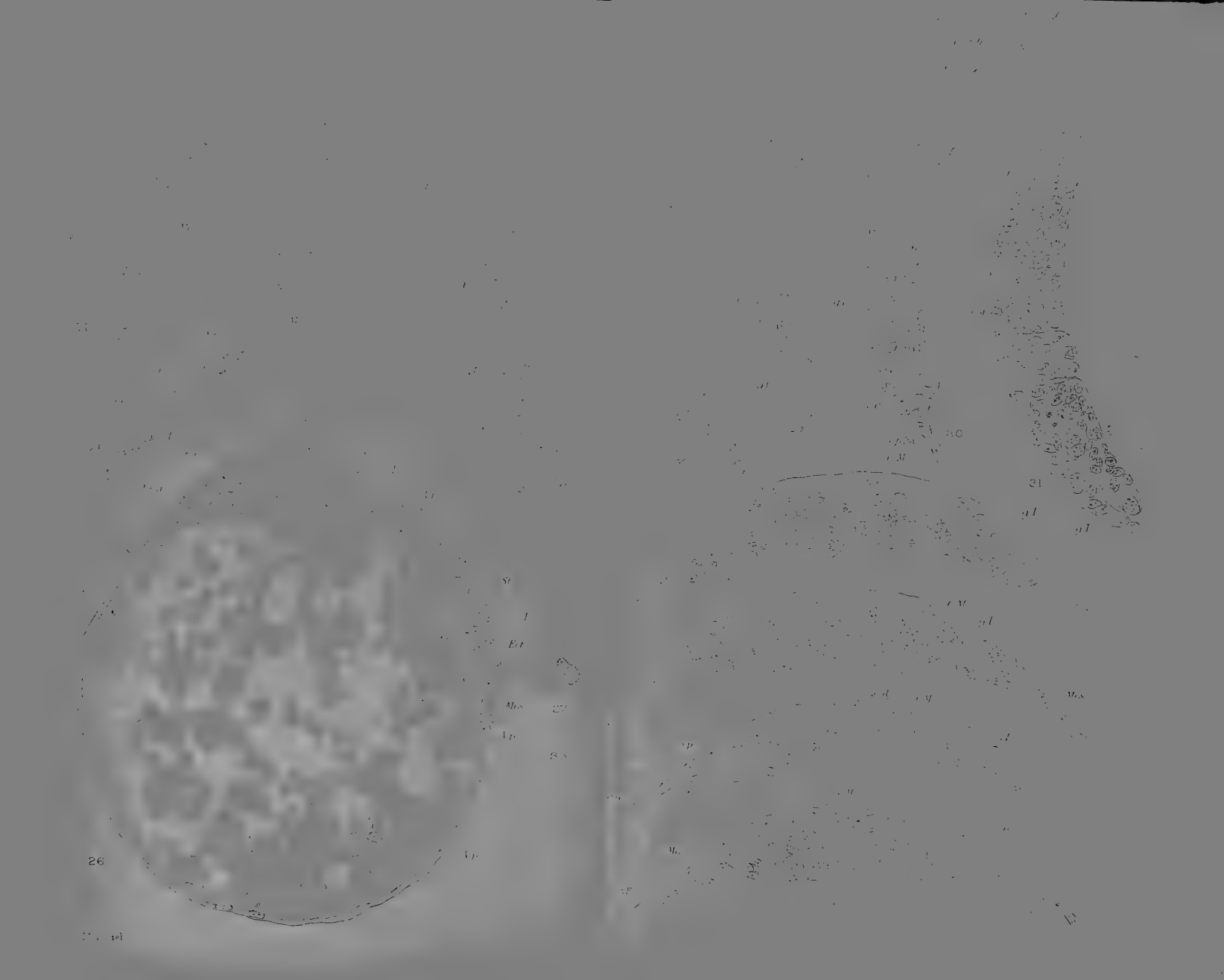
E.S. Goodrich del.

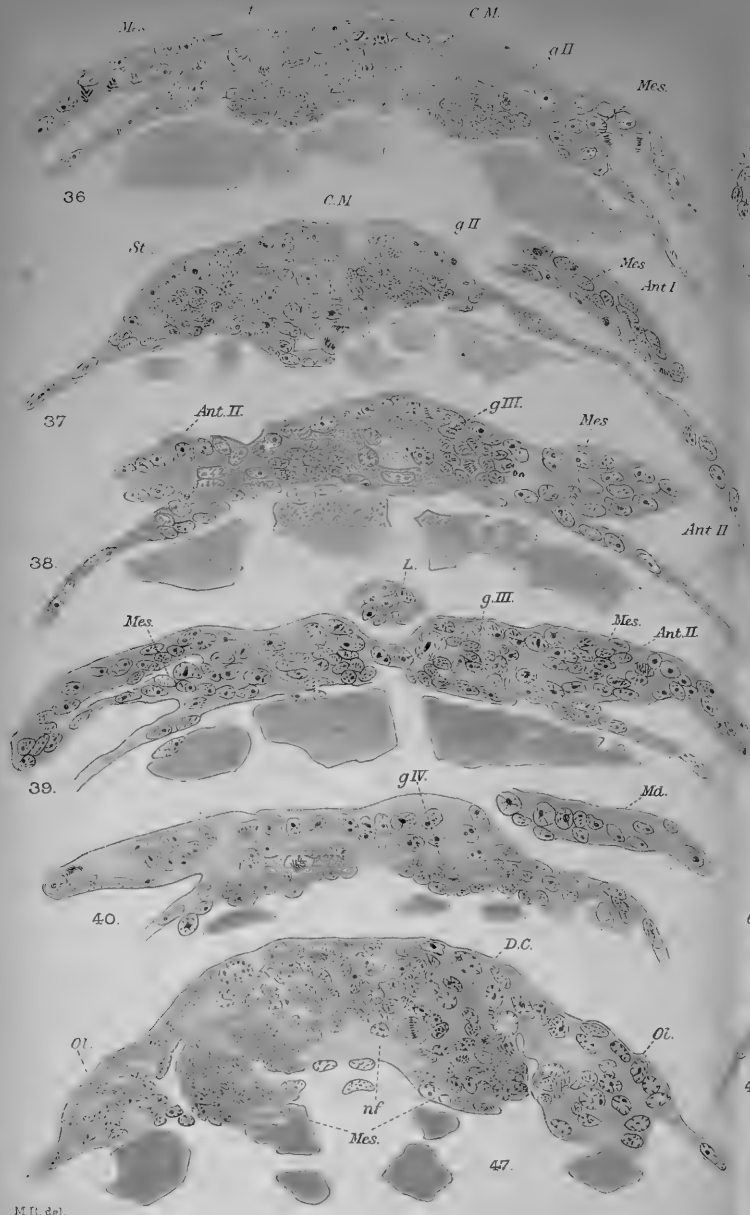
Huth, Lith^r London.

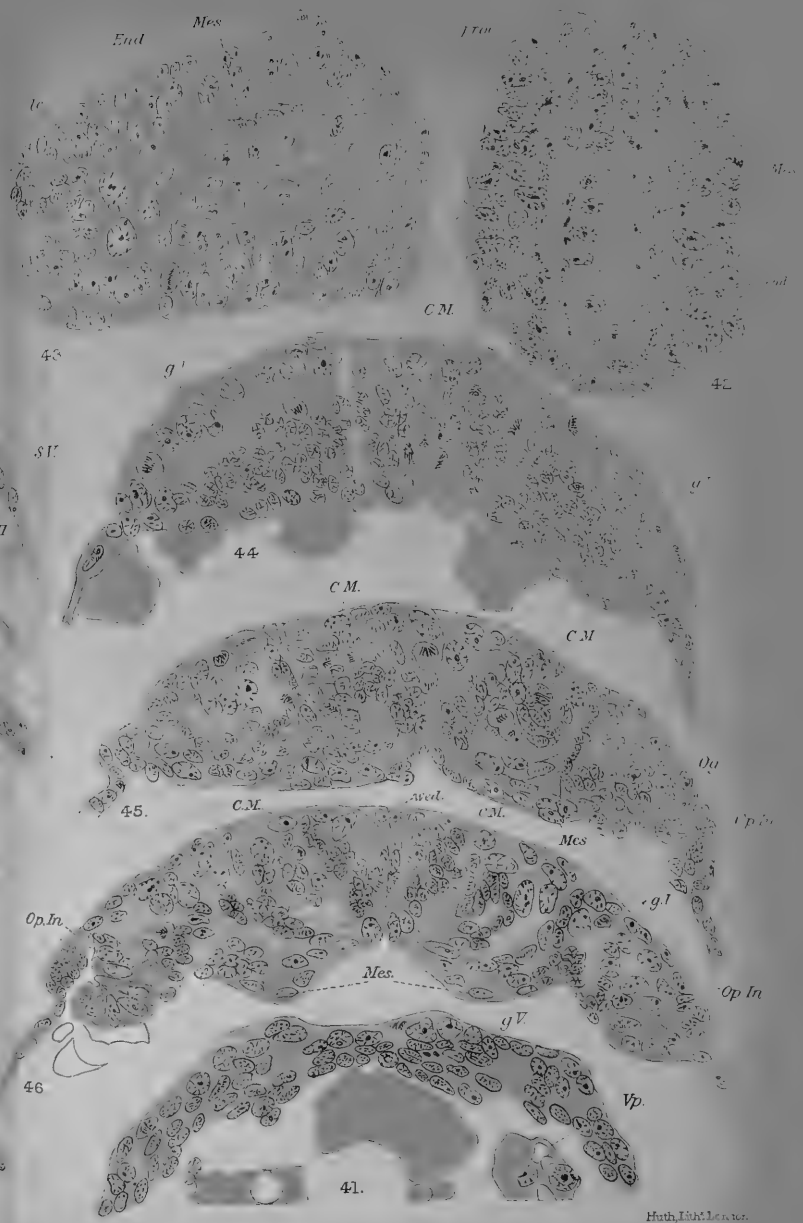



A
B1
C
D
E
F
1
2
3
4
5
6
Ant I
Ant II
Md
Mx I
Mx II
Lb
Vp
Dp



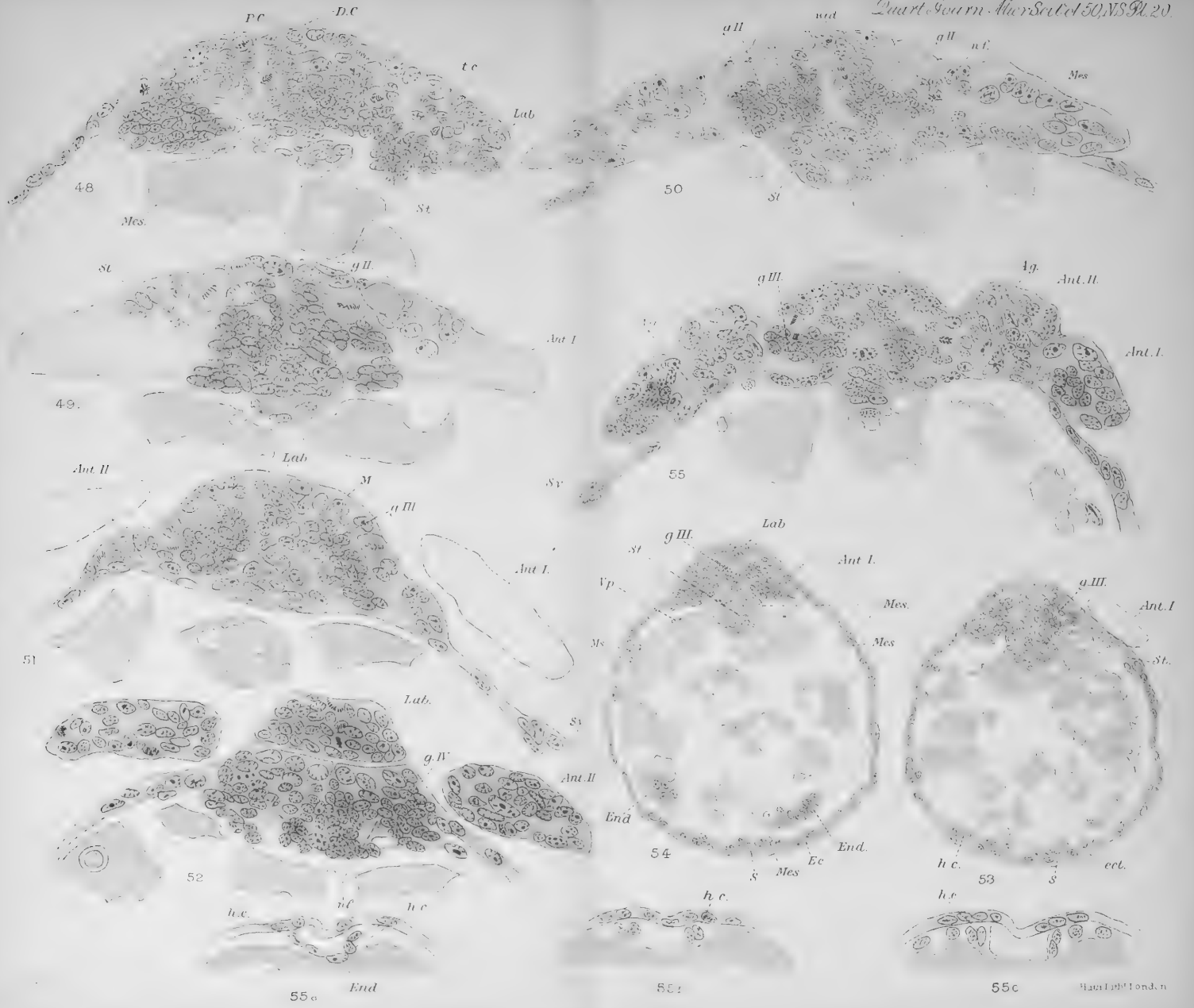


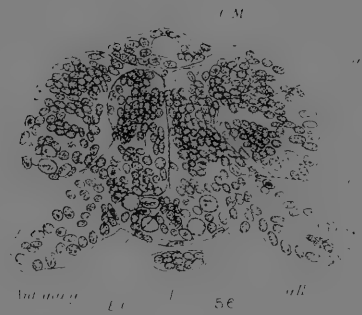


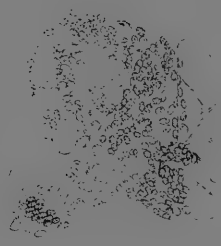


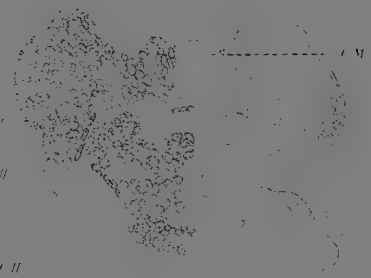
M.H. del.

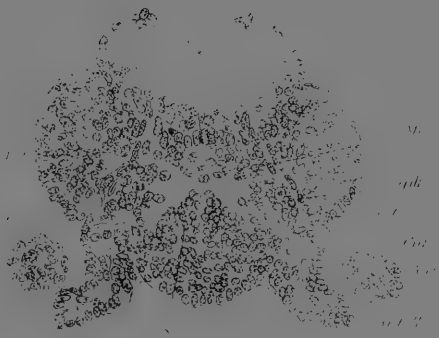
Huth, Lith. Leeds.

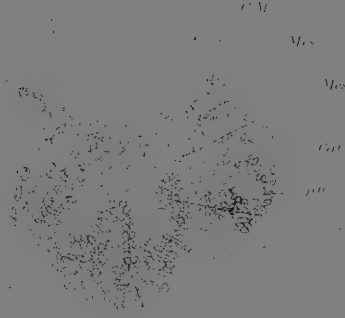


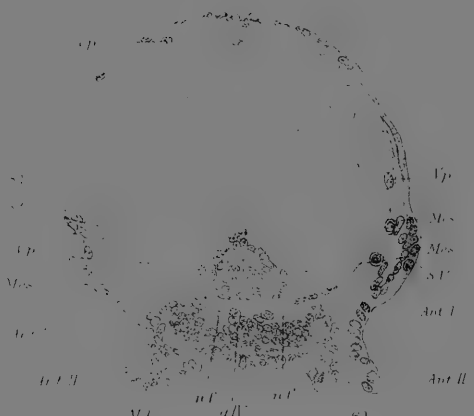


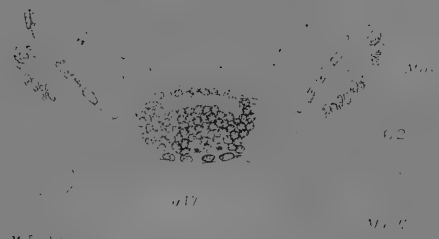


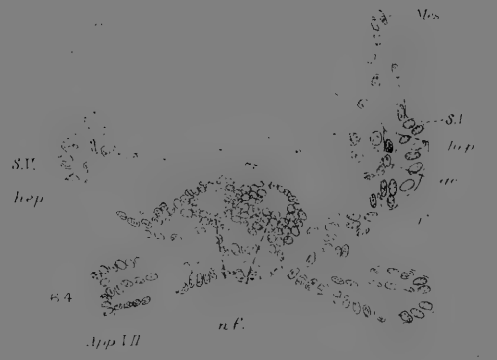




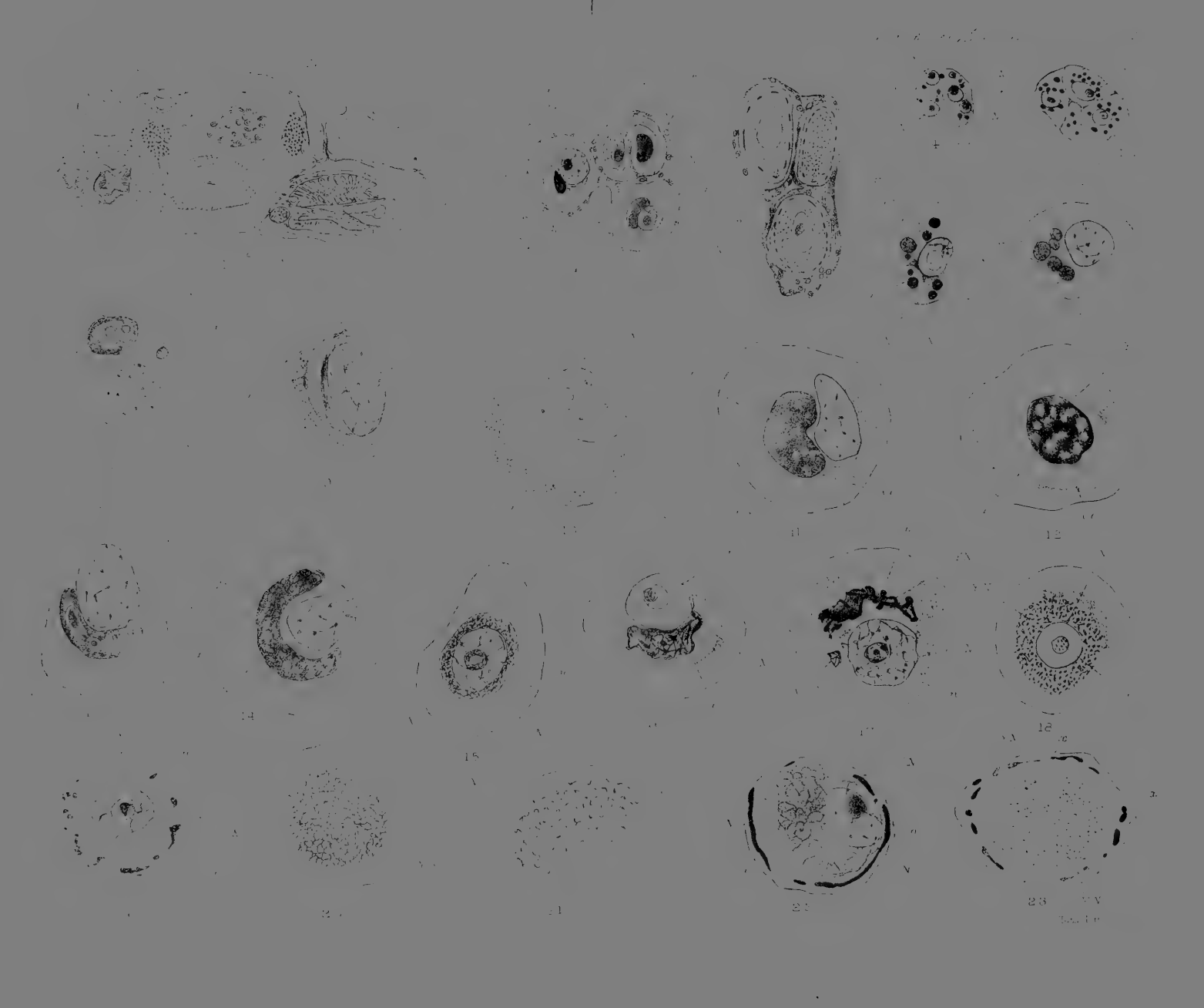


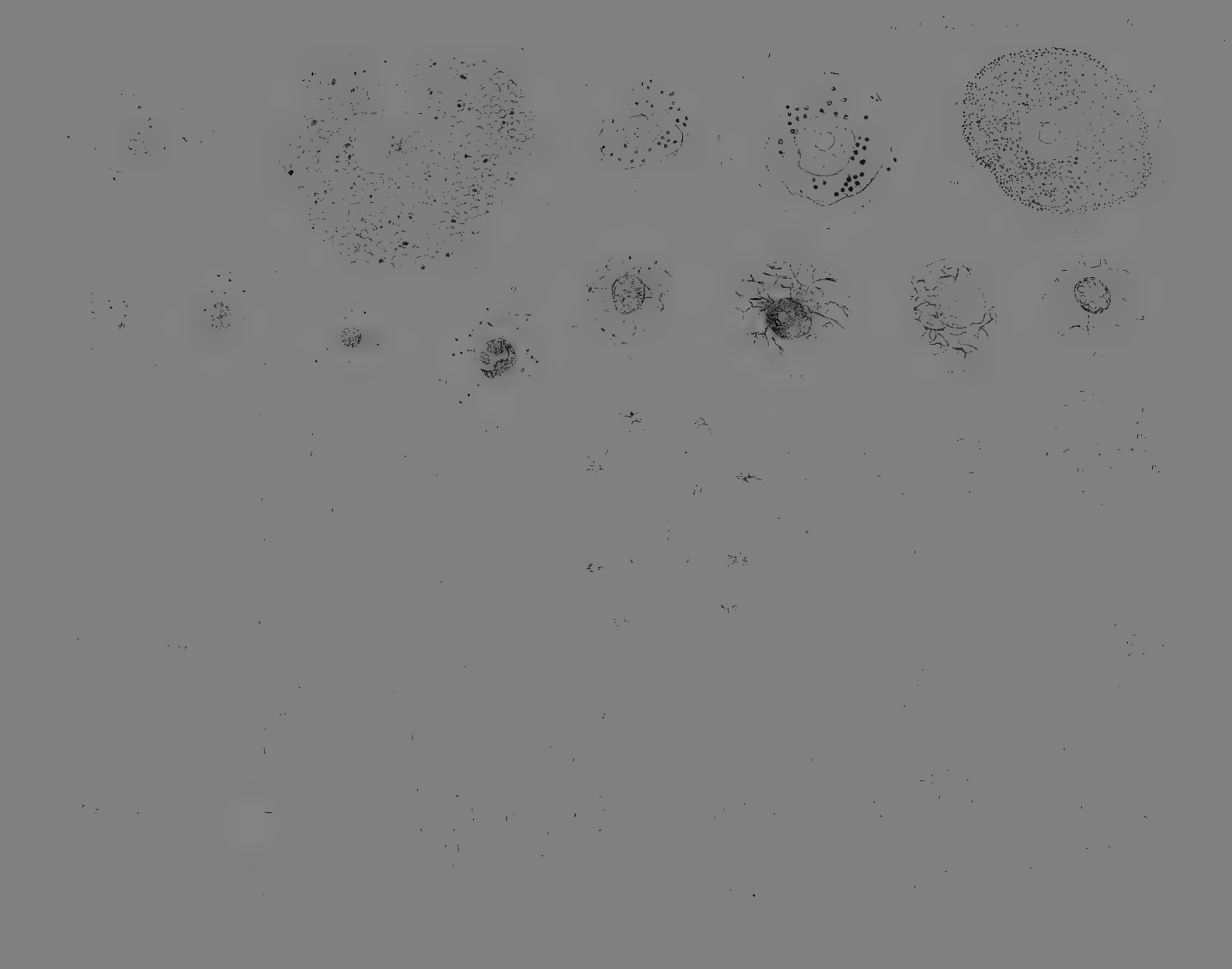


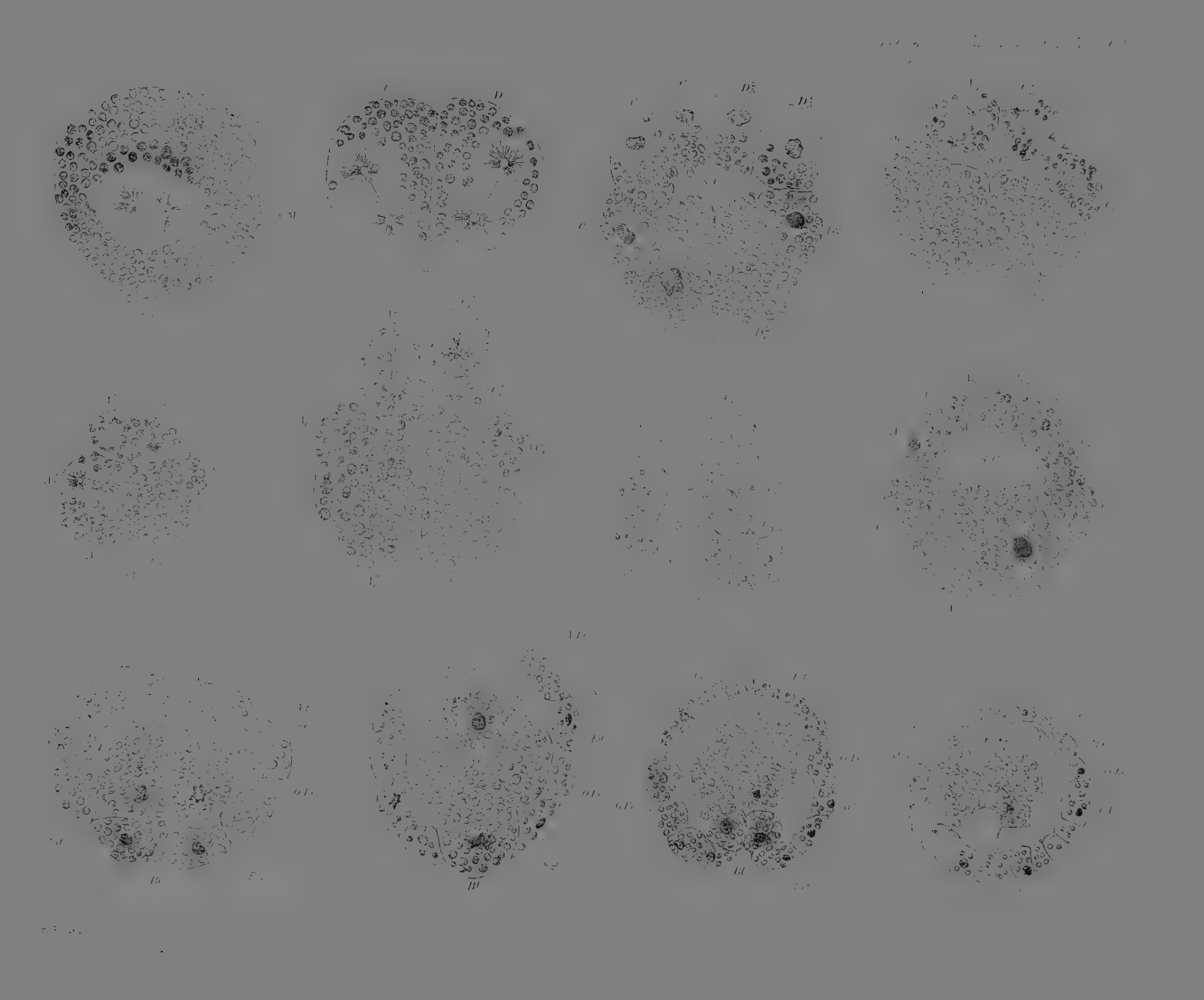


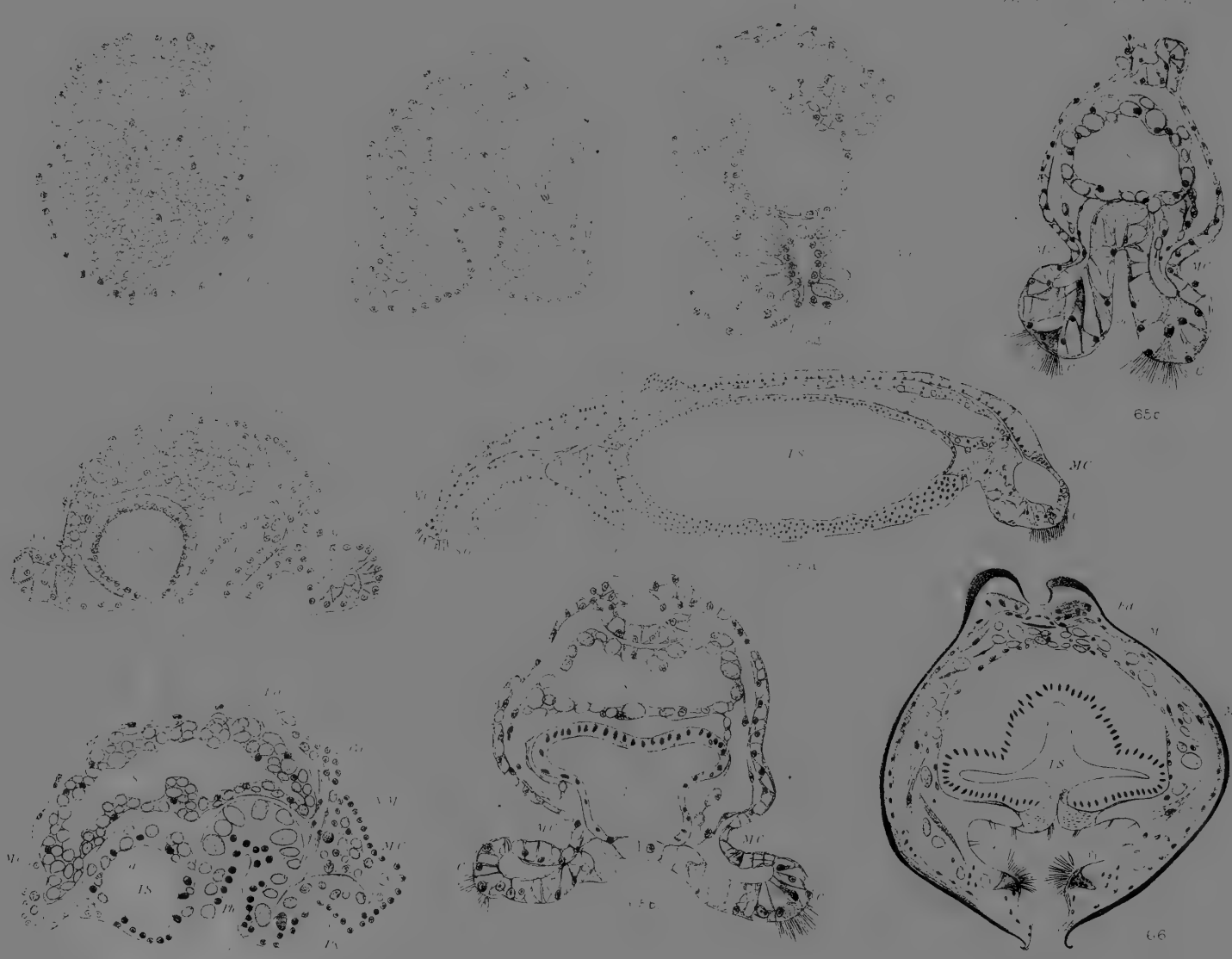


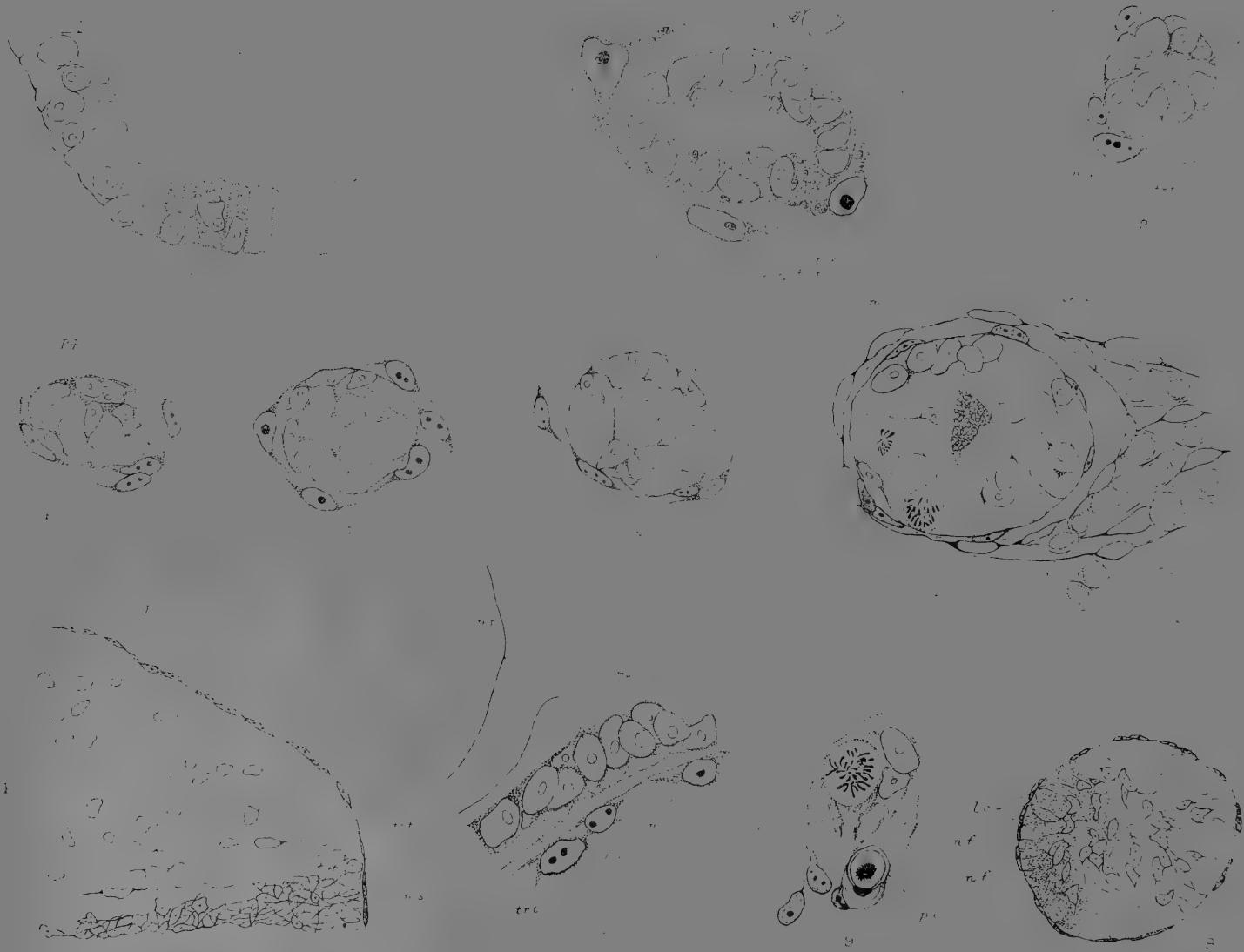
M. L. del.

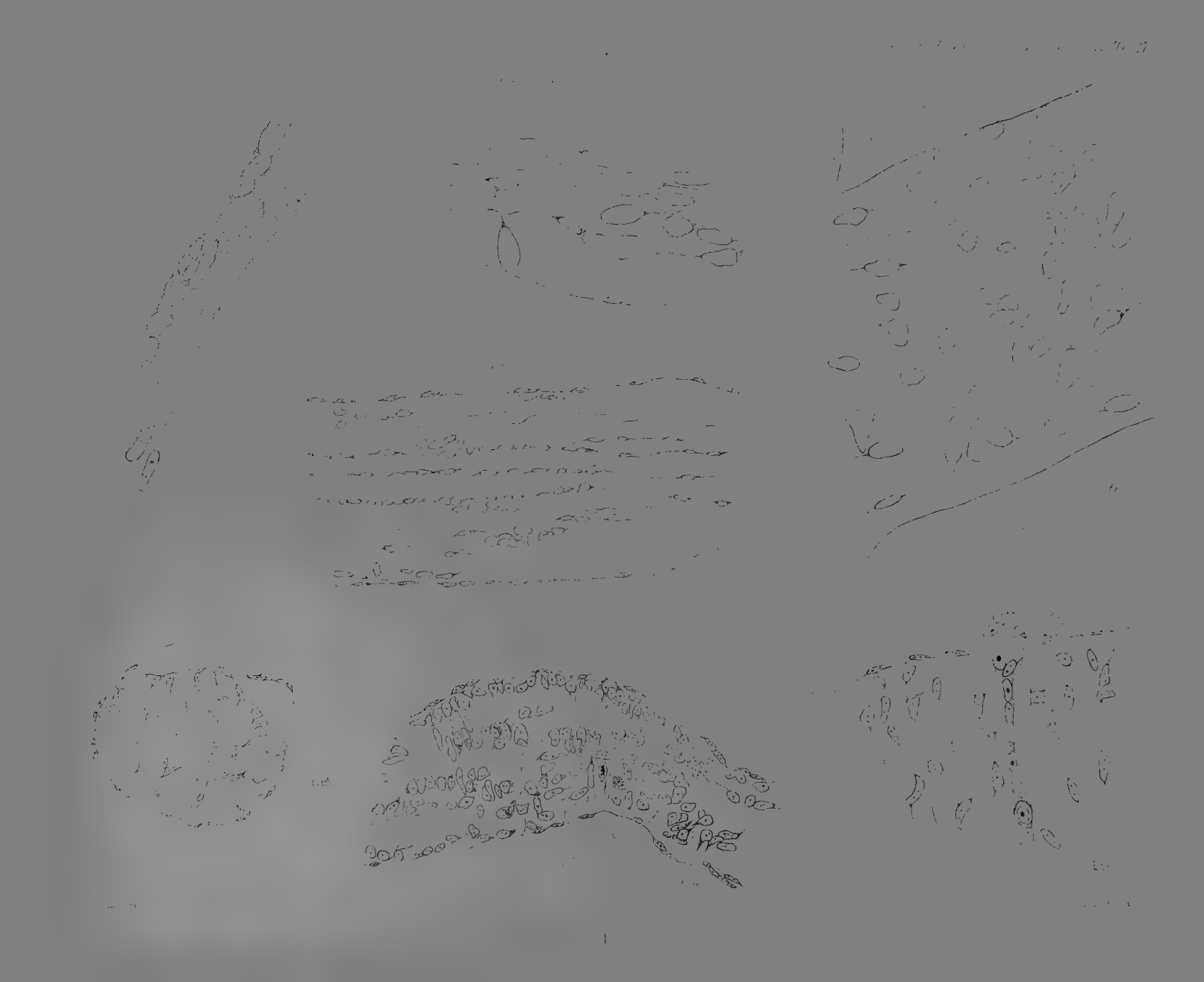


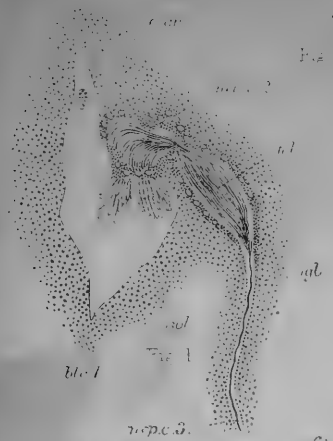


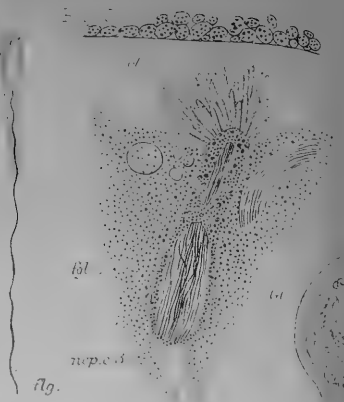


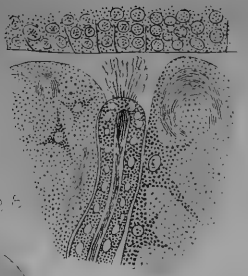

65c
66

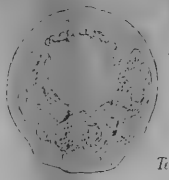


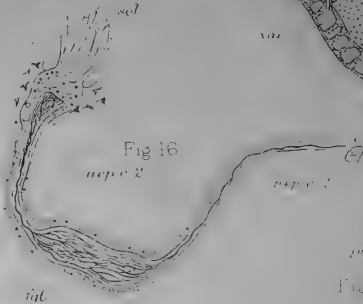


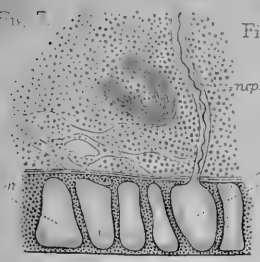


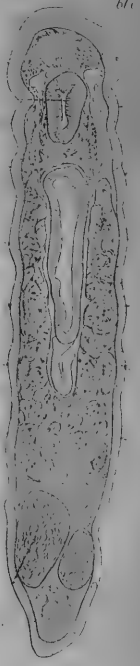


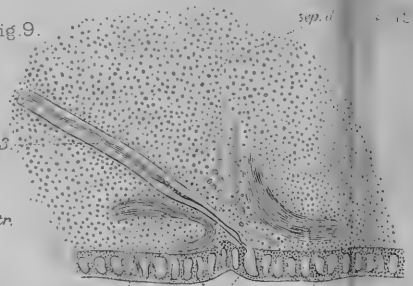


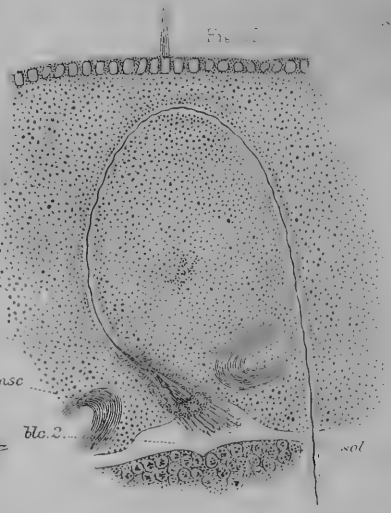


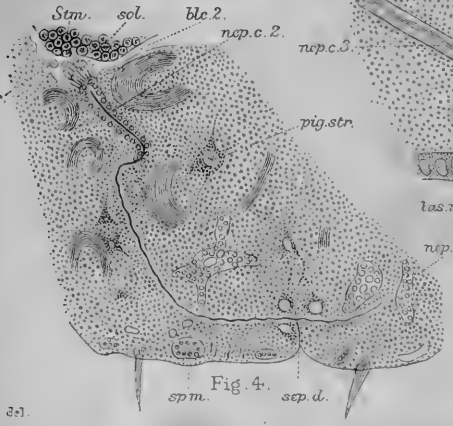












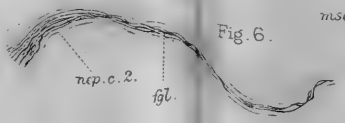


Fig. 4.

Fig. 6.

C. S. del.

Huth. Lith. Lev. Sou

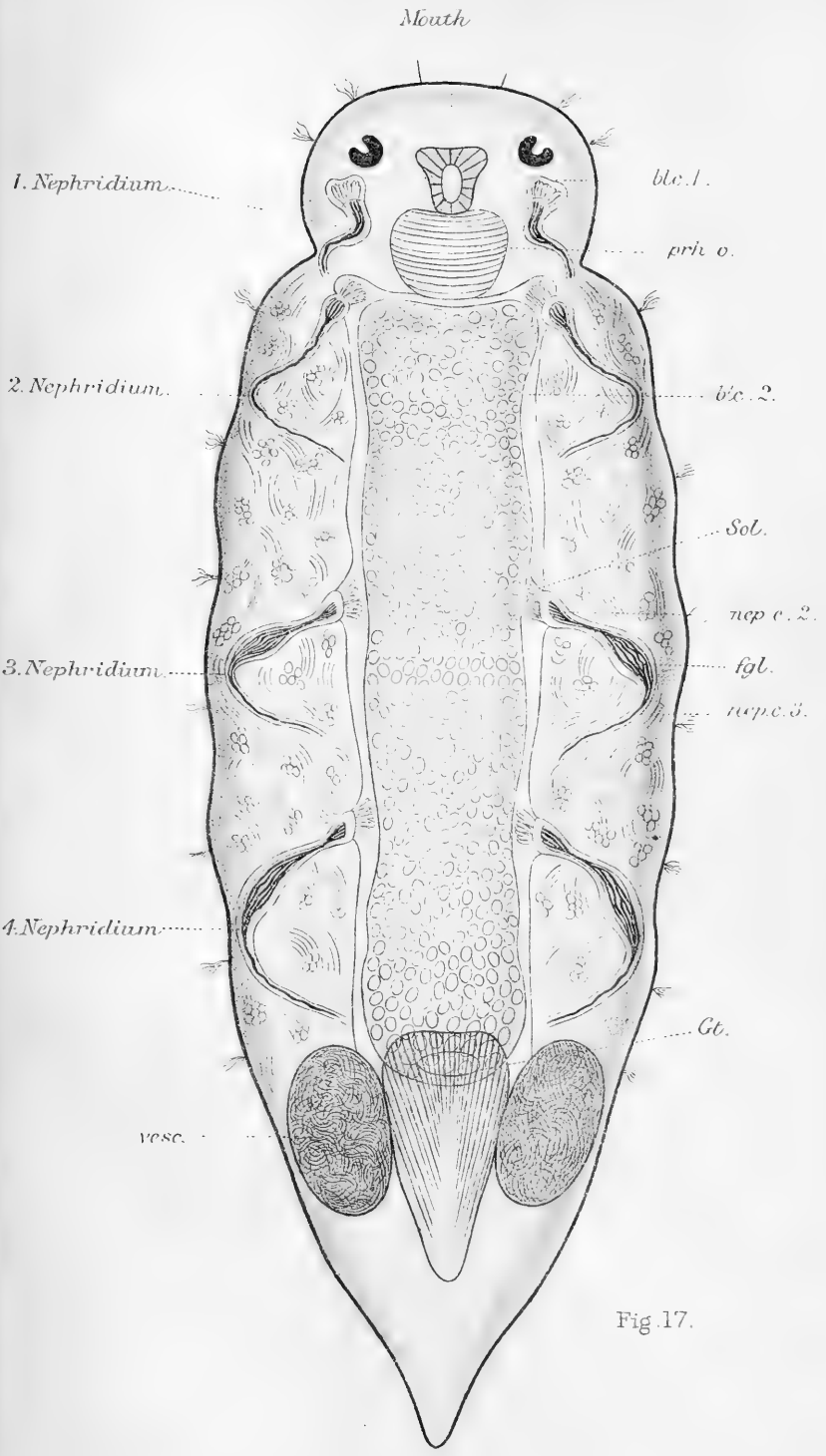
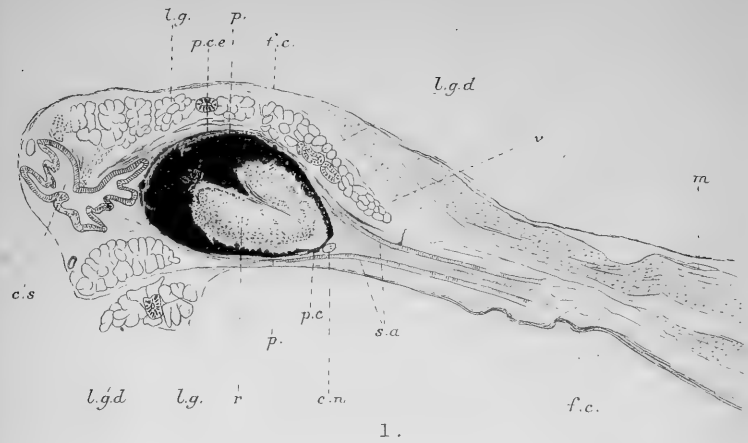
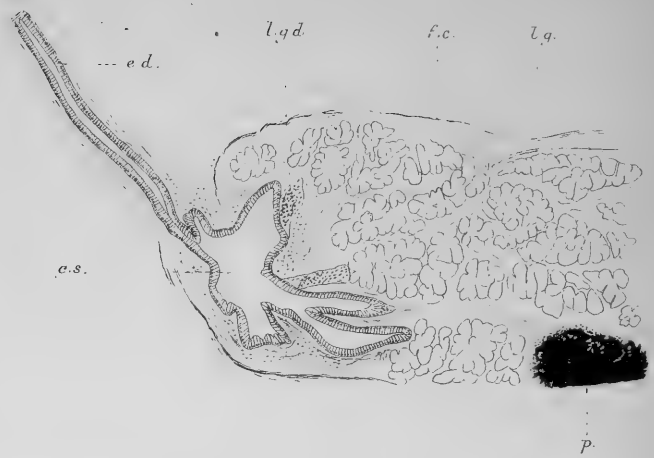

Mouth
1. Nephridium
2. Nephridium
3. Nephridium
4. Nephridium
vesc.
blc. 1.
prh. o.
blc. 2.
Sol.
nep c. 2.
fgl.
nep c. 3.
Gt.

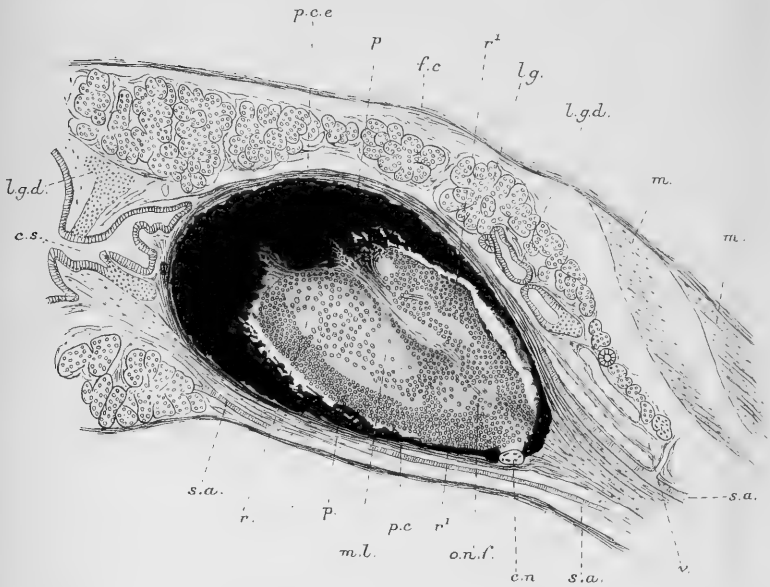
Fig. 17.



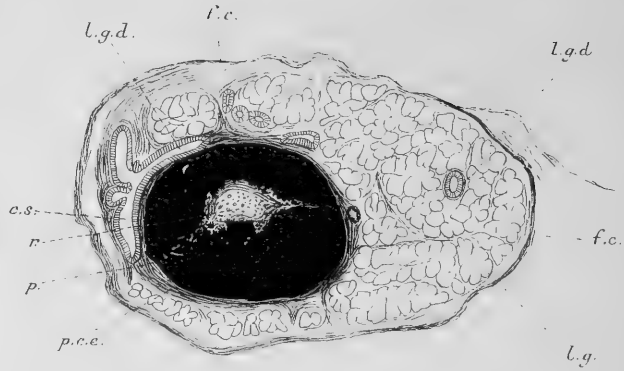
1.



2.



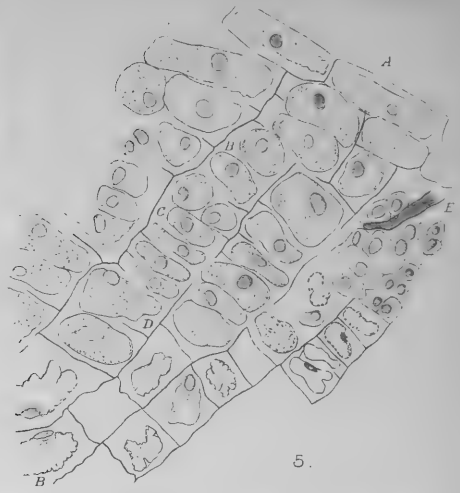
4.

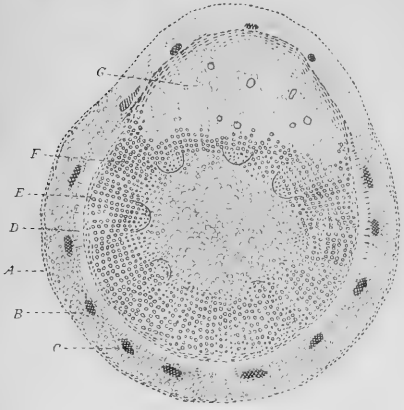


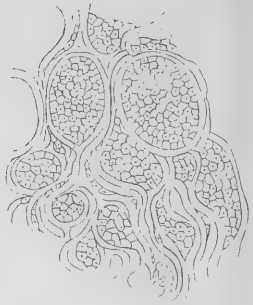
3.

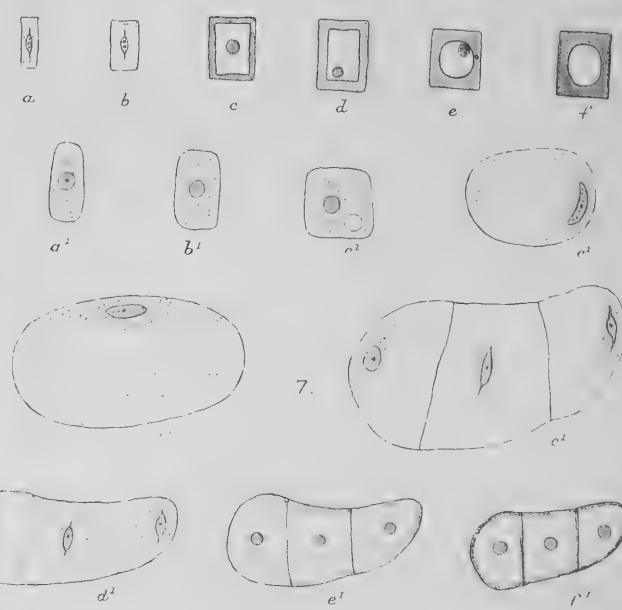
G. Sweet del

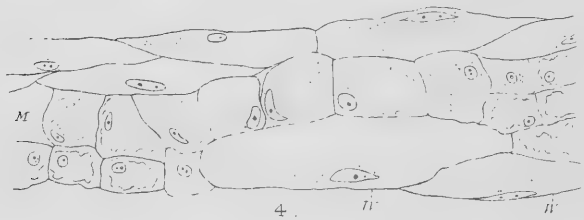
Huth, Lith^r London



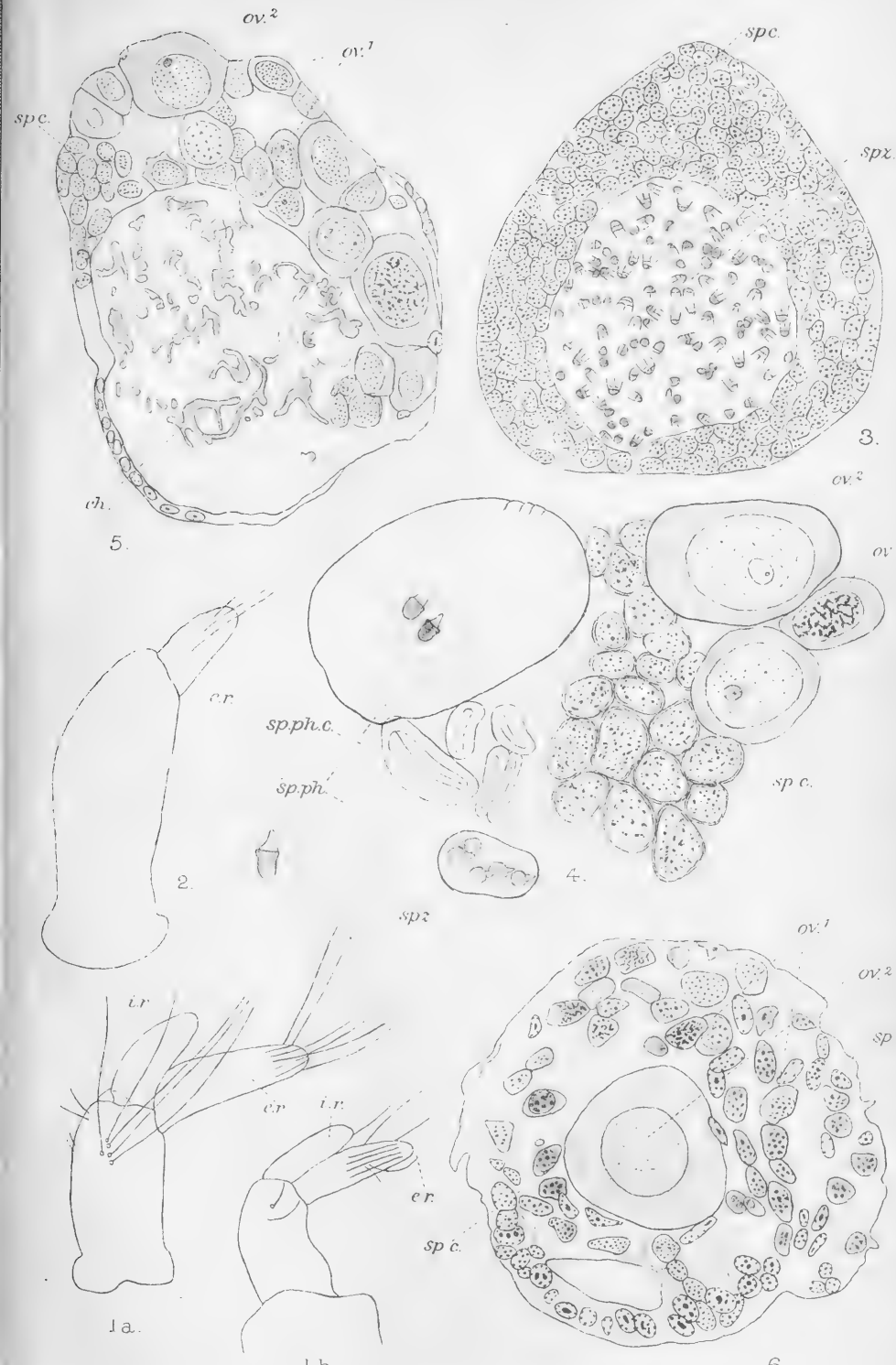








Huth, Lith^r London.



Huth, Lith. London.

Mintern Bros. imp. London

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4

Fig. 5.

E.T.B. del.

Huth, Lith^r London.

MICROHYDRA RYDERI, Potts.