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; HONORARY MEMBER OF THE BIOLOGICAL SOCIETY OF PARIS; 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.

VOLUME 49.—NEW SERIES.

With Lithographic Plates and Text-Figures.

LONDON:

J. & A. CHURCHILL, 7, GREAT MARLBOROUGH STREET.

1906.

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MEMOIRS:

CONTENTS OF No. 196, N.S., FEBRUARY, 1906.

MEMOIRS:

New Series, No. 193 (Vol. 49, Part 1). Price 10s. net.

OCTOBER, 1905.

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1905.

Adlard and Son,] [London and Dorking.

CONTENTS OF No. 193.—New Series.

MEMOIRS:

Notes on the Structure and the Development of the Elephant's Placenta.

By

Richard Assheton, M.A.,

Lecturer on Biology in the Medical School of Guy's Hospital, University of London;

And

Thomas G. Stevens, M.D.,

Demonstrator and Assistant Lecturer on Biology in the Medical School of Guy's Hospital.

With Plates 1—5.

Introduction.

On August 31st, 1902, an elephant belonging to Messrs. Sanger and Sons gave birth to a calf in the gardens of the Zoological Society of London under circumstances related by the Society's Prosector, Mr. F. E. Beddard, F.R.S., in the 'Proceedings' of the year 1902, vol. ii.

The birth of an elephant in this country is so rare an event that only two former occasions have been recorded; and at no previous time has an elephant been born in the gardens of the Zoological Society. The opportunities of investigating the minute structure of the placenta have therefore occurred but seldom; in fact, the only accounts we have of the microscopic features of the elephant's placenta are those by Sir William Turner in 1876, and by Dr. H. C. Chapman in 1880.

The latter author, writing in 1899 (Chapman, **4**), referring to the specimen described by him in 1880, remarks that it is

the sole specimen in existence of an elephant's placenta at full term; that of Owen's being "d'un fœtus de six mois environ d'après mon examen . . . . Je puis dire que l'occasion d'obtenir un placente d'éléphant ne s'est pas encore présentée, même en Orient, ni dans l'Inde ni dans le Siam; j'ai reçu des lettres des hommes qui gardent les éléphants royaux qui affirment que la naissance d'un jeune éléphant est tout à fait inconnue." Though this may be an exaggeration of its rarity, still it is clear that the difficulty of obtaining a placenta is so great that we ought to take full advantage of the present opportunity, especially as the records of previous observations are meagre, and do not afford us any information upon matters which are now of special interest, on account of the advance which has been made in the knowledge of the minute anatomy, and of the development of the mammalian placenta during the last twenty years.

Through the kindness of Mr. Beddard some pieces of the fœtal placenta of the elephant born in the Zoological Gardens came into our hands, and we herewith record the results of our investigations and the interpretation we place upon the structures observed. There can be little doubt that the calf, although it never lived after birth (Beddard, **2**), was born at full term. The actual date of impregnation was not known, but it was believed to have been in April, 1900. Accordingly the birth of the calf was expected in February, 1902. The event, however, did not occur until more than six months later. The histological characters of the placenta, as explained below, also point to the placenta as being fully formed.

Previous Observations.

The fœtal membranes and placenta of the elephant were described by Owen (**13**) in 1857, who obtained from Dr. Morton in India the membranes of a fœtal elephant. These membranes, which had been preserved in "a keg of arrack," were supposed to have been of the period "at about the middle of the period of utero-gestation."

Owen describes the general features of the zonary nature of the placenta, and alludes to the presence of two other villous patches covering the ends of the oblong sac or chorion, which he regarded as being also areas of attachment to the uterus. "The chief points of attachment of the chorion to the uterus are, at the equator, by the annular placenta, and at each pole of the elongated sac by the subcircular villous patches." Owen seems to have made no examination into the more minute anatomy of these patches of attachment.

Twenty years later, however, Sir William Turner received from Professor Flower "a slice of the zone for microscopical examination" of this same specimen, which had been preserved in the Museum of the Royal College of Surgeons. Turner described the result of his examination in his "Lectures on the Comparative Anatomy of the Placenta" on pages 101-102. He describes how he "succeeded in passing some injection into the vessels of the chorion and the large trunks in the stem of the villi," and concluded that "there could be no doubt, however, that in this separated placenta of the elephant a large amount of uterine mucosa was inextricably locked in between the fœtal villi."

A few years later Dr. H. C. Chapman (3) described the birth of an elephant and the nature of the placenta at full term, remarking that the chief points of difference between his description and that of Owen are to be regarded as due to the fact that his specimen was one at full term and Owen's was one of about half the period of gestation. Chapman made some observations on the more minute structures of the placenta, and states that "the examination of the injected blood-vessels in my specimen leaves little doubt that at least one fourth of the girdle-like placenta of the elephant consists of hypertrophical mucous membrane of the uterus."

Our specimen would appear to agree with that of Chapman with regard to the absence of any allantoic cavity.

Owen found in his specimen (about half time) a distinct allantoic cavity or series of cavities, the allantois being "so interposed between the chorion and the amnios as to prevent

any part of the amnios attaining the inner surface of the placenta."

Chapman, however, found no allantoic cavity, but from the position of the fœtal vessels with regard to the inner surface of the placenta, and to certain bodies which he identified with structures named subcircular bodies by Owen, and stated to be upon the inner surface of the allantois, he concludes that the cavity of the allantois has disappeared through fusion of its walls together and to the amnion.

Our specimen similarly exhibited a fusion of amnion with allantois and chorion in the region of the zonary belt, but elsewhere the amnion was fully separated from the endochorion. Nowhere could an allantoic cavity be found.

Chapman also noticed and figured subcircular areas of villous tissue at the poles of the chorionic sac, already described by Owen.

Both authors allude to the morphological interest of those villous patches of connection with the walls of the uterus. Owen writes: "The most important modification in the vascular structures connecting the chorion with the uterus in the elephant is their combination of two forms of the placenta, viz., the 'annular' and the 'diffused,' which forms are restricted in other Mammalia to distinct kinds of quadrupeds."

Neither author gives any account of the more minute structure of this diffuse and probably non-deciduous part of the placenta. It is to be regretted that this portion of the fœtal membranes was not preserved on the occasion of the birth of the young elephant in the Zoological Gardens last year and that no observations were recorded upon this point (Beddard, 2). It is, however, certain that these villous patches if present must have been very inconspicuous, for although not actually looked for, they were not so evident as to attract attention.

There would seem to be some variation in the breadth of the zonary placental area. Owen speaks of its being "partially divided by opposite constrictions into two moieties, one measuring 12 inches, the other 10 inches in

length, and the extreme breadth being 5 inches in each; the connecting isthmus is 3 inches in breadth." Chapman finds that "the placenta preserves the same average width all round; there is no constriction dividing it into 'two moieties' described by Professor Owen."

He also notes another difference, namely, that "the villous processes are as well developed at the edges of the placenta as in the middle." In our specimen the belt was divided by two double constrictions into three chief areas, which were approximately 10 inches in width.

Like Chapman's specimen the whole area was villous and "somewhat broken up into cotyledons as one finds in the human placenta" (Stevens, v. Beddard, 2).

Through the kindness of Professor Stewart we have been able to examine the historic placenta described by Owen and Turner which is now in the Museum of the Royal College of Surgeons.

We find the specimen exactly as described by Owen, with its three points of attachment, the zonary belt and the two villous patches, near the poles. These latter are quite obvious though the villi are short, none exceeding 2 mm. in length.

There is one point which we should like to add to Owen's description.

On page 348 he writes: "A thin brown deciduous layer is continued from the borders of the placenta, from a distance varying from 1—3 inches, upon the outer surface of the chorion."

Chapman says of this in his specimen: "On each side of the placenta there is an indistinct brownish granular layer four inches in width and about a line thick, which runs parallel with the whole circumference of the placenta, and in some places even overlaps it slightly. This same granular matter was found even scattered over the surface of the placenta and was easily rubbed off with the fingers." He suspects it is of maternal origin.

This is present in Owen's; but what we want to draw

attention to is the fact that there are numerous arborescent villi along this region, the smallest of which are exactly similar to the small villi of the subcircular villous patches of the poles.

These villi have the appearance of having come out of their maternal crypts without bringing away any maternal tissue. Nor is there any trace of maternal tissue to be detected among them unless the brown granular matter is maternal; so that this region is as diffuse and non-deciduate as the subcircular polar patches.

Section I.

Histology of the After-birth of the Elephant.

With reference to the macroscopic characters of the fœtal membranes we have nothing beyond the remarks made above to add to the descriptions of Owen and Chapman.

The portions which we have had for microscopical investigation are pieces of the thick zonary placental area and certain curious disc-like bodies which are found scattered over the extra-placental parts of the fœtal membranes and noticed by Owen, who spoke of them as "the subcircular bodies."

This material had been preserved in a saturated solution of corrosive sublimate and kept subsequently in strong alcohol.

In a section taken through the whole thickness of the zonary region of the after-birth we distinguish three well-marked layers or regions which, commencing from the chorionic surface next the fœtus and representing the inner surface of the amnion, are, firstly, a layer of some 2-3 mm. in thickness, composed of fibrous tissue containing fœtal blood-vessels, fig. 1 (A). Secondly, a middle layer, by far the thickest of the three, being 30-35 mm. thick, made up of a close tangle of fœtal and maternal blood-channels, fig. 1 (B), and thirdly, the more maternal surface along which the rupture between after-birth and uterus has occurred, fig. 1 (C), with a thickness of about 8 mm.

We will take these three layers separately. Region A fig. 1 is made partly of gelatinous and partly of fibrous connective tissue, and contains the blood-vessels, arteries, veins, and capillaries having a distinct endothelium. We can find no constant line of demarcation that would indicate the line of fusion between the amnion and allantois or between the walls of the allantois, but there is very generally a split, which may represent one or other of these presumably originally separate organs. At other points there is however undoubted fusion throughout the whole thickness of this region.

Except in the walls of the blood-vessels only a very few nuclei can be found; and of these the greater number lie close to the fœtal surface. The walls of the blood-vessels are greatly thickened and are built up of interlacing bundles of large non-striated muscle fibres embedded in the same sort of gelatinous and fine fibrous tissue just alluded to.

These muscle fibres seem to be somewhat peculiar; they are in some cases very large. They show a wavy outline with elongated nucleus which in transverse section is clearly seen to be subcentral in position. Each fibre has a thicker middle part which tapers suddenly at each end to a long fine fibre.

The middle layer (region B, fig. 1) is made up of flattened plates of fibrous tissue which run from the fibrous layers (A) and branch repeatedly in all directions in an arborescent fashion. Many branches end in small flattened foliations within the region (B), others pass through this layer and end in thicker less flattened terminations within the region (C). It seems possible from the fact that we find very thick trunks in region C that some of these lobate branches may have been torn off and left in the walls of the uterus (fig. 1 *v″*, fig. 3 *fb.*, fig. 15 *v″* and *st. v.*). Wherever these fibrous ramifications are found they contain blood-vessels which in the foliation, i.e., in region B, break up into a fine network of capillaries, and in the lobate terminations, i.e., region C, into a network of vessels which are mostly of rather larger calibre.

In region B there is a complicated system of wide blood channels (*m. ch.*), containing the maternal blood, and forming a series of interlocking loops around and between the foliations which contain the capillaries of fœtal connection (*f. c.*) (fig. 2).

The maternal blood flows in sinuous channels, having very definite walls, whose character we describe in a subsequent paragraph.

Between the fibrous blood-vessel-carrying fœtal plates and their foliations on the one hand, and the sinuous maternal-blood-carrying loops on the other, there is a well marked layer of tissue of a syncytial character which is present throughout, and which cannot from purely anatomical considerations be said to belong to the one or to the other.

For reasons which will appear in the sequel we believe that it is wholly of fœtal origin.

This layer is not indicated in the diagrammatic fig. 1. Fig. 2, which is a careful camera drawing of a part of the region B, fig. 1, represents the histological nature of the parts just named. At *f. c.* one of the terminal foliations of a fœtal villus is thus seen in transverse section, the line ending in the fibrous groundwork, and at (*c*) a fœtal capillary cut across transversely. Let it be noted that the size of the lumen is only just sufficient to accommodate a single red corpuscle. These capillaries, like all the fœtal blood-vessels, are lined by a well marked endothelium. Occasionally a section will pass parallel to the flattened surface of the foliation and show the network of the capillaries (fig. 4).

A maternal blood-channel packed with blood corpuscles may be seen at *m. ch.* These channels are loops of sinuous vessels which open into larger ones as indicated in fig. 2, *m. ch.* These channels have very well defined walls, but the walls are of a peculiar nature, and it is not possible to speak with certainty about them without a knowledge of their development.

The walls are homogeneous, with a thickness of about $\frac{1}{3}$ diameter of a red corpuscle. They stain with the same dyes that stain the fibrous tissue of the fœtal villi, but less strongly. We believe the walls to be quite devoid of nuclei;

although in one or two places nuclei have the appearance of extending into these walls, yet such cases are equally open to other interpretation. (Fig. 11. *End.*)

Cells with deeply staining nuclei can be found on the inside of the homogeneous wall of the maternal blood-channel, which cells lie flattened up against the wall. It is not possible to follow any connecting strands between the cells, but the general appearance is that of a much attenuated endothelium. In parts especially nearer to the fœtal side of the organ there can be little doubt of its endothelial appearance, but in the deeper parts and in the larger channels the matter is more doubtful. (Compare figs. 8 and 13.)

In places one is almost inclined to suggest that these cells are leucocytes flattened up against the homogeneous wall (*v*, figs. 13 and 14).

The thickness of these walls is very uniform throughout the system of loops, but the walls of the wider channels with which the loops communicate are thicker; the outer parts of these show a less firm consistency, and stain rather differently; they are really intervillous spaces, as explained in Section II.

As to the origin of these walls and endothelium containing the maternal blood we can say nothing definite, but we have discussed below some of the possibilities, in our reference to the microscopic details of Owen's specimen. The whole of this region (B) is composed of fœtal vessels and the fibrous tissue which carries them, and these maternal blood-channels with their endothelium—if such it is—together with a third tissue, a syncytial tissue, which everywhere separates the fœtal and maternal vessels.

This syncytial layer, fig. 2 (*cy*), fig. 11 (*cy*), is a loose granular cytoplasm with faintly staining nuclei (*n*), scattered at rather wide intervals.

This syncytial layer at one place appears more closely applied to the fœtal tissues, at another more closely applied to the maternal blood-channels. At almost all points it is crowded with brown and yellow pigment granules, so that in

an unstained section the meandering course of this intervening layer stands out clearly.

It may be noticed that so complicated and complete is the branching of the fœtal plates that a section taken horizontally through the region B is but slightly different to one taken vertically through the same region.

In region C of fig. 1 we find a very different state of affairs. This region is that bordering upon the line of rupture whereby the after-birth has become separated from the walls of the uterus. The general character of this layer may be gathered from fig. 3. Naturally enough the tissues here show signs of damage and are more difficult to interpret. There are, however, certain details of considerable interest which can be clearly defined. There is a complete absence of the "meandering" appearance. The maternal loop-like channels are not visible as such.

The lobate terminations take the place of the foliation described above. These are of much interest. Fig. 12 is a drawing of a section through one of these. Compared with a foliation it may be noticed that the whole is thicker—there is a greater amount of fibrous ground tissue—the blood capillaries are more internally placed (*cf.* fig. 2, *c.*), and are really more of the nature of small arteries and venules than true capillaries. And, lastly, over the surface of the whole there is a well marked layer of almost columnar cells with well marked cell boundaries and nucleus. The layer of columnar cells cannot be found everywhere as clearly as in the upper part of the fig. 12, but traces of it can generally be seen as in the lower surface of the villus in fig. 12.

These villi in some cases seem to have passed through the whole of the after-birth and possibly may have become torn during parturition, and small portions may have been left behind in the uterus. Generally, however, they end as in figs. 3 and 12, lying in a loose detritus of cellular tissue. In this detritus we detect large numbers of cells which have the same general characters as those forming the columnar epithelium just described.

It is in this region C, if anywhere, that one would expect to find a trace of uterine glands and other undoubted maternal tissue. We cannot, however, offer any evidence of the presence of glandular epithelial cells, or indeed of other maternal tissues, except blood cells.

The transition from region B to region C is sudden, and forms a very striking feature. The connection between the fœtal trunks in B and those in C is quite clear. Here and there it is possible to trace main fœtal trunks giving off foliaceous branches in region B, and passing into region C and giving off the more lobate terminations in the latter layer. These terminations, as well as the main trunks, have a tendency to lie horizontally, that is to say parallel with the lines separating region B from region C, and in between these we find layers of coagulum mixed with blood corpuscles and cell detritus.

We regard this space as an irregular cavity or series of narrow chinks filled with blood which bathes the terminations of the villi directly in some places, but at other points it is separated from the trophoblast layer of the villi by the remains of a curious homogeneous material, whose nature we discuss in our Section II. upon a half-term placenta.

In fig. 3 some of these points are illustrated. The larger fœtal trunks are shown at *f. b.*, the smaller branches at *f. c.* The investing columnar epithelium (*tr.*) is seen to be drawn away from the villi in most places, though their relation to the villi is clearly proved by the general distribution of the epithelium and by the position of the nuclei in the cells, which lie close to the fœtal border. The opposite border of the cells has a marked tendency to adhere to the coagulum, which fills up most of the space between the several villi.

This coagulum is partly of a reticulate nature, and is probably true blood clot, but other parts are more solid and contain remains of oval nuclei, and are to be regarded as of either trophoblastic or maternal origin or of double origin, as will be shown under Section II. Fig. 3 illustrates the difference between coagulum and detritus.

We regard this space containing a coagulum and blood corpuscles and cell detritus as one of probably many large blood sinuses into which the lobate terminations hung freely. The characters of the epithelium covering these parts of the villi are those of an epithelium perfectly free from pressure. Moreover, we can find no cells amongst the detritus which we cannot ascribe in origin to these epithelial coverings. There is no trace of any other kind of cell, as, for instance, cells which might line an expanded uterine gland.

We should expect to find communications between this space and the maternal channels of region B, and also evidence of connecting pieces which joined the after-birth to the uterine walls if the condition described above resembles that of the Carnivora as described by Duval (**5**), etc.

In fig. 7 (*x*) we believe there is such a communication. It is a drawing of a vessel just inside region B, which apparently crosses the boundary layer and opens out into the space between the fœtal villi in region C. There is no doubt that this is one of the larger maternal blood-vessels, as it has the same characteristic appearance as all the others already described. It lies within region B because smaller maternal vessels can be seen between it and region C, as shown at *m. ch'.* in the diagram. The fœtal plate which appears to divide B from C is shown at *f. c.*, and it will be noted that the wall of the vessel depicted seems to end abruptly at this line. Up to this point inside the vessel we find the usual red corpuscles and very numerous leucocytes, but projecting from the region C into the vessel a coagulum will be found apparently identical with the coagula seen in various parts of region C.

We think that there can be very little doubt that the epithelium covering the lobate terminations in region C (fig. 12) is continuous with and has a similar origin to the syncytial layer of region B, in spite of the rather different character of nucleus; because of the connection which we can in certain places trace between the two, and of the identity of relation

of the layers to the fœtal villi, and of the constantly lighter staining of the nuclei. The syncytial layer is less evident in the parts of region B nearer the fœtal surface, and close to this surface may perhaps be absent. In fig. 11, which is nearer the maternal surface, the syncytial layer is evident and shows a decided marking out into cell areas. Compare figs. 11 and 12, which are of the same magnification. We find blood-corpuscles abundant between the maternal vessels and the syncytium in the parts close to the maternal surface of region B, and in many places also towards the fœtal surface. The amount, however, diminishes very much the more distant the spot is from the region C, which is the line of rupture. We regard most of this extravasated blood as being probably due to the violence of parturition.

The maternal blood-channels are filled with blood-corpuscles, except the sinus in which the lobate terminations hang, which being open to the torn surface is not distended with blood. It contains coagulum and some corpuscles, together with the detritus as described above.

We can detect no difference between the red blood-corpuscles, which are spherical within the maternal channels, and those in the fœtal capillaries. There are no nucleated red corpuscles. The maternal stream contains many more white corpuscles than can be found within the fœtal vessels.

In the fibrous tissue of the larger fœtal villi there are irregular lymph spaces, which contain some coagulum, and numerous leucocytes (fig. 6), but no red blood corpuscles.

Pigment.

The general brown colour of an unstained piece of the placenta is due to the presence of dark brown or yellow pigment granules. These are of various sizes, and the larger ones are distinctly spherical with clear centres; that is to say, the pigment is deposited somewhat unevenly upon the periphery of minute spheres.

They can be found only sparingly distributed in region A, not at all in region C, but abundantly in region B. Here they may be found in the fibrous tissues of the fœtal villi, and occasionally in the fœtal blood in leucocytes. They occur chiefly, however, in the syncytial layer, which is crowded with them, especially around the nuclei (fig. 11), and in the leucocytes in the maternal blood (figs. 13 and 14) and in the leucocytes in the lymphatic spaces in the large trunks of the fœtal villi. The leucocytes of the maternal blood are nearly always loaded with this pigment.

We find that the pigment is free of iron. When sections are treated with ferro-cyanide of potassium and dilute hydrochloric acid, there is a general blue coloration after a while, the blood-corpuscles and certain parts of the fœtal fibrous tissue being particularly strongly stained. The pigment granules are absolutely untouched by the blue. They are insoluble in ether and alcohol and soluble in caustic potash 1 per cent., and in ammonium sulphide.

There are several points of interest in connection with this pigment, which may be considered to be an excretory product. Fig. 13 is a drawing of a part of a maternal channel lying close against a fœtal villus. The syncytial layer is attached chiefly to the latter, but parts are adherent to the maternal channel wall. The maternal channel contains red corpuscles of different sizes, and large leucocytes, which are loaded with pigment, some sticking to the walls, others free within the blood-stream and some (l^1) apparently undergoing disintegration, the pigment being set free in the stream. Frequently these cells may be found flattened up against the walls, as in fig. 14 (*end*) and, indeed, sometimes it is difficult to say whether they do not penetrate the walls.

Again, it is possible to find all stages between flattened pigment-laden cells as (l) in fig. 14 and (*end*) of the same drawing, which are not to be distinguished from the cells we find in other parts of the channels, and have spoken of as possibly endothelial.

It is likely that the leucocytes play some part in the trans-

ference of this pigment. The appearances seem to favour the idea, namely, that leucocytes convey the pigment, which is formed or deposited by the syncytial layer, into the maternal layer, where they disintegrate, and so discharge their load of pigment.

It seems possible that these leucocytes are derived from the endothelial layer, as suggested by the forms shown in fig. 14, and by the probable method of development as indicated under Section II. of this paper.

Another possibility suggests itself. The large number of leucocytes in the fœtal fibrous tissue alluded to above is very remarkable. These are spherical cells with small, deeply-staining nuclei, and are mostly without pigment granules.

The size and character of the nucleus correspond with the size and character of the nucleus in the pigment-loaded cells of the maternal channels. Do these leucocytes migrate from the fœtal fibrous tissue through the syncytial layer, and there pick up the pigment and pass on to the maternal stream?

It is possible to find in the syncytial layer small nuclei which correspond in size and staining affinity with these leucocytes, which nuclei are certainly not the true nuclei belonging to the syncytium.

It is rather significant that in the immediate neighbourhood of one of these fœtal leucocyte-containing spaces the adjoining maternal channels are more crowded with pigment-laden leucocytes (*v*, fig. 6) than elsewhere.

The Subcircular Bodies.

The position of these peculiar structures was described by Owen and Chapman, and a more minute examination was made by Turner, who correctly describes them "as composed for the most part of fine fibres."

In transverse section figure (9) they are seen to be composed almost entirely of fine fibres, and probably some gelatinous matrix. The fibres are especially abundant, and

arranged as though forming a capsule round the middle region (*cap.*).

There are blood vessels, mostly capillaries, which are more numerous towards the chorionic surface. Only very few nuclei lie embedded among the fibres, and these are chiefly near the surface. Most of the nuclei one sees are nuclei of the endothelium of the capillaries.

Turner evidently regarded these as corresponding to the structures in the mare and pig, which gives rise to the "hippomanes" in the former. On the chorionic surface there are curious small protuberances, with constricted stalks, which are especially vascular (*v*, fig. 10), and are probably minute villi, although in the specimen which we had there is no trace of a trophoblastic epithelium.

It seems to us extremely doubtful whether these structures are comparable to the hippomanes producing bodies. The capsule-like internal structure suggests an origin comparable to the curious recesses described by Hubrecht in the diffuse non-deciduate placenta of a lemur. [Hubrecht in his Spolia Nemoris on Nycticebus Tardigradus.]

Section II.

Histology of the "Half-term" Placenta.

Owen's specimen was supposed by him to be of about half-term, though Chapman considered it to be of not more than six months.

In spite of the fact that it has been over fifty years in spirit, it is possible to make out a good deal from certain parts. The more central parts are less well preserved, and have shrunk, but the edges are remarkably good and of very great interest.

The central part resembles in all essential points the condition already described for the full-term placenta, but between the centre and the edges we find conditions which have every

appearance of being those of the earlier stages in development.

Except for the important absence of the walls of the uterus, we believe we have in this specimen of Owen's all the stages of formation of the fœtal villi and their connection with the maternal blood supply.

Fig. 15 is a figure of the cut surface across the zonary belt of Owen's specimen. On the fœtal surface the sub-circular bodies are to be seen on the zonary belt as well as on the other parts of the chorion (*sub-circ.*). On the maternal side there are several points of interest. On each side of the thicker belt there are many branched villi, and beyond these many very small villi which are not branched. These latter were not discovered until an examination had been made by means of sections (fig. 15). They are, however, just visible by help of a pocket lens, and by a careful search over the whole chorion we have been able to recognise them for a distance of some five or six inches on each side of the zonary belt, and also for some three inches around the two villous patches near the poles.

Over the remainder we believe there are no villi, though we have not been able to examine all parts by sections. This, then, reduces the quite smooth part of the chorion in the half-term placenta to a couple of bands some five or six inches in width, between the poles and the zonary placental belt.

Turning to the maternal surface of the zonary placental belt, it will be seen that on either side there is an area completely devoid of villi and quite smooth.

These smooth bands are covered with the same "brownish granular matter" which is found extending outwards over the branched villi at the sides of the zonary belt alluded to above, as described by Chapman, who suspected it to be of maternal origin. Whether it is so we will discuss later.

Lastly, the more median portion of the maternal surface is on the whole rough, though here and there smooth patches can be made out by a surface examination of the whole

specimen which resemble the lateral zones. The ragged state is due to the projection of villous tufts (V″) of long stalks ending in tufts of villi (V″), of similar stalks apparently broken (*st. v.*), and of very occasional large blood-vessels, which can hardly, from their nature, be anything else than maternal arteries.

On the face of the section the branching fœtal trunks, and the larger blood channels which contain the maternal blood can be easily seen; and a more careful inspection will show that the smooth zones forming the sides of the placental belt are formed by a thin homogeneous material which seems to prevent as yet the fœtal villi gaining access to the maternal tissues along these areas. This material extends across the central zone, but is here broken up by the penetration of the villi (which no doubt fitted into corresponding crypts in the walls of the uterus), and constitutes our region C in the description of the full-term placenta.

The microscopic examination of this smooth area throws a flood of light upon what we have found in the full-term placenta if, as we think, the edges of the zonary belt represent younger phases of development than the more central part, which resembles so closely our full-term specimen. It will be remembered that these smooth bands are not present in the full-term placenta (Chapman, **3**, Beddard, **2**).

The nature and origin of the homogeneous material forming the smooth surfaces of the lateral bands present us with a problem of much difficulty. Probably it is impossible to solve it completely in the absence of the uterine part of the placenta. There would seem to be two layers—one, the thinner, lying next the uterus, in which, or more often on the outside of which, we can find a few large rounded cells with small, dark nucleus (figs. 19 and 20); and the other a thicker layer which generally stains very slightly differently from the thin layer, and contains more or less degenerated nuclei (fig. 23).

Abutting upon, and in many cases embedded in, this are the ends of fœtal villi. These fœtal terminations form a

well-marked layer (fig. 16), in which they lie closely apposed to one another, and only sometimes separated by an intrusion of the homogeneous material just spoken of.

The villi consist of a core of mesoblast with capillaries (which in this region stain deeply, almost black with hæmatoxylin, and give the Prussian blue reaction with ferrocyanide of potassium), and are covered by a typical cubical epithelium, whose cell-boundaries are distinct and whose nuclei lie close to the fœtal border of the layer. The cytoplasm generally stains very slightly. This, however, is only the case when the villi, crowded together, lie against one another. Where they abut upon the homogeneous material the epithelium has a very different appearance. The cell boundaries are less distinct; the nuclei are much larger, and towards the apices of the villi the cells and nuclei are much elongated and the epithelium shows signs of proliferation (fig. 17).

As the cells become separated from their point of origin all trace of cell-wall disappears, and the nuclei get fainter as they are absorbed into the homogeneous material, and ultimately lose all special affinity for nuclear stains, and are stained only by such diffuse stains that colour the homogeneous material, if they do not even totally vanish (figs. 17 and 23).

We may say here that although the nuclei throughout all parts of Owen's specimen stain readily as a whole with such stains as hæmatoxylin, thionin, saffranin, carmalum, yet the preservation is not sufficiently good to allow of any detail of chromatic grains. In no case have we seen an undoubted mitotic figure.

There is no trace of any maternal blood channel outside the inner margin of this area consisting of terminations of fœtal villi.

To what conclusion must we come as regards this homogeneous material? Is it all fœtal: a kind of plasmoditrophoblast? or is it partly maternal: either cell detritus—e. g. uterine epithelium—glandular secretion, or both?

It stains moderately with hæmatoxylin carmalum, eosin,

nigrosin, and deeply with saffranin; it is not affected by osmic acid nor by ferrocyanide of potassium, whereas the mesoblastic portions of the terminations of the fœtal villi are stained an intense blue.

With fuchsin and orange there is a slight differentiation between the outer thinner and inner thicker layers, which are further distinguishable by the presence of the rounded cells in the outer layer and the elongated nuclei in the inner layer (fig. 23).

It seems probable, therefore, that it is of double origin, partly from fœtal trophoblast (the inner thicker portion) and partly from maternal tissues (the outer thinner), in which case the large rounded cells with small nuclei would be of maternal origin.

It is difficult to understand what the function of this layer may be, unless it is to prevent for the moment—for some not discernible reason—the entrance of the fœtal villi into the maternal tissues. The fact that cells are being budded off from the trophoblast seems to indicate a non-absorbent epithelium; that the nuclei of these budded-off cells should die and disappear shows it is not a phagocytic edge that is being formed, and this is also rendered improbable by the smooth regularity of its superficies. The ready response to the iron test noticed above suggests the absorption of iron from this layer. We have searched in vain, however, for blood remains, which, perhaps, one might expect to find if this layer was the seat of destruction of hæmoglobin-bearing corpuscles.

A glance at the cut end of Owen's specimen (fig. 15) will show that the surface of the section is roughly marked out into areas by the longer and stouter branches of the fœtal villi. Within the boundaries of each such area we find on the whole a uniform stage of development, but one area will often show a different state to its neighbours, and roughly speaking the nearer the area is to the maternal surface and to the edge of the placental zone the earlier is the stage represented.

On the supposition that these stages which undoubtedly occur really represent the earlier phases in the normal development through which the more central region has already passed—for the central region resembles in all essential features the full-term placenta—we have here all the stages showing how the maternal blood gains access to the fœtal part of the placenta.

The details of these processes are exemplified as far as we can determine them with our limited material by the sections drawn for figs. 16-24. Fig. 18 shows a more internally-placed area where all trace of cell boundary has disappeared; the mesoblastic portions of the fœtal villi (*f. v.*) stand out clearly, and between these the broken-down trophoblastic syncytium. Nuclei are perceptible, but are less easily stained.

In this, we see several clear spaces (*m. ch.*) apparently within the common syncytium or plasmodium formed by this breaking down of the trophoblast of adjoining villi. These spaces have exactly the appearance of having been produced by the bursting in of a stream of fluid containing corpuscles and so crushing up the fine reticulum of the plasmodium, thereby forming the well defined non-cellular walls so characteristic of the full-term maternal channels.

The original nuclei of the trophoblast can be seen as fairly large oval highly staining nuclei lying on either side of, but usually well away from the newly formed channels. Possibly they are less numerous than in the supposed earlier stage, but of this we are not sure. The nuclei of the corpuscles which float in the fluid in these channels are much smaller and are more deeply stained.

Whence are these nuclei? The two alternatives are that these corpuscles are derived from certain of the nuclei of the plasmodium, dividing and causing a local liquefaction comparable to the process described by Hubrecht in the placenta of Tarsius spectrum (Hubrecht, **9**) or they have had some more foreign origin and have been brought into their present position in a stream driven along where resistance is least by the pressure of the maternal blood behind it.

We incline to the latter hypothesis. Firstly, because there are no cells or nuclei in the plasmodium which seem to be the parent cells of these corpuscles. Secondly, at the heads of these new channels the contained corpuscles are not, as far as we can judge, undergoing multiplication, while we can find places, in the larger channels, where they are certainly being formed. Thirdly, we can find no intermediate stages between the unbroken plasmodium and the clear space containing separate corpuscles.

As favouring the other view there is the fact that there are no non-nucleated red corpuscles at all in these newest channels. We find them only in the larger or older channels. Although we cannot speak positively about the origin of these corpuscles, we have some evidence of their immediate formation.

In the larger channels there are large numbers of red non-nucleated blood corpuscles, and large numbers also of nucleated corpuscles, some with more than one nucleus and quantities of bodies of all sizes which stain deeply which seem to be fragments of nuclei (fig. 22).

Also near the margin are numbers of large cells, sometimes elongated and almost endothelial in position, which in some cases appear to be breaking up, and in others to have more than one nucleus. We regard these as the parent cells of the nucleated corpuscles within the channels, but the question, which must remain unanswered for the present, is what is the actual origin of these mother cells? We do not find them in the small channels, but we can trace them back into the largest channels and even into the large vessels (fig. 25), which occasionally occur and are evidently the connecting vessels between the placental blood system and the vessels of the uterus, which we are inclined to consider maternal (fig. 15, and fig. 1 *b*. *U. V. A.*). An hypothesis not without some evidence to support it would be to suppose these cells to be of maternal endothelial origin, and to become gradually washed along the newly formed channels, adhering for a while (after the manner of white blood corpuscles) to the

sides of the larger vessels (figs. 22 and 24), and growing and multiplying.

In the smallest newly formed channels there is clearly no endothelium. We see the non-cellular wall as described in the first section, and think it probable that the endothelium therein described is formed by these migrating large cells just mentioned. On our hypothesis this apparent endothelium would be of maternal origin, though different in nature to the endothelium, both of the maternal channels of the carnivore and of the rodent type of placenta.

There are still traces of this proliferating endothelium in some few of the larger channels in the full-term placenta.

In some places there are characters which seem to suggest the former hypothesis, but they afford evidence even less convincing than what we have just advanced on the other side.

In fig. 21 an interesting section is illustrated wherein the new channels and their blood contents are seen to be in chinks between the trophoblast layers of adjoining villi. This is very clearly recognisable in the larger circular channel (*M. C. H.*) in the wall next to the mesoblast of a villus, and in the long, narrow channel at the bottom of the drawing.

In these the trophoblast has hardly changed from its cubical character which is typical of the villi near the smooth edge (v. fig. 16), and the nuclei and cell divisions are distinctly recognisable.

We found on examining the rough surface of the placenta of Owen's specimen that we could only make out a few of the vessels which connect the placental circulation with the vascular system of the uterus. Of these, which were less than half a dozen in number, we were able to take a piece of one. It is in a bad state of preservation, but examination of it by section enabled us to be sure that it is a blood-vessel, and probably arterial. Its endothelium undoubtedly shows signs of proliferation (fig. 25).

These vessels pass through region C before giving off branches in region B.

In fig. 15 certain long villous tufts may be seen. These are villi which have passed through the homogeneous layer and presumably have penetrated into the uterine mucosa (or into glands). Some, we believe, have been torn off and left in the uterus (fig. 15, *st. v.*) (this, we believe, has also occurred in our full-term specimen), others have come out from their crypts (fig. 15, V″).

It is extremely difficult to make out any epithelium covering these villi. They are much branched and have small pieces of something identical or similar in appearance to the homogeneous material, which perhaps contains cells. Anyhow there is no definite epithelium. The mesoblastic core, which is thick and lobate, contains a more or less central vessel (artery ?) and a number of small capillaries arranged round the periphery—separated by a large amount of connective tissue.

The pigment, which is so obvious a feature of the full-term placenta, is here hardly recognisable. A few fine granules may, however, be found over most parts.

The branched villi (fig. 15, V V) close to the edge of the placental zone have a covering of trophoblast similar to that on the crowded villi of the zonary belt. They also are covered by the curious homogeneous layer which at the apices of the villi seems to be receiving nuclei from the fœtal epithelium.

The only difference between such a villous patch and the villi of the placental zone is that in the former the villi are less numerous and less crowded; in the latter they are much crowded. It is not difficult to conceive how an increase in size and number of villi such as those on the border (fig. 15, V V) might lead to a close tangle of fœtal villi originally diffuse and non-deciduate, especially if (as seems to be the case) they were prevented from penetrating the uterine tissues. Into this tangle a bleeding at the surface of the uterus might percolate, and so give rise to the system we have described above, by flowing at first between the villi (figs. 16, 21, 24) and ultimately perhaps within the substance of a syncytium (fig. 18).

We must leave for the present any further consideration of

the fate of the cells L. C. (figs. 21, 22, 24) and *bc.* (figs. 21, 20), etc., which we have spoken of as "endothelial cells," "maternal cells," "blood cells," as we are doubtful whether the condition of preservation is sufficiently satisfactory in Owen's specimen to enable us to come to a reliable conclusion upon so difficult and important a point. We think we have strong evidence that they are derived from the endothelium of maternal blood-vessels. There are also appearances which resemble stages in hæmatopoietic areas, as described for other mammals (e. g. Hubrecht, **9**), but we certainly have been unable so far to follow the process.

There are no non-nucleated blood corpuscles in the small developing channels; which fact points strongly to the conclusion that there is no circulation as yet in these channels.

The cells floating in the fluid in these channels are of various shapes; squamous, fusiform, or subspherical. The cytoplasm of the smaller ones is clear, and the nuclei, which are nearly spherical, stain deeply.

We have assumed in our conclusions that the processes observed in the lateral regions of the half-term placenta are essentially similar to the processes by which the middle region, which resembles the full-term condition, is formed.

We are probably justified in this supposition since we know that at full term the lateral smooth regions have disappeared, and the whole breadth is similar to the central part.

Conclusions.

Those who have devoted any time to the study of the development of the placenta know well enough how difficult a task it is to determine the origin of certain of the tissues of the fully formed organ, even when a rich series of developmental stages is procurable.

Since we have only the fœtal part of the placenta at its final and one other stage before us, it follows that there must be many points upon which we cannot speak with certainty. It is perhaps a hazardous undertaking to attempt an interpretation

of the facts as given above, but seeing how improbable it is that more suitable material will be obtainable in the immediate future we must risk the attempt.

As a result of our investigation, certain questions at once present themselves. Do our investigations confirm Turner's conclusion that the elephant's placenta is deciduous? What is the origin of the syncytial layer; does it belong to the fœtal or maternal tissue? What is the origin of the walls of the maternal channels; are they fœtal or maternal? How does the placenta compare as regards its minute structures with other known placentas?

Turner defined a deciduate placenta as one in which there is a "shedding of the vascular part of the maternal placenta during parturition." He came to the conclusion that the elephant's placenta is without doubt deciduate.

The placenta, as will be gathered from our description above, is at first sight more like the type of placenta found in those orders called deciduous than that of the true ungulates, lemur, etc., and known as non-deciduous. There can be no doubt about the shedding of at any rate part of the maternal vascular system. But for all that it is impossible to speak with certainty, except as regards the blood, from so scanty a supply of material; and it is not absolutely certain that any maternal tissue except the blood, which obviously comes away in large quantities, is lost during parturition. In the after-birth the great mass of tissue is without doubt fœtal.

Of the three regions, A, B, and C, of our description of the full-term specimen, region A is wholly and region C mostly fœtal. That this is true of region A needs no explanation.

As regards region C the matter is less obvious. We find, however, some tissue which we must regard as a remnant of the homogeneous material described in Section II. on the Owen placenta. This contains nuclei, which are probably of the thick inner layer of that material and therefore trophoblastic; and very occasionally we think we can detect one of the rounded cells with small nucleus which we have ascribed to maternal tissue.

There also (though not shown in our section fig. 3) lie the arterial vessels which connect the maternal system with the vascular system of the after-birth as suggested in our diagram fig. 1 (*b*).

We can, however, find no trace of glandular epithelium or of other tissue of any kind except blood-corpuscles and coagulum, which do not show strong evidence of fœtal origin.

The detritus which lies between the villi of this region is composed of coagulum, blood-corpuscles, and cells, nearly all of which are in every respect similar to those covering the fœtal villi.

In region B we find some evidence of maternal tissue besides blood, but it is by no means conclusive. We have no doubt that—on the hypothesis of the epithelial layer covering the villi being trophoblastic—the maternal blood circulates at first between the trophoblast layers of adjoining villi; (that is to say, really between the fœtus and the mother), and ultimately probably within a plasmodium caused by the breaking-down of the trophoblast cells. The evidence is clearly against the endothelium of these channels being an ordinary maternal capillary endothelium, though we think it is for the reason given above very likely the product of cells derived from maternal blood-vessel endothelial cells. If this is not so, then the only maternal tissue in region B is the blood.

The placenta as regards its zonary belt is deciduate. Owen wrote: "The most important modification in the vascular structures connecting the chorion with the uterus, in the elephant, is their combination of two forms of the placenta, viz. the annular and the diffused, which forms are restricted in other mammals to distinct kinds of quadrupeds." And even in the zonary belt there is a combination of deciduous and non-deciduous placentation. The lateral bands of this belt are formed of long branched tufts which seem to form no interlocking system of blood-vessels destined to be thrown off at birth. Their appearance in the half-term placenta (fig. 15) tempts the suggestion that they form an edging which tacks

down, so to speak, the lateral bands of the belt whose smooth maternal surface indicates but slight means of attachment.

A similar function amongst others must be assigned to the long villi which pierce region C and enter the uterine walls.

Although there is an obvious resemblance between the elephant's placenta and the zonary placenta of the Carnivora, and even between the meandering character which we have described, and the labyrinthine lamellæ of the dog or cat's placenta, yet we believe that this resemblance is superficial—for in reality the two types are very different.

Bearing in mind the possibility of affinity between Ungulates, Carnivora, and Proboscidea through some such forms as Phenacodus, it is especially interesting to compare the placenta in these three types.

Firstly, the elephant's placenta differs from that of the dog in having a distinctly non-deciduous part, which resembles closely the diffuse type of the mare.

The carnivorous placenta is a broad central band leaving the two poles bare, while in the elephant the central band is comparatively narrow, and the poles are villous, leaving two broad bare zones between the poles and the central deciduous belt.

There is in the carnivora nothing comparable to long villi which pass through the "coagulum" region and extend deep into the maternal tissues, and are, so far as the projecting parts of the villi are concerned, non-deciduate.

There is no sign in the elephant's placenta of anything like the angio-plasmode. The mode of vascularisation of the after-birth is quite different in the two types.

In the Carnivora the trophoblast advances into the maternal tissues according to Duval and surrounds the maternal capillaries. Other authors, Strahl, Fleischmann, Heinricius, etc., give different accounts, but in no case is there anything comparable to the conditions we find in the elephant, where the trophoblast is comparatively inactive and extravasated blood from the maternal system seems to force its way between the mother and fœtus and ultimately into the trophoblastic

plasmodium. In this it resembles far more closely the process as it is known to occur in Rodents, Insectivora, Tarsius, Primates, etc.

There is, however, this striking difference. In the Rodents, Insectivora, Tarsius, etc., the vascularisation of the trophoblast by the maternal blood occurs before the advent of fœtal capillaries. In the elephant the fœtal villi are fully developed with their capillaries before any trace of maternal vascularisation occurs.

In a recent interesting paper in the 'Philosophical Transactions of the Royal Society,' by C. W. Andrews, on "The Evolution of the Elephant," the writer discusses the probability of affinity between the Proboscidea and the Sirenia, and gives as one of the points of resemblance the supposed fact that the placenta in each group is non-deciduate and zonary. The author would seem to have derived this statement from Flower and Lyddeker's "Mammals," where the statement occurs that the elephant's placenta is non-deciduate and zonary, which is misleading, for the zonary part is incomparably the more important, and is, as Turner and Owen asserted, deciduate.

Turner very clearly emphasises these differences in his paper on the placenta of the Dugong. In discussing the possibility of affinity between the two groups, he says, "In this connection, therefore, it is interesting to observe that, as regards its form, the placenta, both in the elephant and the dugong, is zonary; though they differ in this very important particular, that in the elephant the zonary placenta is deciduate, in the dugong it is almost entirely, if not entirely, non-deciduate."

There is an interesting point in Turner's description of the dugong's placenta which suggested the explanation of the very large thick villi with broken stems, many of which pass obliquely through our region C (fig. 3, *f b*, fig. 15, V″).

Turner writes of the dugong (p. 655): "But my description has shown that, in addition to the multitude of short villi and shallow crypts, the dugong also possessed a small

proportion of larger villi, which were implanted in larger and wider and more deeply seated crypts, passing for some distance in an oblique direction subjacent to the layer of short crypts."

In the specimen of the dugong described by Turner the fœtal placenta had been torn away from the maternal tissues in such a way that the layer of short villi were drawn out intact from their uterine crypts, but the longer, less numerous villi had been broken off and left within their crypts.

Turner regarded this as abnormal, and believed that in normal parturition the longer villi would also come out, perhaps bringing some of the uterine tissues with them. He does not seem to have thought it likely that they should be naturally torn off and left, and absorbed by the maternal tissues, which is an alternative by no means improbable. Such an absorption of fœtal tissues by the uterine tissues is common enough—as, for instance, in the case of the placenta of the mole, of Perameles (Hill), mouse (Jenkinson), etc. A similar absorption may frequently be noticed in rabbits, where embryos, if too numerous, are crowded out, die, and become absorbed together with the placenta. Such also is the probable fate of the long villi of the elephant and dugong.

Summary.

1. The full-term after-birth of the elephant consists of a chorion from which spring many much-branched villi, which spread out in all directions into plate-like branches. These end in (a) proximal foliaceous terminations, in which the fœtal blood vessels ramify, which interlace with a complicated system of much larger blood channels filled with maternal blood, having well-defined but non-nucleated walls; (b) more distal lobate terminations, which are covered by a well-marked columnar or cubical epithelium — presumably the trophoblast — which are partly embedded in a kind of coagulum or detritus, and partly appear to hang loosely in irregular blood spaces without walls; (c) the stems of still

more prolonged villi, which have been torn off and probably left embedded in the walls of the uterus; (d) a few torn ends of blood-vessels.

2. The main trunks of the villi and their foliaceous terminations are everywhere separated from the maternal blood-channels by a syncytial layer, which is continuous with the epithelium covering the lobate terminations, and is presumably trophoblastic.

3. The half-term placenta originally examined by Owen in 1850 shows, in its more central region, characters which are essentially similar to those of the full-term specimen, and goes far to prove the existence of longer villi which penetrate deeply into the uterine mucosa. The lateral areas of the zonary belt exhibit many most interesting previous conditions. We are able to see in these the simple terminations of the fœtal villi covered with a single layer of trophoblast separated from the uterine tissues by a layer of material partly maternal and partly of fœtal origin.

There is no process of growth round existing maternal capillaries to form an angio-plasmode, nor apparently any phagocytic action on the part of the trophoblast. The vascularisation of the after-birth is effected by the invasion of the trophoblast by extravasated maternal blood, which flows at first in intercellular and intervillous passages which form the larger channels of the after-birth maternal vascular system, and then makes its way along intra-cellular or intra-syncytial canals through a plasmodium produced by the breaking down of the trophoblast of two adjoining villi.

We think the evidence is in favour of considering the corpuscles floating in this invading stream, which contains no red non-nucleated corpuscles in its more advanced portions, to be of maternal rather than trophoblastic origin.

4. The tissues of the full-term placenta contain pigment granules, which are deposited chiefly in the syncytial layer. This we regard as an excretory product; it is almost quite absent from the tissues of the half-term specimen. Leucocytes, either of maternal or fœtal origin, seem to be concerned

in the transference of this pigment into the maternal blood stream.

5. The subcircular bodies of Owen we find as described by him and Turner, though we note the presence of minute villi on their outer surface.

6. We confirm the opinion of previous writers that the zonary band in part is a "deciduous" form of placenta, although there is not much maternal tissue except the blood. It is not correct to speak of the after-birth being composed of a "much hypertrophied mucosa layer of the uterus."

7. The placenta of the elephant shows by its long villi, which tend to remain embedded in the uterus wall, a resemblance to the condition found in the Sirenia; by the villous patches at the poles and other villi which come out from the uterus, either with or without their trophoblastic covering, but with no maternal cells attached, a resemblance to the ungulata vera of the Perissodactyl type; by the invasion of the trophoblast—if such it is—by the maternal blood stream, a resemblance to the Discoplacental type, although the actual manner by which this invasion occurs would seem to be—so far as our very limited material affords us opportunity of observation—unlike anything hitherto described.[1]

8. The resemblance, at first sight obvious enough to the zonary placenta of the carnivora, is superficial. The elephant's placenta differs from that of the carnivora in (a) consisting of three areas of attachment instead of one, two of which are wholly in the non-deciduous type, the other partly deciduous, partly non-deciduous. (b) There is nothing formed comparable to an angio-plasmode. (c) The maternal capillaries do not directly become the maternal vessels of the after-birth.

[1] There is a very marked resemblance to the conditions found in the sheep's placenta during the final stages of development. This I hope to describe in detail in a paper now in preparation.—R. A.

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EXPLANATION OF PLATES 1—5,

Illustrating Mr. Richard Assheton's and Dr. Thomas G. Stevens's paper, "Notes on the Structure and the Development of the Elephant's Placenta."

Complete List of Letterings.

A. Arterial vessel or placental afferent. *bc.* Blood cells (derived from maternal endothelium?). *bl.* Maternal blood corpuscle. *cap.* Capsule-like structure. *co.* Coagulum. *cy.* Syncytial layer—trophoblast. *cycy.* Syncytium formed by fusion of two adjoining trophoblasts. *end.* Endothelial-like cells. *f.b.* Fœtal blood-vessels. *f.c.* Fœtal capillaries. *f.v.* Fœtal villi. *i.* Deeply staining iron-containing tissues. *L. C.* Large cells presumed to be of maternal origin. *l.* Leucocyte. *l'.* Leucocyte undergoing disintegration. *Mc.* Maternal cells. *M. C. H.* Large maternal channel inter-cellular. *m. ch.* Small maternal channel intra-cellular. *Mes. f.* Fœtal mesoblast. *m. f.* Nucleus of non-striated muscle fibres. *n.* nuclei. *pl.* plasmodial remnant. *shr.* Space caused by shrinkage. *sh. v.* short villus. *st. v.* Stem of villus broken off and presumably left in the walls of the uterus. *Sub. circ.* Subcircular bodies of Owen. *tr.* Trophoblast (probably). *U.* Walls of the uterus. *U.V.A.* Uterine blood-vessel (arterial). *U.V.V.* Uterine blood-vessel (venous). *V.* Branched villus of non-deciduous border. *V'.* Villus of deciduate portion. *V''.* Villus of zonary belt which penetrates into the walls of the uterus. *V'''.* Minute villi on the chorion beyond the zonary belt. *w.* Non-cellular wall of the maternal blood-channels. *x.* Point of communication between the maternal blood-channels of region B inside the space of region C. *z.* Layer of homogeneous material containing degenerating nuclei of trophoblastic origin. *zz.* Layer of homogeneous material containing no trophoblastic nuclei, but occasional rounded cells (of maternal origin?).

PLATE 1.

Fig. 1 *a*.—A diagram of a section through the full-term zonary placenta of an elephant to illustrate the relative position of the several regions A—D. Region A is the chorion from which villi pass towards the walls of the uterus. Some pass through the whole thickness of the after-birth, others only extend a short distance. Region B, which forms the great bulk of the after-birth, is made up of fœtal villi and channels containing maternal blood. Region C is a much looser layer in which the fœtal villi are lying freely in irregular blood-spaces. There are no walls to these blood-spaces. This region is traversed by a few blood-vessels which carry blood probably towards the

fœtal placenta. Some of the ends of the villi in this region are covered by a plasmodial remnant (*pl.*). The region lettered D indicates the probable relation of the uterine surface to the after-birth. The longest villi penetrate into this maternal tissue (V″).

Fig. 1 *b*.—A diagram of a similar region to show the probable relation of the blood channels to the maternal vascular system. A is an arterial vessel carrying blood from the uterine vessels U V A to region B, where it circulates in the channels round the villi and collects into large efferent vessels, which open into irregular spaces in region C, whence it is supposed the blood passes into maternal veins, U V V.

Fig. 2.—A camera drawing of a portion of a section taken vertically through the placenta showing the features characteristic of region B. The wide maternal channels (*m. ch.*) are filled with corpuscles. The mesoblast tissue of the fœtal villi and their branches are coloured darkly. Many fœtal capillaries are seen cut across transversely (*f. c.*). Between the fœtal mesoblast and the blood-channels containing maternal blood an irregular layer of syncytium (*cy.*) is seen which can almost without doubt be called trophoblast. × 340.

Fig. 3.—A camera drawing of a piece of region C, from a section taken vertically through the placenta. The large fœtal blood-vessels (*f. b.*) in the large trunks of the villi and the small terminations are clothed by a columnar epithelium (*tr.*), probably the trophoblast. These epithelia have shrunk away in many places and adhere to a coagulum, which is partly blood-clot and partly remnants of a trophoblastic plasmodium and cell detritus. × 75.

Fig. 4.—A section of a foliaceous termination of a fœtal villus in region B cut parallel to its broad surface, showing the network of fine capillaries. × circ. 200.

PLATE 2.

Fig. 5.—A group of leucocytes in a space among the tissues of a fœtal villus. × circ. 200.

Fig. 6.—A section taken across a fœtal villus cutting a fœtal vein and artery transversely. This shows the large spaces in the mesoblastic tissues filled with leucocytes. Adjoining this is a maternal blood-channel of moderate size also crowded with leucocytes. × 95.

Fig. 7.—A camera drawing of a section through the boundary between region B and region C. At the point *x* a communication between the vascular channels of region B and the irregular blood-space of region C is shown. × 75.

Fig. 8.—A piece of a maternal blood-channel from region B to show the sinuous baggy nature of these channels and the endothelial character of cells found lining the walls (*end*). × circ. 200.

Fig. 9.—A transverse section through one of the subcircular bodies. *ch.* chorionic surface; *all.* allantoic surface; *f. c.* capillaries. × 95.

Fig. 10.—A piece of the chorionic surface of the above showing minute vascular villi; *f. c.* capillaries. There is no trophoblast. × 200.

PLATE 3.

Fig. 11.—A highly magnified drawing of a small piece of region B to show the characters of the syncytial layer (*cy.*). It is taken near to region C, and shows the syncytial layer more distinctly than would a piece nearer to the fœtal surface. The syncytium is distinctly marked out into cell areas. The distribution of the pigment granules is shown, and also the very definite walls to the maternal blood-vessels. × 920.

Fig. 12.—A highly magnified drawing of the terminal portions of fœtal villi in region C, showing the larger size of the capillaries and the more columnar character of the investing epithelium, not here a syncytium. Compare this with fig. 11. × 920.

Fig. 13.—A small piece of a maternal blood-channel (from region B), and an adjoining fœtal villus *v'*. This is to show the leucocytes (*l*) in the channel crowded with pigment granules. Some near the middle seem to be disintegrating, or at any rate getting rid of their load of pigment *l'*. × 920.

Fig. 14.—Another piece similar to that of fig. 13, but showing the flattening-up against the wall of these pigment-collecting (?) leucocytes. × 920.

[All the above are from the full-term placenta. Those that follow are from Owen's specimen, namely the half-term placenta.]

Fig. 15.—This is a drawing of a section through the whole zonal belt of Owen's specimen. The comparatively small area over which the villi penetrate is clearly seen, leaving nearly two-thirds of the maternal surface smooth. Note also the bands of non-deciduous villi on either side V, the minute simple villi V‴, and the large trunks V′ with their long tufts V″. At *pl.* the homogeneous material, and at 16 the region of the crowded together terminations of the villi abutting upon *pl.* Nat. size.

PLATE 4.

Fig. 16.—A piece just within the homogeneous material layer. It shows the ends of fœtal villi covered with well-defined cubical epithelium. Between these in some places small pieces of detritus and of the homogeneous layer have been enclosed. × 300.

Fig. 17.—An apex of one of the villi abutting against the homogeneous layer. The cells of the trophoblast seem to be passing out into the plasmodium when they lose their affinity for staining and appear to die. × 400.

Fig. 18.—A rather more internally placed area. Here the trophoblast has degenerated into a syncytium, and the adjoining syncytia fuse. In the left-hand corner an advanced stage is illustrated. Within the syncytium certain spaces are recognisable (*m. ch.*) in which numerous cells are floating (*b. c.*). × 325.

Fig. 19.—Some of these large rounded cells mentioned under fig. 23, seen on the maternal surface outside a sharply defined membranous layer. × 400.

Fig. 20.—Similar cells just within the membranous layer. × 400.

Fig. 21.—A piece taken from about the spot where the line 16 ends in fig. 15. Here the new blood-channel is clearly seen to be between the trophoblast cells, not within them. × 400.

Fig. 22.—A large maternal channel derived from inter-cellular (inter-villous) space. The walls are well marked, and on their outer side are supported by the columnar cells of the trophoblast. Compare fig. 24. Inside are many blood-cells, and along the border large cells which give rise to smaller ones. Are they maternal in origin? × 490.

PLATE 5.

Fig. 23.—A piece of the limiting homogeneous layer, showing one apex of a villus abutting against it. The nuclei passing off into it are seen getting less and less distinct as they pass further away. Beneath this layer is an outer layer destitute of the above-mentioned oval nuclei, but some tissues containing loose rounded cells with small nuclei probably of maternal origin. × 400.

Fig. 24.—Part of a large intervillous space forming one of the chief blood-channels for maternal blood. At *f. c.* the fœtal villus with its capillaries; at *tr.* the trophoblast layer shrunk away from its mesoblast and forming the wall to the large blood-channel *M. C. H.* in which many large cells (*end*) and masses of fragments can be observed. × 400.

Fig. 25.—A portion of a transverse section of one of the few blood-vessels found attached to the rough median area of Owen's specimen. The endothelium has the appearance of being in a state of proliferation. It is assumed to be a maternal artery. × 400.

The Development, Structure, and Morphology of the Scales in some Teleostean Fish.

By

H. W. Marett Tims

(King's College, Cambridge).

With Plate 6.

Introduction.

The interest attaching to a precise knowledge of the structure and development of the scales of fishes is twofold. Not only may it throw light upon the affinities between groups of existing fishes and their evolution, but it helps to elucidate the problems involved in regard to tooth-genesis; for there can be but little doubt that scales and teeth are homologous structures.

It was from the latter point of view more particularly that I was led to an investigation of the subject, which I commenced during the summer of 1902, in the Gatty Marine Laboratory at St. Andrews, where, through the kindness of Professor McIntosh, F.R.S., I was permitted to work.

In the first instance I confined myself to an examination of the structure and development of the scales in the Gadidæ, especially in G. virens and G. callarias, of which I obtained fairly complete series from Professor McIntosh's rich stores. I further examined specimens of G. minutus, G. æglefinus, G. merlangus, and G. pollachius. Some of the results obtained were laid before the Zoological Section of the British Association at its meeting at Belfast in the form of a preliminary communication.

Since that time I have further examined material supplied

to me from the Marine Laboratory at Millport, as well as a specimen of Gadus argentina kindly given to me for examination by the late Professor G. B. Howes, F.R.S.

From my own experience and from the expressed opinion of other workers in the same field, a succinct account of the earlier literature is a much needed desideratum, and this I have endeavoured to furnish, the more recent and generally known papers being omitted.

Historical.

The first to note the presence of markings on the surface of the scales of fish was Petrus Borellus in 1656 (**2**). He thus describes them in 'Observatio,' xxxvii (p. 23): "Squamæ piscium apparent si aspiciantur, lineis orbicularibus multis distinctæ, et in parte qua cuti adhærent radiis ac punctis multis transcurrentibus eas divisa." In his accompanying illustrations he figures these lines and points over a limited area of the scale only, which is quite in accord with the condition found in some scales, though he makes no reference to this point in the text. This observation is of interest as being one of the earliest results of microscopic investigation. Nine years after this (1665), R. Hooke (**10**) published still further details upon the same subject. He seems to have investigated the matter from a more comparative standpoint, for though he describes the scales of the sole and dog-fish only, giving illustrations of the former, he says he has examined the scales in "multitudes of others, which it would be too long to enumerate." This early account of their structure is sufficiently interesting to merit quotation. He writes (p. 162): "This skin I view'd, was flead from a pretty large Soal and then expanded and dry'd, the inside of it, when dry, to the naked eye, look'd very like a piece of Canvass, but the microscope discovered that texture to be nothing else, but the inner ends of those curious Scolop'd Scales, which on the back side, through an ordinary single magnifying glass, look'd not unlike the Tyles on an house.

"The outside of it to the naked eye, exhibited nothing more of ornament, save the usual order of ranging the Scales into a triagonal form, only the edges seem'd a little to shine, the finger being rubb'd from the tail-wards towards the head, the Scales seem'd to stay and raze it. But through an ordinary magnifying glass, it exhibited a most curiously carved and adorned surface, each of those (formerly almost imperceptible) scales appearing much of the shape [as shown in his figure], that is, they were round and protuberant, and somewhat shap'd like a Scolop, the whole scale being creas'd with curiously wav'd and indented ridges, with proportionable furrow between, each of which was terminated with a very sharp transparent bony substance, which, like so many small Turnpikes, seem'd to arm the edges," of which he further on says "every other of these are much longer than the interjacent ones." He noticed that the scales were but partially imbedded in the skin, and remarks that "the texture or form also of the hidden part . . . . seems to consist of a great number of small quills or pipes, by which, perhaps the whole may be nourished; and the side parts consist of a more fibrous texture, though, indeed, the whole scale seem'd to be of a very tough grisly substance like the larger scales of other Fishes."

With reference to "the Scales of the skin of a Dog-fish (which is us'd by such as work in Wood, for the smoothing of their work, and consists plainly enough to the naked eye, of a great number of small horny points) through the microscope appear'd each of them curiously ridged and very neatly carved; and indeed, you can hardly look on the Scales of any Fish, but you may discover abundance of curiosity and beautifying; and not only in these Fishes, but in the Shells and crusts or armour of most sorts of marine animals so invested."

The next writer upon the subject was Leeuwenhoek in 1685 and again in 1698. He noted the presence of scales and fins in the eel and apprehended that this discovery "is new, at least to persons of the Jewish nation (for to this day

they deem this delicate Fish to be unclean, and hold it as an abomination to them)," according to the Mosaic law, which regards as such "whatsoever hath no fins or scales in the waters" (Lev. xi, 12). Consequently he figures the scale in this fish, a figure which is highly creditable. Moreover, he noted the imbrication of the scales and the fact that they varied in size in different regions of the body. He makes the assumption that the scales may be taken as an index of the age of the fish, for he describes the appearances thus: they were principally "composed of a kind of globules or little balls . . . lying in rows contiguous to each other" which "produced the appearance of divers circles or rings on the face of the scale. And although I did not observe these scales to be exactly alike, yet the circles or rings seemed to me to be of the same number in all of them, whence I was led to conclude, that the scale had been every year augmented by the addition of one circle, and consequently that, as there were seven circles in this scale, this Eel was probably seven years old." A similar thing to that which "is evident in trees" or "shown in horns whence we gather that as many knots or rings as are found on the cow's horn, so many years of age is the animal."

On the authority of Mandl, Leeuwenhoek is stated to have subsequently abandoned this view of the rings on the scales as affording an index of age.

An examination of the scales was made by means of sections and the appearances as seen under the microscope are represented. He concludes that a scale is formed in each year, each succeeding scale being somewhat larger than its predecessor and "glued" to its under surface. From the description given, one is led to the conclusion that Leeuwenhoek regarded the rings as the edges of succeeding scales, though he does not expressly state it. This point is of great interest in comparison with Williamson's work (**20**) on the ganoid scales in 1842. That this view was not accepted at the time is shown by his remark, that "this assertion of mine is however violently contradicted, because many people think that

I cannot by any means prove what I affirm"; he consequently proceeded to demonstrate his facts by means of sections through the scales.

The paper ends with some very interesting remarks on the longevity and causes of death in fishes.

During the eighteenth century many other writers made short, but unimportant, references to this subject, among them being Réaumur, Petit, Schaeffer, Ledermüller, and Fontana, an admirable review of the literature of this period being given by Mandl (**15**). Réaumur gave the name "Argentin" to the translucent material on the surface of scales, and a rough chemical analysis of it was made subsequently by Heinrich Rose, and his results incorporated in a paper by Ehrenberg (**5**).

Kuntzmann (**13**) recognised that the variation in scales may be of generic value, and from his observations he was led to classify the scales of fishes into seven groups, which include, but under different names, the Ctenoid, Cycloid, and Clupeoid of to-day.

The first investigator to seriously deal with the subject in a truly scientific manner was Agassiz in his work 'Recherches sur les Poissons Fossiles,' in 1834. He here makes use of the scales as a basis for his classification of fishes into the Placoids, Ganoids, Ctenoids, and Cycloids, the latter including the Clupeoids. While mentioning that the scales are contained in pockets of the skin and are not retained in position by means of blood-vessels, as Leeuwenhoek appears to have believed, he nevertheless adopted the idea of the latter, slightly modified, in regarding each newly-formed scale as being successively attached to the lower surface of the preceding scale.

The next paper of importance is that of Mandl, already referred to, in which the histological structure of the scale is examined in a more thorough and detailed manner. He drew attention to the fibrous substratum of the scale, which he accurately figures. The surface is described as composed of "corpuscules," which Mandl recognised as being quite

separate from one another. Radiating from the centrum or "foyer" are longitudinal lines, and connecting these transversely are what he calls the cellular lines. He likens the upper surface of the scale to cartilage, the cells of which he regards as being the "corpuscules." Many of Mandl's interpretations will not hold at the present day, but from the point of view adopted in the present paper the isolation of the individual corpuscles is of considerable interest. In addition to this, he came much nearer to a correct interpretation of the peripheral growth of the scale than had been done by any previous observer.

In 1873 Baudelot contributed a lengthy paper to the 'Archives de Zoologie Experimentale.' He examined the scales of a great number of fishes, furnishing elaborate statistics and instituting comparisons relating to the various measurements of scales, the number of concentric markings, etc. Beyond this he adds but little to the knowledge of the morphology of the scale. His descriptions of the patterns upon the upper surface agree in the main with that given by Mandl, but using different terminology. Baudelot, however, recognised the calcified nature of the upper layer and its growth by the deposition of calcareous molecules in the membranous zone forming the border of the scale. He also gives a short account of the actions of various chemical reagents, the results of which I have been able to confirm.

Following upon this we have the classical works of Johannes Müller, O. Hertwig, Peters, Williamson, Hofer, Klaatsch, and Nickerson. These are all too well known to need any further reference as to the advances in our knowledge of this subject which each has contributed.

It must not be supposed that the foregoing pretends to afford in any way a complete bibliography, the more important of the earlier works alone having been dealt with in chronological sequence, in order to furnish a short historical sketch of the subject under consideration.

Development.

The first trace of the scales in Gadus callarias appears when the animal is between 3 and 4 cm. in length. The skin consists of an epidermis, which is very readily detached, and which, in the majority of preserved specimens, is altogether wanting. This is a fact of some importance which should be borne in mind, since the absence of this layer in much preserved material is doubtless the cause of the discrepancy in the accounts given by writers as to the part played by the epidermis in the formation of the scale. In a freshly caught fish it can be seen, as a soft, thin, almost gelatinous membrane, which tends to become separated when the animal is put into any other than its natural medium. A surface view of this membrane is shown in Fig. 1, taken from an animal 11 cm. in length, Fig. 2 representing a section through the same membrane in a somewhat older specimen. It consists of rounded or slightly fusiform cells, crowded together, each cell having a relatively large and well-marked nucleus. Opening on to the free surface are large numbers of spherical glands, the openings of which are proportionately small. Chromatophores are distributed throughout the thickness of the epidermis as well as on both its inner and outer aspects.

On comparison with the epidermis of Lepidosteus, though there is a certain general resemblance, there are points of marked difference. In this animal, Nickerson (**16**) describes two kinds of glandular structures—one, the larger, oval in shape, with the long axis perpendicular to the surface of the body, and which are irregularly distributed; the other, considerably smaller, more numerous and spherical, the majority of which occur "near the surface, where many of them open." Nickerson remarks that the latter "do not appear to have been recognised by previous observers." He thinks that they are the true mucin secreting glands, the former having "the function of secreting some component of slime other than mucin."

From a comparison of the epidermis in the two forms, the glands present in the cod correspond to the mucin-secreting, the larger being unrepresented, slime not being present in the cod. When viewing the surface the rounded ends of the glands are visible, and give to the tissue the appearance of ordinary vegetable parenchyma. Lying upon the surface of the cells are numerous chromatophores, distributed with a considerable degree of regularity. Comparison with the gar-pike shows that the presence of the larger glands in the latter cause a more reticulated appearance, the wider meshes representing their cavities.

Beneath this layer is a thin, but well-defined basement membrane, corresponding, I take it, to Huxley's protomorphic layer, the readily detached epidermis being consequently ecderonic, the tissue lying subjacent to the basement membrane being enderonic.

The enderon is clearly divisible, even from an early stage, into two layers, an outer composed of elongated, nucleated, closely-set cells, the boundaries of which are distinguishable with difficulty; so numerous are they, that this might almost be designated as the "nuclear layer." It stains deeply with borax carmine, offering a marked contrast to the next or deeper layer of enderon, in which the tissue is more fibrous, the fibres running longitudinally. The nuclei here are more sparsely scattered, larger, and more elongated than in the previous position. Beneath this and separating it from the muscle is yet another layer of pigment cells, which vary considerably in size and are very unevenly distributed. The conditions are represented in Fig. 3.

Comparison with the dermis of Lepidosteus (**16**) shows the same general disposition of parts, though differing in details. For example, I have not been able to distinguish the more complicated interlacement of fibres noted by Nickerson.

In the cod the scales arise in the more superficial nucleated layer of the dermis, their first indication being local aggregations of cells, as evidenced by their nuclei. A

horizontal slit-like cavity forms in the interior, the floor of some of the larger spaces being raised into a small conical elevation projecting upwards into the cavity. In none of my specimens has this papilla been much more marked than is represented in Fig. 3. In the majority, I have been unable to detect any such elevation. Whether when present they are merely artefacts, or whether they are to be regarded as very diminutive homologues of the dermal scale papillæ of Selachians may be open to doubt. If so comparable, it might be objected that more than one such elevation ought to be found in each cavity. It is possible that this may be only a central one, and that more may arise at a little later stage, a stage which so far I have not been fortunate enough to meet with.

The limits of the cavity are sharply defined from the surrounding tissues by the still closer arrangement of the nuclei; this is rather more evident on the floor of the space. In the latter situation there appears to be a very thin layer immediately bounding the cavity, from which nuclei are absent, the layer having a homogeneous aspect. The nuclei in connection with the scale cavities are those of the "Scleroblasten" of Klaatsch, the thin homogeneous layer being the first indication of the scale. Nickerson writes (loc. cit., p. 121): "The fact that in Selachians the scale is formed over the surface of the papillæ, while in Ganoids (Lepidosteus) and in Teleosts it arises in the midst of the mass of cells forming the elevation is a fundamental difference not to be overlooked." This account of the development of the Teleostean scale is evidently drawn from the description given by Klaatsch. The description and figures given by the latter of the early conditions seen in the trout do not correspond in detail with what I have just described in the cod. Here the scale, if I have interpreted the appearance aright, arises as an apparently homogeneous layer covering the surface of what may be regarded as the dermal papillæ, precisely comparable to the early process of scale formation in the Selachians. If such be the case, then the "fundamental difference"

between Selachians and Teleosteans of which Nickerson speaks does not exist.

It is possible that the difference between the observations of Klaatsch and those just related may be more apparent than real. If the vertical extent of the scale-cavity were reduced, which quite conceivably might be the case in correlation with the varying size of the scale, then the roof and floor of the cavity would be approximated and the scale-anlage lying on the surface of the latter would give the appearance as if arising in the midst of the mass of cells. Such, I believe, from a comparison of the figures, is a very possible explanation of the differences.

Following upon this stage is the laying down of the calcareous material. I am not in a position to say whether this appears upon the surface of the homogeneous layer as a kind of secretion, or whether the homogeneous layer itself becomes impregnated, but I incline to the latter view. One point is, however, certain, namely, that the calcareous material does not form an uninterrupted layer, but is in the form of minute isolated platelets, the external surface of each of which bears a minute pointed and backwardly directed spine; in other words, each is a minute microscopic placoid scale: this is shown in Fig. 4. These are placed upon a basal membrane which stains with borax carmine, and which can be seen extending upwards between the contiguous ends of adjacent denticles.

This condition has been entirely overlooked by previous observers, the reason being not far to seek. The skin has been treated with an acid, either for the purpose of decalcifying, or following the routine method of placing in acid alcohol after staining with borax carmine. I have been careful to avoid all trace of acid in the preparation of the specimens, and thus prevent any decalcification. Naturally, the sections are somewhat liable to be torn, and are difficult to cut, but the results are sufficiently satisfactory to leave no doubt as to the existence and mutual relationships of these minute placoid scales. In order to test this explanation, I

took portions of the skin of the same animal and from the same region of the body, and treated it with borax carmine and acid-alcohol. The sections obtained showed precisely the characters figured by Klaatsch. The scales are seen on section to form an elongated continuous plate from which project several small pointed elevations at the caudal and cephalic ends. Owing to having been decalcified, the organic basis of the placoid scale has become stained, like the substratum below and between them, and thus gives the appearance of continuity. Though Klaatsch figures these tooth-like elevations, he does not appear to have recognised their morphological value. Indeed, in his lengthy memoir I have not noticed any particular reference to them, beyond the general statement (p. 196): "Der Spitzentheil der Placoidschuppe ist mit wenigen ausnahmen völlig reducirt. Die Oberfläche der Teleostierschuppen geht neue Komplikationen ein, welche zu mahnigfachen Reliefbildungen führen."

The scale continues to increase at its periphery by the addition of more denticles, so that meeting and overlapping the adjoining scales they become imbricated. As a result of this growth, that part of the dermis superficial to the scale cavity becomes stretched and thinned. Accompanying this increase in size of the scale, there is a progressive conversion of the subjacent nucleated layer into fibrous tissue, which thus in course of time presents a laminated appearance, certainly as many as five layers coming to be ultimately formed. I would draw attention in passing to the fact that this conversion into fibrous tissue is from above downwards—that is, it follows the direction of growth which Huxley maintained to be the character of enderonic tissues. The bearing of this point will be discussed subsequently.

As a result then, of the continuous growth of the scales in a closed cavity, there is stretching of the less rigid tissues of the roof, and at the same time the "pull" exerted by it would tend to crowd the denticles together, and also to keep the growing margins of the scale upturned. This mechanical

factor is, I believe, of considerable importance in producing the modification in shape which the denticles undergo in their further development.

By the continued growth of the scale, the posterior margin reaches the surface of the derma, lying between it and the superposed epidermis. It is more than probable that this is the cause of the tendency of the epidermis to become separated. As far as I have observed it, there is no corresponding elevation of the epidermis, the scale lying altogether beneath it. It remains throughout in the scale-pocket (Schuppen tasche, Klaatsch), which becomes stretched over the superficial surface of the scale, following all the irregularities. It is this pocket which mainly acts in retaining the scale in position. In larger scales—for example, in the herring—the pocket becomes still more stretched and attenuated, possibly even ruptured, and this would account for the ease with which the scales may be detached in the Clupeoids.

Structure of the Fully-formed Scale.

I have been unable to find in the literature any adequate account of the fully-formed Teleostean scale. They have been described as "variously sculptured," "ringed," "marked with concentric and radiating lines," etc., but all such descriptions are misleading and fail altogether to interpret their true structure. From the following account it will be seen that the cycloid scale is a complex structure. It consists of a fibrous substratum which preserves the general form of the scale. Upon the upper surface of this are placed a number of calcified plates, or "scalelets" as they may be termed. Covering the entire upper surface is a very delicate membrane distinct from the thickened epidermis which clothes the surface of the body generally, and which is in reality the thinly stretched outer wall of the scale-pocket above mentioned. The fibrous stratum may best be revealed by placing the scale for some minutes in

20 per cent. hydrochloric acid, which dissolves away the greater part of the scalelets, leaving only their outline in organic material, which may easily be removed by scraping with a scalpel. The same result may be attained merely by scraping without the previous addition of acid, but more force is necessary and there is consequently a greater tendency to a tearing and disarrangement of the underlying fibrous lamellæ. This sub-stratum consists of distinct lamellæ of very delicate fibres, having the appearance of ordinary white fibrous tissue, though when torn the individual fibres tend to curl after the manner of the yellow elastic variety. They are extremely resistant to the action of carmine or hæmotoxylin, but are readily stained with eosin or picro-nigrosin. The bundles of the superficial lamella are excentrically arranged, each bundle corresponding with a ring of the superposed scalelets. Subjacent to this is a layer the bundles of which interlace with each other at right angles, each running diagonally to the long axis of the scale. Between these two lamellæ, and also underlying the latter, the fibres appear to form reticulate but much thinner strata. How far these last are to be regarded as definite layers, or merely the results of "teasing out," I have not been able satisfactorily to determine. The number of lamellæ present varies in different parts of the scale, being most numerous at the centre, while at the periphery the superficial excentric layer alone seems to be present. In the scales of a large common cod about $2\frac{1}{2}$ feet in length five layers are to be seen at the centre in transverse section.

The fibrous strata were noted as long ago as 1839 by Mandl (14), and again by Baudelot (1), but seem to have escaped the notice of some recent writers. Mandl, though not describing the arrangement of the lamellæ, appears from his accompanying illustration to have observed them correctly. Baudelot, on the other hand, describes the layers as being very much more complicated than I have detected. He says: "Le trajet de tous ces faisceaux ou plans fibreux est extrêmement compliqué et des plus difficiles à

démêler'' (p. 165). The summary of his observations may be given in his own words: "Chaque plan fibreux n'offre pas le même texture dans toute son étendue; dans sa portion centrale, c'est-à-dire celle qui correspond au centre d'accroissement, il est formé de fibres entre-croissées à angle droit; dans sa portion périphérique, il se compose de faisceaux fibreux, entrelacés sous divers angles et décrivant, soit des arcades, soit des courbes de diverses natures. Toutes les fibres du bord pérephérique semblent se perdre dans la couche extérieure de l'écaille ou elles prennent une direction plus ou moins parallèle à celle des crêtes concentriques" (p. 174). This description accords in the main with my own observations. It is possible that the scales of the perch, upon which Baudelot chiefly founded his assertions, may present a greater complexity, more particularly, as will be shown subsequently, there is reason to believe that degenerative changes are in progress in at least some species of cod. So far as I have been able to observe no difference exists in the general arrangement of the fibres in any of the species of cod examined.

Sections through the scale merely show the various fibrous lamellæ. I have been unable to detect the presence of any corpuscles; if they exist, they must be very minute. Neither have I met with any of the calcareous masses described by Williamson. There are, however, numerous delicate vertical tubes traversing the substance of the scale and which probably subserve a nutritive function. They are particularly in evidence beneath the centrum of the scale. They may possibly represent Williamson's Lepidine tubules, or they might be regarded as Volkmann's canals, though the absence of corpuscles would tend to negative the latter interpretation.

Upon the upper surface of the fibrous layer are placed the translucent calcified scalelets, which, in the recent condition, are closely invested by the delicate outer wall of the scale pocket. If the scale be stained while this membrane is in situ the whole surface becomes uniformly tinted. On removal of the membrane the scalelets are quite unstained,

though each individual is mapped out by lines of coloration where the membrane persists in the interstices between the more elevated scalelets. The only reagent which I have as yet tried which seems to act readily upon the scalelets is hæmalum, and this is not so marked as in the younger stages.

Scalelets vary in shape and arrangement in different species of cod, more particularly in the lateral fields. The common cod and the coal fish furnish examples of the two most widely separated types. In the common cod (Fig. 6) the scalelets consist of a basal plate quadrilateral in shape. Those in or near the mesial line of the scale at either end are very nearly square, while in the lateral fields they are rhomboidal. Near to the peripheral margin of each scalelet there is a well-marked transverse ridge, which gives to it the appearance of a square envelope with a straight flap not fastened down, while the extreme peripheral and central margins are slightly bent upward, and are in contact with the adjacent margin of the scalelets immediately in front and behind. Towards the growing edge of the scale they are not always in such close contact, and a thin transverse line of colour between them may be seen in stained specimens. The appearances are more readily understood in sections through the scale, and may be represented diagrammatically (Pl. VI, fig. 7).

The same placoid pattern can still be recognised, but each scalelet is considerably expanded laterally. The transverse ridge near the peripheral margin is due to the centrally directed but minute spine; the anterior and posterior margins of the basal plate are upturned, the lateral being straight. The spines are most marked in the posterior field. The centrum or "foyer" consists of a flattened plate of calcified material, oval in shape, with an irregularly serrated margin. From its appearance in section and on surface view and from the conformation of its margin, I believe it to be formed by the fusion of a number of basal plates, the spines of which have entirely disappeared. There is no indication of any markings such as would suggest that, previous to fusion, there had

been any upturning of the central and peripheral margins of the scalelets. This appears to be easily explained. The centrum is the first part of the scale to be formed, while there is comparatively plenty of room in the scale pocket; with increase in size the walls of the pocket become stretched and exert an upward "pull" all round the margin of the scale; this leaves an impress on the marginal scales in process of formation, and would account for the up-turning at the peripheral margin, while the pressure thus exerted in a centripetal direction would push the central margin against the peripheral border of the scalelets immediately in front and bring about the same result, but, as shown in the diagram, to a lesser degree. At the growing edge of the scale the young scalelets appear to arise from the lateral wall of the scale pocket, for they have at first a direction perpendicular to the surface of the scale.

The type of scale above described, or but slight modifications of it, is that most generally met with in the Gadidæ. In G. virens, however, there are differences which render the scale easily distinguishable (fig. 5). The scalelets have a tendency to become triangular in the lateral fields, while in the anterior and posterior fields, though quadrilateral, they differ markedly from those of the common cod, as will be seen by reference to the figures. Moreover, there is no transverse ridge visible. Under a high power the scalelets are very clearly imbricated. On transverse section, there is no indication of any spine, the attached border is implanted in a kind of socket in the upper surface of the fibrous layer, and I think there can be but little doubt that they represent merely the basal plates of the placoid scale, all trace of spines having disappeared. The centrum appears to be similar to that previously described. One or two other points remain to be mentioned. In individual scales of all species a partial fusion of adjacent scalelets may be seen. In every instance which has come under my notice, this fusion has been a lateral one. While this condition would appear to be the exception in most species, in G. minutus it might almost be said to be the rule, for I have not observed

any scale in which it was absent, usually the fusion being considerable. This would seem to be a point of some importance, for if we imagine a lateral fusion of the small scalelets to take place throughout, we then approximate to the typical clupeoid scale, which is composed of excentric imbricated rings.

On the other hand, if the spines present in the scalelets of the G. callarias be more pronounced and slightly more perpendicular in direction, we have the spines of a ctenoid scale. In the latter the spines are present only in the posterior field, in the same position as they are most evident in the cod. The earlier tendency to disappearance in the anterior field is probably due to the imbrication.

One further point: the radiating lines of scalelets at a certain distance from the circumference become replaced by double lines. This is probably in adaptation to the increased circumference of the scale. Still nearer the margin one of the four again bifurcates (if the scale be placed with the anterior pole towards the observer, it seems to be always the line to the right which thus becomes divided). This is only marked in the posterior field, though elsewhere the same takes place, but is neither so striking nor does it appear to be of such regularity.

Clupeoid Scale.—The scales of the adult herring, sprat, and pilchard have been examined, but, with the exception of some unimportant differences, such as relative size, there is but little to distinguish between them; consequently, the description of the herring scale here given applies equally to the sprat and pilchard.

The excentric markings seen on the clupeoid scale is limited to the anterior portion, the posterior uncovered part being quite smooth, each excentric line roughly describing a semicircle. Neither in stained nor unstained specimens are any radiating lines visible such as are seen in the scales of the cod. Further, the excentric series of markings presents a considerable degree of uniformity in passing from the centre of the scale to the periphery. In all the scales examined

there have been lines of intermission which become more visible on staining. This is due to the deficiency of the calcified upper portion of the scale allowing the underlying fibrous (staining) stratum to be visible.

Though these lines of intermission occur with a certain amount of regularity, I do not think they can be regarded as delimiting the different periods of seasonal growth of the scale, for two reasons. In the first place, they are not always of equal number in scales removed from the same situation in the same fish; and secondly, they do not always extend around the anterior moiety of the scale, but are often interrupted so that the excentric (calcified) lines of one area pass into those of the area next to it. I believe these lines of intermission have little, if any, morphological value.

All markings are absent from the posterior uncovered portion of the scale, the calcified layer being smooth and of extreme thinness.

Examination of a section through the scale shows that the excentric lines in the anterior part are due to minute ridges arising from a continuous basal calcified layer, each ridge having an inclination towards the centre of the scale.

The fibrous portion of the scale has an arrangement similar to that previously described for the cycloid scale, but in one section the perpendicular tubules are slightly larger.

Sections through the long axis of the scale in a young herring show the same placoid condition as in the young cod, the individual scalelets being much more minute. The projecting spines in the median line of the scales are backwardly directed, as in the Elasmobranchs; that is, they have the same inclination as that of the excentric markings in the adult scale. These placoid scales are limited to the anterior portion of the scale, the scalelets of the posterior portion being merely elongated flattened plates without any spines.

I cannot say whether they have been present and have disappeared, but I have never seen them, though I have examined fish of various ages, and I am inclined to think that that portion of their philogenetic history has disappeared altogether from their ontogeny.

As development proceeds, the basal portions of all the scalelets fuse to form a continuous plate, except at the lines of intermission. The spines appear to fuse laterally with their neighbours, thus giving rise to the excentric markings which have been seen to be in reality projecting ridges.

Comparison with the scales of the cod show certain points of marked difference. In the latter individual scalelets remain distinct in certain species—for example, in G. virens and G. callarias; though in G. minutus, as has been said, there is a considerable amount of lateral fusion; while in all species there is an entire fusion, with a total absence of spines in the region of the centrum. The same influences seem to have been at work in the clupeoid scale, and to have carried their results still further. The condition found in the centrum of the cycloid scale is identical with that seen in the posterior uncovered portion of the clupeoid, while in the anterior part fusion has taken place to a greater extent, the basal portions of the scalelets fusing throughout to form a continuous sheet (except at the lines of intermission), while a lateral fusion involving the spines has given rise to the continuous excentric ridges.

There is one point of marked difference in comparison with the scales of such a form as G. virens. In the latter the appearance of excentric lamination is due to an imbrication of the scalelets, the spines having more or less disappeared, whereas in the clupeoids there is no such imbrication, the excentric markings being produced by the spinous portions of the scalelets.

What have been the determining causes of these differences in the various scales, or for the retention and disappearance of the spines in the anterior and posterior portions respectively of the individual clupeoid scale, I am unable to say. The disappearance on the exposed portions of the latter cannot be due to friction, or it would equally have affected the exposed part of the cycloid scale. I am inclined to think that the retention of the spines in the covered areas may have the effect of retaining the scales in position, since in the

clupeoids, where the spines are but very feebly developed, the scales are readily removed, whereas ctenoid scales are the most difficult to separate, and in them the spines are most marked. In this respect the cycloid pattern occupies an intermediate position.

Amongst the species of cod whose scales have been examined, those of G. callarias retain the most primitive condition, while those of G. minutus seem to be converging towards the clupeoid pattern. But both cycloid and clupeoid type agree in this fundamental particular—that they both arise as a number of minute scalelets of the placoid variety.

Morphology.

It now remains to discuss briefly the morphological bearings of this interpretation of the scale structure. Hitherto the Teleostean scale has always been regarded as a morphological entity. This will be evident from the following quotation from the important memoir of Klaatsch (**12**). On page 174 he writes: "Die Teleostierschuppe entspricht der Basalplatte der Placoidschuppe"; and again: "Jede Teliostierschuppe einer Placoidschuppe entspricht."

From a consideration of the facts set forth in this paper one is led to the opposite conclusion, namely, that the scale of the Teleostei is a compound structure, the morphological unit being the individual scalelet, each of which is the homologue of a single placoid scale of the Selachians. When I first enunciated this view at Belfast I was unaware that a similar interpretation had been placed upon the Ganoid scale by O. Hertwig (**6**), an interpretation which both Klaatsch and Nickerson refuse to accept. This view is, I believe, quite new in regard to the Teleosteans, and the fact that a similar interpretation has been placed upon the ganoid scale by so high an authority as Hertwig is a fact of the utmost value in its support.

The union of a number of placoid scales upon a single fibrous basis would lead to the formation of a teleostean

scale in which the spines might persist only in the anterior covered field, as in many scales of the ctenoid type; a further suppression of the same would lead to the cycloid pattern. The tendency to lateral fusion of the scalelets so frequently seen in the latter would give rise to the scales of the clupeoids, the degree of fusion being seemingly correlated with the relative increase in size of the scale. A still further stage of suppression is seen in the herring, where not only all vestiges of the spines are wanting, but the scalelets themselves have almost disappeared from the uncovered posterior field.

The phylogenetic order of the scales would, therefore, appear to be placoid, ctenoid (?), cycloid, and lastly, clupeoid, and as far as the Gadidæ are concerned ontogeny recapitulates the phylogeny. I hope shortly to have completed researches into the development of the ctenoid type.

Within the limits of the Gadidæ one meets with unmistakable signs of fusion of the scalelets in G. minutus and G. æglefinus, which, in the earlier stages, are quite distinct. A further advance in the same direction gives the clupeoid scale. One reason for regarding the cycloid scale as a ctenoid in which the spines have almost (G. callarias) or entirely (G. virens) disappeared has already been given. There are, however, certain other facts which tend in the same direction—for example, the fact that the scales on the blind side of Arnoglossus are cycloid, while on the ocular side they are ctenoid. Further, the opercular bones in the turbot and many other Pleuronectids are heavily armed in the young, whereas they are smooth in the adult. Again, the ctenoid scales in the dwarf variety of the plaice (Pl. pseudoflesus) may be taken as another indication in the same direction.

On the other hand, Dunker (4) states that in the male plaice after maturity the cycloid may develop into ctenoid scales, and that the same may happen in flounders only 2–3 cm. in length. I do not wish to pass lightly over any point adverse to the views here expressed, but I may be permitted to refer to the opinion of an independent reviewer,

Mr. Stead (**17**), who throws considerable discredit upon the conclusions, and upon Dunker's want of care in the establishment of his facts.

Cunningham, in dealing with the ontogeny of the plaice (**3**), seems to imply that the cycloid precedes the ctenoid condition, but it is not evident upon what grounds he rests the implication. Holt has shown (**9**) that the scale tubercle of the adult turbot is developed from a simpler and more cycloid form in the young. It is, however, by no means certain that this scale tubercle is the homologue of the ctenoid spine, and not a special development.

Wiedersheim (**18**) states that the cycloid scale is the more primitive, but without stating the grounds upon which the assertion is made. Hofer (**7**) is of the same opinion, basing his conclusion both on developmental history and on palæontology. With respect to the latter evidence this writer takes into consideration only the Physostomes of the Jurassic, whereas the earliest Teleostomes date back certainly to the Lower Devonian. The embryological evidence put forward by Hofer appears to me to be inconclusive, based on supposed histological homologies, and from the point of view taken in this paper is open to the objection that the scale and not the scalelet is taken as the morphological unit.

Klaatsch (loc. cit.) claims a polyphyletic origin for the cycloid scale. It is beyond the limits of the present paper to enter into a general discussion as to all the ganoid, dipnoan, and other scales, but I may say in passing that such a complication appears to me to be unnecessary, and that the various scales, so far as I have knowledge of them, can be reduced to modifications of a single primitive placoid pattern.

In consideration of the eminence of the authorities just quoted, one cannot but speak with the greatest diffidence and regard this question as at least "non proven," though as yet I have been unable to find any direct evidence against the view of the ctenoid ancestry for the cycloid scale.

If the interpretation, that the individual scalelet and not the whole scale is the ultimate morphological unit be accepted,

it follows that many of the deductions of previous authors from the evidence of the scales alone will necessarily have to be modified.

As above stated, Hertwig has made a similar suggestion as to the morphology of the ganoid scale. To this Klaatsch objects owing to the great number and indefinite arrangement of the spines in the Selachians. Neither of these objections appears of much weight, since the number of scales must have been very considerably greater in the larger and more primitive Selachians, and with a reduction in body size the spines would tend to be more crowded, but not of necessity in the first instance to have undergone any very great numerical reduction. Secondly, the tendency to a fixation of the scales in situ upon a fibrous basis would impede the movements of the body. In order that this might be prevented as much as possible without impairing their other exoskeletal functions the scales have assumed a definite shape which has consequently interfered with the regular linear arrangement of the scalelets. If the disposition of the latter be examined, not from the point of view of excentrically arranged lines, it will be seen that those on the lateral fields still preserve a very fairly definite antero-posterior series, while in the anterior and posterior areas this plan is but little disturbed.

I pass to a consideration of the points involved in the account of the early stages of the development. It has been seen that the teleostean scale arises and remains throughout its existence as a dermal structure. This at first sight appears to present a difficulty in homologising the scalelet with a placoid scale or with the scales of Lepidosteus. In the development of the two last-mentioned the greater part of the spine is dermal; it is only the enamel tip which is epidermal in origin. This in Lepidosteus is only subsequently added when the dermal spine has reached the lower boundary of the epidermis. It is also at this time and from the same source that the scale receives its layer of ganoin. In the Teleosteans the spines are so much reduced in size

that they do not, during the period of their growth and development, attain that superficial position. Consequently, neither enamel nor ganoin is formed upon them. This does not offer to my mind any serious obstacle in establishing their homology ; it is simply that they have not reached to the same level, and as a consequence the epidermis has had no opportunity of sharing in their formation. This view finds support in the opinion to which Nickerson inclines, that the enamel is not secreted in Lepidosteus until at least a part of the spine has been formed. The development of the teleostean scale shows that it is entirely a dermal structure; and regarding the line of demarcation between the dermis and epidermis as Huxley's protomorphic layer, it follows that the entire scale must be regarded as of enderonic origin, and that the scale is covered throughout its existence by the superficial layer of the scale-pocket, which is also enderonic. The successively formed layers of the fibrous substratum are added to the deeper surface, the direction of growth being from without inwards, precisely in the direction which Huxley regarded as being indicative of the growth of enderonic tissues. The calcified layer, the first part of the scale to be formed, does not increase in thickness after being once formed, there being merely an increase in size and alteration in pattern of the individual scalelet. As to the presence of the true ganoin in the teleostean scale I cannot but agree with Nickerson in thinking that Klaatsch is in error, and that in the scales of the Gadidæ no such material is present.

Huxley (**11**), following Williamson, regards fish-scales as "essentially tegumentary teeth," and of this there can be but little doubt, but the further conclusion of these writers, that the scales are formed by a calcareous deposit in a deep layer of the ecderon, I think erroneous. Presumably what they interpret as the deep layer of the ecderon I have regarded as the superficial nuclear layer of the enderon. It is difficult to establish a difference now that the importance of the basement membrane is no longer upheld; but from the marked difference in the histological characters, and judged

by Huxley's own criterion of exogenous or endogenous mode of growth, I think the entirely enderonic origin of the whole teleostean scale must be admitted. It would seem that a difficulty had presented itself to Huxley, since he regards the nature of the deeper layers of the scale as uncertain; for he writes (**11**, p. 386) that it is "an open question whether the deep layers of all scales are produced by a continuation of the process" (i. e. of a calcification of the ecderon), "or whether in some cases a deep truly enderonic structure may be added to this superficial ecderonic constituent to constitute the perfect scale. A process of the latter kind would, at any rate, find its parallel in the eventful union of the teeth of many fishes with their jaws, and in that of the plates of the Chelonia with the vertebral elements."

The view which I have advanced seems in no way to invalidate the conclusion as to the homology between teeth and scales. In the former there are both dermal and epidermal constituents as represented by the dentine and enamel cap; the same is seen in the scales of Selachians and Lepidosteus. In the latter the dermal portion grows upwards to reach the epidermis and there receives its enamel addition. In the teeth of the higher vertebrates the process has become more specialised, and the enamel germ grows inwards to meet the uprising dermal papilla. In the Teleosteans conditions are similar to those obtaining in the Selachians, but owing to the smallness of the spines and their failure to reach the level of the epidermis, no enamel tip is consequently formed.

There remains yet one further point to which I would refer, namely, that raised by the recent work of Hoffbauer and Stuart Thomson (**17**). These writers believe that there is an annual growth of the scale in rings, which therefore furnish an index of the age of the fish. Thomson likens this annual growth to the annual rings in the stem of a Dicotyledon. Neither the idea nor the simile are by any means new; both were, as has been shown, originally suggested by Leeuwenhoek in 1685.

The same idea has also from time to time been referred to by other writers. During the course of my observations I have been led to note carefully the appearance of the scales endeavouring to estimate the ages of the fish with whose scales I was dealing, and the length of which only was known to me. In young fish the rings are quite easily to be recognised, which Thomson regards as the rings of growth of the first summer and first winter. Such a scale he figures in his paper.

It may be noticed in passing that the point of the first bifurcation of the lines of scalelets at the one pole marks what Thomson regards as the period of first summer growth, and that the further bifurcation previously mentioned as being present in some scales corresponds with the limit of first winter growth. This is about the farthest point to which I am able to follow. With the increasing size of the scale, I confess I am quite unable to detect that regular series of alternating broad and narrow bands such as Thomson describes. From the scale of a G. Callarias $2\frac{1}{2}$ feet in length, one of which is represented in fig. 6, I cannot make any estimate as to the age. If this be so in the cod, I find still greater difficulty in the case of the herring and other Clupeoids, concentric markings upon which are not merely irregular, but to many the term "labyrinthine" might almost be applied. As a method of practical value, it seems to be of but little value, at any rate in my hands.

Apart from this, however, there seems to be many theoretical objections. Klaatsch and others have pointed out that the scales do not appear simultaneously all over the body. They commence to appear just behind the pectoral fins in the neighbourhood of the lateral line. From this point the appearance of the scales radiates, those in the tail region being last to appear. It appears, therefore, that in instituting comparisons of age, the scales should always be taken from the same region, and that region stated. Of course, if the extension of scales over the whole surface of the body is rapid, then the limit of error will be so small as to be practically negligible. Until we have some data as to the rate of extension

in different fishes, it would appear that scales taken from different regions of the same fish might yield different results; this according to Baudelot (1) is precisely the case. He states that many fish present scales of different varieties: "L'existence simultanée d'écailles cténoïdes et d'écailles cycloïdes surdes points du corps différents, a été constatée par moi dans les Trigla Lineata, Sargus Rondeletii; Perca Fluviatilis, sur divers pleuronectes (Pl. sola, Pl. flesus, etc.) chez plusiers scorpions. Le même fait a été observé chez le Pelamys sarda par Péters, et sur l'apron par L. Vaillant. Le Thon possèdè aussi deux sortes d'écailles distinctes. Les écailles de la carêne ventrale de l'Alose et du Rareng, les écailles de la ligne latéral d'un grand nombre de poissons (Trigle), présentent aussi une conformation particuliere" (p. 432). With regard to the number of concentric rings, the same author writes: "En comptant les crêtes concentriques dans chacun des champs de l'écaille, on constate que le nombre de ces reliefs n'est pas le même pour chacun d'eux." Again: "Le nombre des crêtes est susceptible d'offrir les plus grandes variations dans les écailles d'un même poisson," citing as examples the perch and the pike (p. 436). In speaking of the increase in size of the scale by the addition of peripheral rings, he says: "Ajoutons enfin que l'accroissement n'a pas lieu au même degre pour tous les écailles d'un même poisson." A large number of tables are furnished by this writer giving the number of concentric lines in the different fields; many vary as much as six or eight, and in some instances ten.

From these considerations, number would seem to be very unreliable as an index of age, and from personal observations I am of opinion that their relationship to one another is equally untenable.[1] A further point must not be lost sight of in this connection; it is the possibility of the shedding and replacement of the scales. I have been assured by prac-

[1] Since writing the above Mr. Stuart Thomson's more detailed paper has been published ('Journ. Marine Biolog. Assoc.,' vol. vii, 1904). I see no reason, however, for modifying the opinion which I have already expressed.—H. W. M. T.

tical fishermen of considerable experience on the east coast of Scotland that it is well known to them that such is the case, more particularly in fishes after spawning, analogous to the moulting of birds after the breeding season. I am told that it is very evident in the herring. As yet I am not in a position to speak definitely, but the examination of sections of the skin in a fully grown herring certainly suggests such a possibility. That a replacement should occur seems probable, for in such fish as the herring, in which the scales are so easily removed, it would be but a very natural provision.

It might be urged that even should it be proved to occur, it would not entirely overthrow the view of age-index, since the new scales might begin with the same number of rings as that at which the older ones ceased, after the manner of the antlers in deer. Such a view appears to me improbable, but it can only be settled by accurate investigation.

The conclusions arrived at in this paper further establish the view of a true selachian ancestry for the Teleosteans, as well as affording indications of the affinities existing between the Teleostei themselves. On the other hand, I think it points in the direction of breaking down the separation of the Anacanthini as defined by Johannes Müller, and of which other evidence is not entirely wanting.

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EXPLANATION OF PLATE 6,

Illustrating Mr. H. W. Marett Tims's paper on "The Development, Structure, and Morphology of the Scales in some Teleostean Fish."

Fig. 1.—Surface view of epidermis of G. virens, showing the chromatophores and the openings of the glands.

Fig. 2.—Section through the same.

Fig. 3.—Section through the dermis of G. virens (4·6 cm.) after treatment with acid alcohol.

Fig. 4.—Section through a scale of G. virens (5·6 cm.). From a specimen to which no acid had been applied.

Fig. 5.—View of the external surface of a scale of G. virens (9 cm.).

Fig. 6.—Ditto, G. callarias (2½ feet).

Fig. 7.—Section through scale of G. callarias.

On the Propagation, Structure, and Classification of the Family Sphæromidæ.

By

H. J. Hansen, Ph.D., F.M.L.S.

With Plate 7.

I. Introductory Remarks.

Three years ago H. F. Moore ("Rep. Porto Rican Isopoda," in 'U. S. Fish Comm. Bull.' for 1900, vol. ii, p. 172, 1901) wrote on the Sphæromidæ: "No attempt is made to furnish a key to the genera, owing to the extreme confusion that exists in this family, and it is doubtful if the following two species are properly assigned generically. The dissimilarity of the sexes has frequently misled authors into placing them in widely separated genera, and, while this has not been done in the present case, the limitations of the genera are so indefinitely established that the author has not been able to satisfy himself of the generic affinities of the species described." It may be added that Moore, in reality, refers both his species to genera to which they do not belong. But his critical remarks quoted are correct, and convey an idea on the state of things; other authors have complained in a rather similar way, and the extreme difficulty in arriving at some clearness has probably been felt by every carcinologist who has attempted to name or describe a number of animals belonging to the family Sphæromidæ.

During a stay in Messina and Siracusa in 1893 I collected

especially marine animals of various orders and classes; of Sphæromidæ I gathered a large number of specimens, most of them belonging to the genera Sphæroma (Bosc) and Cymodoce (Leach). In attempting to name the material of Cymodoce, I soon felt that the first thing to be done was to separate the adult males, which proved to belong to three species, then to refer immature males and the females to their respective adult males. The literature could not help me, but, fortunately, the number of specimens of nearly all stages of all species was so rich that the task could be carried through. During this examination I observed that the adult females had neither eggs nor young in the marsupium, but that the brood could be discerned through the skin of the ventral surface of the thorax; the young occupied internal pouches, as had been shown by Leichmann to be the case in Sphæroma rugicauda (Leach). Furthermore, I observed that in the same adult females of Cymodoce the proximal half of the maxillipeds is strongly expanded, forming large ciliated plates not found in immature specimens or males, and that the end of the mandibles is light-coloured, while it is dark-brown or black in other specimens; a subsequent dissection showed that the three anterior pairs of mouth-limbs and the distal half of the maxillipeds in egg-bearing specimens of Cymodoce have been so strongly reduced that the animals cannot eat, while the proximal half of the maxillipeds has been exceedingly expanded; in Sphæroma the mouth-parts are similar in both sexes and in young animals.

These facts and other features were discovered ten years ago, but a publication was postponed. During a stay in London in 1902 I looked through the large collection of Sphæromidæ in the British Museum, wrote numerous notes, and figured some details; most of the specimens examined being types or co-types for species established by Leach, Say, White, Miers, and Haswell, this perusal has been of great importance for my study. The next year I began to work out a revision of the genera of Sphæromidæ. The U. S. National Museum, and especially Dr. Chas. Chilton in New Zealand, favoured

me—as loan or present—with a good number of forms, for which I am most grateful. I have drawn more than a hundred figures, but seeing that further material must be procured, and that for this reason and other obligations, years must pass away before I can finish a more detailed paper, I think a preliminary abstract of the main results, together with brief diagnoses of the genera, and notes on reference of species, may be useful to my fellow-students. Nearly every year new species are described and new genera established; the latter are, in most cases, imperfectly defined, and the species are frequently referred to genera to which they do not belong. Though most preliminary communications—to put it very mildly—contribute more to the swelling of the literature than to advancement of science, I hope yet that this paper may be considered by zoologists as an exception from the rule.

During the preparation of this paper I received further aid from other sides. From the authorities of the Zoological Museum in Berlin I obtained some forms of much interest; Professor E. L. Bouvier, Director of the Entomological Department of the Museum in Paris, lent me an important typical specimen; Mr. A. Viré, the ardent explorer of the cave-fauna in France, has presented me with two valuable forms; Dr. Joh. Thiele, at the Berlin Museum, and especially my friend Dr. W. T. Calman, at the British Museum, answered queries on certain structural features in various animals. I beg the authorities of the Zoological Museums in London, Washington, Berlin, and all the gentlemen named, to accept my sincere thanks for their aid.

The number of forms seen by me is very large. Twenty-eight genera (not counting mere synonyms) have been established by earlier authors; of these I have been able to examine material preserved in spirit of all but three; of one (Ancinus) of these three I saw an exsiccated specimen, and the two genera not seen by me seem to be of slight importance. That I have seen numerous new species is a matter of course; many of them have been inspected, but not being able to give illustrations here, I establish as few as possible,

describing in all only two new species as types for new interesting genera and adding some remarks on an old quite imperfectly known form. For various reasons I cancel two genera; some of those still maintained are of slight value, but I did not think it proper to withdraw more than absolutely necessary. I must establish seven new genera, six of which are types of importance. Most of the species hitherto established are enumerated, but I did not wish to mention every species of Sphæroma and Cymodoce scattered in the vast literature. The enumeration is undertaken in order to refer the species to the genera to which they really belong; a perusal of my notes on such genera as Sphæroma, Cymodoce Næsa, Cassidina will convey an idea of the extreme confusion as to classification in nearly the whole literature. Rather frequently the descriptions—especially when accompanied with figures—of species unknown to me are sufficient for reference, but in several cases this must be doubtful; in too numerous cases—especially when the species in question differ as to shape of the proximal joints of the antennulæ or of the end of abdomen from the type of that genus to which they have been referred in the literature—is it unfortunately impossible to say anything on the real relationship, because figures and especially descriptions are too incomplete.

Only in very few cases titles of papers are given; if such references to literature had been inserted everywhere in the systematic "notes" the bulk of this paper would have been very much increased. The synonymy of several species of Sphæroma and Cymodoce is extremely intricate, and is omitted. It is scarcely necessary to say anything on my treatment of characters and classification; every student who will take the trouble to read the three following chapters and look through the diagnoses of sub-families, groups, sections, and genera may easily perceive the principles of classification. Yet it may be added that in Isopoda—and in other orders of Arthropoda—I dislike a modern tendency manifesting itself in splitting up orders into a very large number of families; wishing to procure a view of the relationships, I collected at

an earlier occasion Cirolanidæ, Ægidæ, etc., as sub-families of the Cymothoidæ (sens. lat.), and to-day I cancel the family Limnoriidæ, referring it as a sub-family to the family Sphæromidæ.

II. On the Propagation.

Even among a very large material of Sphæroma (Bosc) and Cymodoce (Leach) it is next to impossible to find a single specimen with eggs or young in the marsupium, though it is generally easy to find numerous specimens with the marsupium well developed. It is, in my opinion, a testimony of the want of study of the family that this curious feature has been overlooked by all authors excepting Leichmann, who observed and explained it in one species of Sphæroma, but did not examine any other form of the family. I shall now give a very brief abstract of some selected points of Leichmann's paper, adding a few remarks, and then proceed to my own observations on numerous other genera of the family; it may, however, be added that some interesting questions I am certainly able to point out, but, for want of sufficient material, not to solve in any satisfactory way.

Leichmann published a preliminary note in 'Zoologischer Anzeiger' for 1890—the chief paper, "Beiträge für Naturgeschichte der Isopoden," in 'Bibliotheca Zoologica,' 1891. He studied specimens of Sphæroma rugicauda (Leach) gathered near Dantzig. He describes and figures the marsupial lamellæ as so small that the lamellæ from the two opposite sides do not touch each other with their margins. This statement is quite incomprehensible. I have examined specimens of the same species from the coasts of Denmark, even from Vordingborg at the Baltic, and in animals carrying brood the lamellæ from the two halves always overlap each other considerably. An erroneous determination is excluded, as S. rugicauda is the only species of the Sphærominæ known from the Baltic and even from Denmark; furthermore, in S. serratum (Fabr.) and in the other species of the genus in its restricted sense (see below) I have always found the

lamellæ overlapping each other. But Leichmann has made the important discovery that the eggs are enclosed and developed, not in the marsupium itself, but in four pairs of pouches; the openings to these pouches are rather large transverse slits found on the lower surface of thorax at some distance from the mesial line between the sternites, the first pair of slits between the second and third, the last pair between the fifth and sixth sternites. According to Leichmann these pouches are large, elongated, two-branched invaginations of the ventral skin of the animal; they proceed upwards and a little inwards, terminating beneath the tergites near the mesial line. The eggs are laid in the usual way; from the marsupium they must instantly be transported into the internal pouches, because it is impossible to find any specimen with eggs in the marsupium. The eggs are proportionately large, their diameter being ·44 mm., but the young ready for leaving the pouches are exceedingly large, measuring 1·44 mm. in length, ·65 mm. in breadth, and ·22 mm. in depth; the volume of such a young one is therefore between four and five times (Leichmann thinks five times) larger than that of an egg; the mother measures only 5·2 mm. in length and 2·9 mm. in breadth. Leichmann states that the larvæ perform lively movements within the pouches a long time before they leave them, which takes place through the eight slits. He has observed that generally two larvæ slip out, not simultaneously, but shortly after each other; they remain a short time, rarely more than an hour, in the marsupium. But frequently a considerably longer time passes away before the birth of the two next larvæ, so that the entire act takes up some days. This abstract may be sufficient; the question as to the nutrition of eggs and larvæ is omitted in this preliminary paper.

In nearly one third of the genera of the family adult females are unknown to me; of a few genera I have seen only a single female with the marsupium well developed; but, at least without dissection, no brood could be detected. Marsupial plates I have seen in representatives of the two small sub-families, and in all sections of the large sub-family Sphærominæ but

one, viz., Cassidinini. Their number is always three pairs; they belong to the second, third, and fourth pairs of legs. In three genera—Exosphæroma (Stebb.), Isocladus (Miers), and Zuzara (Leach)—all belonging to the hemibranchiate Sphærominæ, they are so small that they are far from reaching each other from the two opposite sides; in all other genera they overlap each other at least somewhat, and generally considerably, or sometimes very much along the mesial line. In the forms with brood of the section Cassidinini seen by me the marsupial lamellæ are wanting; this curious feature is discussed in the sequel.

Of the sub-family Limnoriinæ Limnoria lignorum (Rathke) has been examined. The number of eggs is rather moderate (twenty-nine were found in one specimen); the eggs are enclosed in the marsupium itself. The volume of each full-grown young one is very considerably larger than that of an egg; the marsupium containing such larvæ is accordingly exceedingly distended, more than twice as deep as in a female with eggs recently laid. The marsupial lamellæ are exceedingly large; the marsupium covers the whole lower surface of thorax.

Of the sub-family Plakarthriinæ, a single small female of Plakarthrium typicum (Chilt.) has been examined. The marsupium reaches nearly to the base of abdomen, but its lamellæ overlap each other only very moderately. It contains in my specimen five very large eggs still nearly circular; there is plenty of room for their development in the flat marsupium. Judging from the shape and the biology of the animal, this shape of the marsupium is scarcely much altered during the development of the brood. The third sub-family, the Sphærominæ, present various modes of development of the brood.

Of the hemibranchiate Sphærominæ I have seen adult females of eight genera; of two genera, Hemisphæroma (n. gen.) and Cassidinella (Whitel.), they are unknown, but the former genus is closely allied to Sphæroma (Bosc); Cassidinella seems to be only a sub-genus of Cymodoce

(Leach) and it is therefore most probable that, as to propagation, they agree respectively with Sphæroma and Cymodoce. Sphæroma rugicauda (Leach) is mentioned above; S. serratum (Fabr.) has the same number of pouches with large slits, and all species of the genus in its restricted sense (see below) probably agree closely with each other. I examined a rather large specimen of S. serratum with the young nearly full-grown, being greyish with black eyes; I counted ninety-one young, which occupied by far the largest part of the inner space of thorax and, besides, a good deal of abdomen, as the internal organs of the body, excepting musculature, were scarcely discernible. In the other genera of hemibranchiate Sphærominæ, as in Sphæroma, the brood is developed in internal pouches; but, nevertheless, various deviating features are observed. In Cymodoce pilosa (M.-Edw.) five pairs of large slits—first pair between first and second, last pair between fifth and sixth sternites—are observed; the slits are placed at some distance from the mesial line. Of Bregmocerella Grayana (Woodw.) I have seen two females with the marsupium well developed, and the mouth-parts metamorphosed as in Cymodoce. One of them has no eggs; on the lower surface of thorax I found five pairs of small, very low sub-cylindrical tubercles placed, as are the slits in Cymodoce, at some distance from the mesial line, each tubercle with a minute aperture on the end. In the other female the black eyes of a rather small number of young are visible through the quite membranous ventral skin, on which it is possible, with some difficulty, to find the same thickenings with their central hole. That these tiny apertures correspond with the slits in Sphæroma and Cymodoce is certain, but it is difficult to understand how the eggs can pass in, and quite incomprehensible how the young are able to pass out through them. I suppose that at the birth of the young the skin must split at the apertures, but perhaps some other resource may exist. As mentioned above, the marsupial lamellæ are small and far from reaching each other at the mesial line in Exosphæroma (Stebb.),

Isocladus (Miers), and Zuzara (Leach). In a specimen with marsupium, but without brood, of an undetermined species of Exosphæroma from Victoria, I find, very distant from the mesial line and rather near the base of the marsupial lamellæ, four pairs of low tubercles at the hind margin of second to fifth sternites; each tubercle has a small longitudinal slit at its outer side. Being acquainted with this structure, it was possible with 30 degrees of enlargement to find in Zuzara integra (Hasw.) at least three pairs of nearly microscopical rounded apertures in the same situation as the small slits in the Exosphæroma mentioned, but in some specimens with brood of Exosph. lanceolatum (White) and Isocladus spiniger (Dana) it was impossible to discern apertures with any reasonable degree of certainty, though they must be present. While the structure and the wandering of eggs and young are easily understood in Sphæroma and Cymodoce, the minuteness of the apertures of the pouches in the other genera mentioned is a serious difficulty, perhaps connected with some undiscovered structural feature.

Among the eubranchiate Sphæromine some genera, viz. Scutuloidea (Chilt.), Paracerceis (n. gen.), and Cassidinopsis (n. gen.) have their brood in internal pouches, but the number and position of the apertures has not been examined. Of Dynamene (Leach) (sens. strict)[1] I have seen three females of two European species. The marsupium, which covers the entire lower surface of thorax, is filled either with eggs or with young not arrived at maturity; the marsupial lamellæ, especially the posterior pair, are exceedingly large. The whole arrangement is nearly as in Limnoria lignorum (Rathke); the number and size of eggs and young evidently differ little from those in the last-named species. Næsicopea

[1] Not being able to decide whether Næsa (Leach) or Dynamene (Leach) ought to be used for the European genus, I applied to my friend the Rev. T. R. R. Stebbing, who is specially versed in such questions. He sent me, most courteously, a very detailed exposition, but as he added that he was working on Sphæromidæ, and his results are to be published, I accept his decision that Dynamene must be preferred, and refer the reader to the proofs to be found in his future paper.

(Stebb.) (N. abyssorum [Bedd.]) is so closely allied to one of my European species of Dynamene that the same arrangement is to be expected. In Cerceis (M.-Edw.) (an undescribed species rather allied to C. tridentata (Hasw.) has been examined) the marsupium and the development of the brood is completely as in Dynamene; Haswellia (Miers) is so closely allied to Cerceis that the development is in all probability quite similar.—In Cymodocella a somewhat different arrangement is found; some specimens of C. egregia (Chilt.) have been examined. The marsupial lamellæ are only so long that they overlap each other rather little with their ends. The brood is developed anteriorly in the marsupium, posteriorly in an enormous external pouch; the upper wall of this pouch is the ventral surface of thorax behind the origin of fourth pair of legs, while its lower wall is a rather thin lamella fixed inside the base of the four posterior pairs of legs and in front of abdomen, with its free margin extended between the base of the two legs of fourth pair. That this wall is a folding of the skin from behind goes without mention. At least one half of the eggs or young are found in this pouch; the other portion is covered by the marsupial lamellæ, which also, seen from below, overlap the front part of the wall mentioned. In a female I counted thirteen rather large oblong eggs. From want of females with brood of Amphoroidea (M.-Edw.) and Dynamenella (n. gen.) nothing can be stated on the propagation in these genera.

Of the twelve genera belonging to the platybranchiate Sphærominæ I have been able to study the propagation in only five genera, but these are fortunately representatives for the four sections constituting the group.

Of the section Campecopeini Parasphæroma prominens (Stebb.) has been examined. The marsupial lamellæ overlap each other somewhat at the mesial line; the marsupium is empty, the brood being enclosed in pouches, the entrances to which are longitudinal slightly oblique slits situated at the base of first and second pairs of marsupial lamellæ. As far

as could be ascertained with transmitted light the number of young is very low—about eight; one of them was removed and proved to be large.

Of the section Monolistrini Vireia berica (Fabiani) has been examined. The marsupial lamellæ are very large, but not quite as large as in Dynamene; the brood is formed in the marsupium itself; the eggs are very large, the young nearly ready for birth exceedingly large, and their number very low. The genera Monolistra (Gerst.) and Cœcosphæroma (Dollf.) are so closely allied to Vireia that their propagation is in all probability completely as in the latter genus. Of the section Ancinini, Ancinella profunda (n. gen., n. sp.) has been studied; the structure is nearly as in Cymodocella. An enormous external pouch occupies the lower side of the four posterior thoracic segments; its aperture, which is directed forward, is as broad as the marsupium, and its front end is near the posterior margin of third segment. The space of this pouch is somewhat larger than that occupied by the brood in the marsupium itself. The marsupial lamellæ not only overlap each other very considerably, but also cover about the front half of the wall of the pouch. In one female I found fourteen, in another eleven large oblong eggs.

Of the section Cassidinini I have seen two females with brood and three adult females without brood of Cassidinidea ovalis (Say), besides one specimen with brood of a new species of Leptosphæroma (Hilg.) The structure met with in these forms differs in the most astonishing degree from that observed in any other section, but as it is very difficult to understand and the animals very small my material is insufficient, and I can make out only a part of the features. With transmitted light it is easily seen that the specimen of Leptosphæroma has eight oblong somewhat curved eggs (or rather half-developed young) apparently enclosed in a marsupium, which occupies almost the whole area between the thoracic legs, but is slightly vaulted and not visible from the side, because the lower side of the animal is rather concave; in Cassidinidea the "marsupium" is somewhat more

vaulted than in Leptosphæroma, in the two specimens mentioned with about ten or twelve large half-developed young. In the females of these two genera it is, however, impossible to detect even the slightest vestige of marsupial lamellæ. In Cassidinidea a transverse lobe is observed occupying nearly the area between third and fourth pairs of thoracic legs; its free anterior margin is situated about in the transverse line between the two legs of third pair, while laterally it is curved backwards, originating at the insertion of fourth pair; in Leptosphæroma this lobe is somewhat shorter. This lobe is the front end of the lower wall of an external pouch occupying, as in Ancinella, somewhat more than the posterior half of the lower surface of thorax, but the wall is much thicker than in the last-named genus, in accordance with the fact that it is not overlapped by marsupial lamellæ. The anterior part of the incubatory chamber seems to be a rather similar pouch, which is smaller, closed in front, and without any free lobe behind. But now we come to a serious difficulty. I lifted the free lobe mentioned, which at its base seems to be rather firmly connected with the posterior margin of the lower wall of the front part of the incubatory chamber; I could not with any certainty discover apertures in the junction between the two walls, but pulling more vigorously on the free lobe, the junction named was broken, and a broad entrance to the incubatory chamber was formed. The posterior half of this chamber is a pouch formed as in Cymodocella and Ancinella, but what may the anterior half be? Is it formed by a folding of the skin from in front backwards—as the posterior half is formed by folding in the opposite direction—or by the fusion of the marsupial lamellæ with each other and with the lower surface of thorax along the insertions of the legs? I think the first alternative to be the right interpretation, but I cannot understand the fact that the posterior margin of its wall seems to be connected with the upper surface of the lower wall of the posterior pouch at the base of the free lobe. The animals examined are very small, and my material quite insufficient

for solving the problem ; I suppose, however, that the same structure is found in Chitinopsis (Whitelegge) and in Cassidina typa (M.-Edw.), and the latter form being comparatively large, a study of a rich material of females in various stages will be the best material for a future study of the anomalous and interesting mode of construction of the incubatory chamber in the section Cassidinini.

The perusal of the preceding pages will convey an idea of the astonishing variation met with not only in the family Sphæromidæ but even in the sub-family Sphærominæ as to the structure of the chamber for the development of the brood. Let us give a brief abstract. In some genera, as Limnoria, Dynamene, and Vireia, the room is formed only by the usual lamellæ, which are very or exceedingly large; in Plakarthrium the same arrangement is found, but the lamellæ are of moderate size. In Sphæroma and Cymodoce the brood is developed in four or five pairs of pouches proceeding into the animal and opening with rather long transverse slits at some distance from the mesial line, while the marsupial lamellæ overlap each other; in Bregmocere la we find the same arrangement, but the openings of the pouches are minute; in Exosphæroma, Isocladus, and Zuzara the marsupial lamellæ are small and far from reaching each other at the mesial line, while the apertures of the inner pouches are small or minute, situated near the base of the lamellæ, or even impossible to discover. In Parasphæroma two pairs of apertures of internal pouches are longitudinal slits at the base of the lamellæ. In Cymodocella and Ancinella the major posterior part of the incubatory chamber is formed by a single external exceedingly large pouch with a very broad aperture directed forwards, while the anterior part of the chamber is formed by the marsupial lamellæ. In Cassidinidea and Leptosphæroma the marsupial lamellæ are wanting and the chamber is formed by a posterior and an anterior external pouch united with each other.

But the structure is still more complicated and varied. In the following chapter it is shown that in a little more than

two thirds of the genera the mouth-parts are similar in both sexes and in immature specimens, but in nearly one third of the genera the adult females have the basal half of the maxillipeds exceedingly expanded, being adapted for producing a current of water through the marsupium, while the distal part of the same appendages and all other mouth-parts are strongly reduced. One is apt to suppose that this metamorphosis must be associated with one of the modifications of the incubatory chamber, but it is far from being so. Some instances may be enumerated. Vireia and Dynamene have a normal chamber formed only by the very large lamellæ, but the mouth-parts are normal in the females of the former, exceedingly metamorphosed in those of the latter genus. Sphæroma and Cymodoce have marsupial lamellæ of the same size, but in the former genus the mouth-parts are normal, in the latter metamorphosed. The metamorphosis or non-metamorphosis of the mouth-parts is, on the contrary, connected with and even dependent on the shape of the end of abdomen, as will be shown in Chapters III and V.

III. Metamorphosis of Mouth-Parts in Females of Several Genera.

In all genera the mouth-parts in adult males and immature specimens of both sexes of the same species are always completely alike. In the sub-families Limnoriinæ and Plakarthriinæ and in the major part of the genera of the sub-family Sphærominæ the mouth-parts in females with brood are similar to those in the males, but in some genera the mouth-parts in such females are metamorphosed in a very peculiar way. In Limnoria (Leach), Sphæroma (Bosc), Exosphæroma (Stebb.) Isocladus (Miers), Zuzara (Leach), Cymodocella (Pfeff.), Cassidinopsis (n. gen.), Parasphæroma (Stebb.), Vireia (Dollf.), Cassidinidea (n. gen.), Leptosphæroma (Hilg.), Ancinella (n. gen.), and Plakarthrium (Chilt.), the mouth-parts of females carrying eggs or young are—according to my investigations—shaped as in immature speci-

mens or males; of four other genera, viz. Dynamenella (n. gen.) Amphoroidea (M.–Edw.), Campecopea (Leach), and Tecticeps (Richardson), I have seen females with the marsupium well developed but no brood was perceived, and in all the mouth-parts did not deviate from those in the males. I venture to state that among the genera of which females with brood or marsupium are unknown to me, at least Hemisphæroma (n. gen.), Monolistra (Gerst.), Cæcosphæroma (Dollf.), Cassidina (M.–Edw.), Chitinopsis (Whitelegge), and probably Spelæosphæroma and Ancinus (M.–Edw.), have the mouth-parts similar in males and in females with brood. Of Cymodoce (Leach), Cilicæa (Leach), Cilicæopsis (n. gen.) and Bregmocerella (Hasw.), Dynamene (Leach), Paracerceis (n. gen.), and Cerceis (M.–Edw.) the females carrying brood have the mouth-parts metamorphosed; I have examined at least one species of each of these genera, of some genera two, three, or more species, always with the same result. I am confident that in Cassidinella (Whitelegge), Næsicopea (Stebb.), and Haswellia (Miers), the female mouth-parts will in the future be found to be altered in the same way.

Let us now look at the differences between the mouth-parts of an egg-bearing female and a male (or an immature specimen) of one of the European species of Cymodoce. In the male the major distal portion of the incisive process of the mandibles (fig. 1 *a*) is dark brown or black, lacinia mobilis is well developed, with a plate on the left mandible, the molar process is thick and moderately long (fig. 1 *b*). In the egg-bearing female the incisive process is rounded and yellowish, which shows that it is less hard, lacinia mobilis has disappeared (fig. 2 *a*), while the molar process is very low, scarcely developed, and without equipment for trituration. The female maxillulæ (fig. 2 *b*) have been altered in a corresponding way; the distal half of the inner lobe is much narrower than in the male (fig. 1 *c*), its end rounded and the stiff setæ lost; the outer lobe has gained a number of fine hairs, but its end is rounded and of the strong terminal spines at most a rudi-

ment and generally nothing remains. The lobes of the maxillæ (fig. 2 *c*) have lost all their numerous setæ found in the male (fig. 1 *d*) and in immature specimens, and the bifid outer lobe has been shortened. Besides, all these mouth-parts have the muscles considerably or much reduced; but the muscles to the mandibular palps, still shaped as in the males, have been preserved. The hypopharynx has been very reduced (fig. 2 *e*), being only about half as large as in the male (fig. 1 *f*). The maxillipeds are still more interesting; in the female with brood (fig. 2 *d*) the four distal joints have been reduced in size, especially the lobes are much shorter and have lost all the setæ found in other specimens (fig. 1 *e*); the lobe from second joint has lost its distal setæ, but the two proximal joints with the epipod are, on the contrary, expanded to such a degree that their joint surface is between twice and three times larger than in the male of the same size; some of the muscles in the palp have been reduced in size and all are lighter in aspect, while the musculature moving the expanded proximal portions is well developed. As in Cymothoidæ the first joint of the female maxillipeds has a thin free ciliated plate directed backwards; the second joint is shorter than in the male, but much expanded outwards, and the free outer margin furnished with long plumose setæ not found in the other sex. We can therefore not say that the mouth-parts as a whole have been reduced in adult females; the proximal half of the maxillipeds has, on the contrary, been developed as a special instrument for producing a current of water through the marsupium, while the distal half of the maxillipeds and the outer mouth-parts, the mandibular palps excepted, have been strongly reduced, and are even unfit for use. The direction of the current must, of course, be observed in living animals; judging from various reasons, I am, however, convinced that it goes from behind forward.

The genera in which the females with brood have the mouth-parts metamorphosed are enumerated above. The alterations are essentially as in Cymodoce, but it must be men-

tioned that in Cerceis and especially in Dynamene (Næsa) bidentata (Mont.) I find the differences between mouth-parts in adult females (figs. 4 *a*–4 *e*) and other specimens (figs. 3 *a*–3 *d*) still more astonishing. In both genera more than the proximal half of the lower outer surface of the female mandibles is so completely fused with the skeleton of the head that even a suture cannot be detected when the mandible with the adjoining firm portion of the head is taken out and examined under the microscope, while the outer margin itself of the mandible protrudes above the skeleton mentioned and is indicated on figs. 4 *a* and 4 *c* by dotted lines. Furthermore, the distal half of the mandible has not only lost every vestige of an incisive dark-coloured part, lacinia and molar process, but it shows a very different shape (figs. 4 *a* and 4 *b* as compared with fig. 3 *a*), being distally rounded, with fine and short hairs at the margin. Maxillulæ and maxillæ have not only lost all setæ or spines, but have been much reduced in size (figs. 4 *c* and 4 *d* as compared with figs. 3 *b* and 3 *c*). The maxillipeds (fig. 4 *e*) have the expansions from epipod and from first and second joints much larger than in Cymodoce, while the lobe from second joint has been strongly reduced in size, the joints of the palp somewhat reduced but yet with some short setæ on the lobes.

It is easy without dissection to perceive whether the maxillipeds of an egg-bearing female belonging to this family have been altered or have preserved their normal size and shape. The question whether the mouth-parts have been metamorphosed can generally be decided without difficulty by looking at the end of the mandibles, whether they are very dark or yellowish. But an anomaly must be mentioned here. Of ten females with marsupium of Cymodoce pilosa (M.-Edw.) eight had all their mouth-parts altered as described above, but in two specimens the curious feature was observed that the maxillipeds and maxillæ had been completely metamorphosed, while the alterations in the two anterior pairs of appendages were less complete. In one of these specimens the end of the mandibles had kept their dark colour and the

outer lobe of both maxillulæ their spines, while lacinia mobilis, etc., had disappeared; in the other specimen only a little of the dark colour on the end of the mandibles and the spines on one of the maxillulæ were preserved.

Giard and Bonnier have shown that in the Bopyrinæ the females have the first joint with its epipod and second joint of the maxillipeds strongly expanded and adapted for producing a current of water. Schiödte and Meinert pointed out that in the Æginæ (Æga, Rocinela) the marsupial plates cover the entrance to the mouth, so that egg-bearing females cannot take any nourishment; females with marsupium have never been found on fishes, but are not uncommonly captured with dredge or trawl. In 1890 the present author showed that in the Æginæ and in all other Cymothoidæ, sens. lat. (Cirolana, Corallana, Æga, Nerocila, Cymothoa, etc.) the adult females have the two proximal joints—with the epipod—of the maxillipeds strongly expanded and evidently adapted for the same purpose as the corresponding part in female Bopyrinæ, but in no form any real reduction of the other mouth-parts was observed. In several genera of Sphæromidæ we have a similar expansion of the proximal half of the maxillipeds, but their distal half and all the other mouth-parts are reduced in a most peculiar way, and so strongly that the animals cannot take any food at all. Such metamorphosis of the mouth-parts in females carrying brood is, as far as I know, without parallel, not only among other Arthropods, but among animals of every other series.

Finally, there is the question as to the systematic value and biological bearings of this metamorphosis. In Limnoriinæ, Plakarthriinæ, and probably in all platybranchiate Sphærominæ (I have examined females with brood of representatives for the four sections constituting this group) the mouth-parts are similar in both sexes; in all these animals the end of abdomen has either a rather shallow notch (Plakarthrium) or a notch not visible from above (Campecopea) or, generally, no notch. The hemibranchiate Sphærominæ are naturally divided into two sections, Sphæromini and Cymodocini; in

Sphæromini the females have no notch at the end of abdomen and the mouth-parts normal as in the males, while in Cymodocini the same sex has a distinct, most frequently bilobed notch at the end of abdomen, and the mouth-parts metamorphosed; it may be added that no other distinguishing character between the two sections could be discovered. In the eubranchiate Sphærominæ the case is more difficult. In this group the end of abdomen is a little emarginate in one genus, Cassidinopsis (n. gen.), in all other genera furnished with a notch of very different shape; in some of the genera the female mouth-parts are normal, in others highly metamorphosed. Nevertheless, there is evidently a connection between the presence of metamorphosis of the mouth-parts and the development of the abdominal notch. In the female Dynamene bidentata (Mont.), and especially in another species of the same genus, a species constituting a transition stage to Næsicopea (Stebb.), the mouth-parts are metamorphosed and the abdominal notch very deep and looking much upwards (it is, besides, widened at the bottom and very constricted in the distal part); in Cerceis (M.-Edw.) the notch is rather deep and turned upwards, in Paracerceis (n. gen.) moderately large and deep and turned backwards, but the end of abdomen is somewhat produced. In both these genera the mouth-parts are metamorphosed. In Scutuloidea (Chilt.) the notch is less deep than in the preceding genera, in Cassidinopsis emarginata (Guér.) only a rather slight emargination is found; in both these genera the mouth-parts are normal; in a female of Amphoroidea falcifer (Thoms) with the marsupium well developed, but without brood, the mouth-parts are normal and the notch as in Scutuloidea. Difficulties are found in Cymodocella (Pfeff.) and Dynamenella (n. gen.); in the former genus the notch is very well, though peculiarly, developed, and the mouth-parts normal; in Dynamenella the female notch is about as in Paracerceis, but the mouth-parts seem to be normal. For want of material I cannot further prosecute this topic, but in spite of the difficulties mentioned it can be stated that in the

genera with the notch rather feebly or very feebly developed the mouth-parts are normal, in the genera with the notch rather deep or very deep and looking upwards the mouth-parts are metamorphosed, while in a few genera with the notch looking essentially backwards and at least of moderate depth the mouth-parts vary as to the feature in question. Considering the whole family, we arrive at the result that in all forms with the abdominal notch shallow or wanting in the females the mouth-parts are not metamorphosed; in the large majority of forms with the notch well developed, and in all forms having either a rather deep or very deep notch looking essentially upwards, or a notch divided by a mesial process, the mouth-parts are metamorphosed; while only at most two genera with the notch well developed remain as being—at least for the present—apparent exceptions from the rule. Some remarks on the significance of the notch and on the remarkable connection between the shape of the end of the abdomen and the development of the mouth-parts in egg-bearing females are set forth in Chapter V.

IV. Sexual Differences.

In most genera the adult males are larger, sometimes even much larger, than the females, in some nearly of the same size; in Cassidinidea ovalis (Say) I have found the ovigerous females larger than an adult male. Of Plakarthrium typicum (Chilt.) I have seen several specimens of very different sizes from the same locality; among the smaller specimens I found an adult male and a female with the marsupium complete, while a considerably larger specimen had rudimentary marsupial lamellæ.

The adult males of all genera, Dynamene (Næsa) (Leach) and Ancinella (n. gen.) excepted, possess an oblong or very elongate, generally narrow, flat stylus, the "appendix masculina," proceeding from the inner margin—either near its base or sometimes at the end—of the endopod of plp.^{2}; [1]

[1] On the following pages some abbreviations are generally used, viz. plp.^{1}, plp.^{2}, plp.^{5}, for first to fifth pairs of pleopods, endp. for endopod, exp. for exopod, urp. for uropods.

this stylus is in reality (compare my paper on the "Asellota," 1905) the second joint of that endopod. On endp. of plp.[1] no trace of an auxiliary stylus is found; in Campecopea hirsuta (Mont.) I found a short process, not marked off by articulation, proceeding from endp. of plp.[3] near its end, while the appendix on plp.[2] is exceedingly long, and originates at the base of endp. Of three European species of Dynamene (Næsa) (Leach) I have inspected in all several adult males, but in none of them an appendix masculina was found, and the inner margin of the endopod of plp.[2] is simple, not thickened. In adult males of Ancinella profunda (n. gen., n. sp.) no appendix masculina is found, but the inner margin of endp. of plp.[2] is considerably thickened, with a longitudinal groove on the inner side of this thickening; in the female this margin is of normal inconsiderable thickness without any groove.

At least in the sub-family Sphærominæ, the appendix masculina does not appear before the animals are nearly full-grown, but it is easy by another character to distinguish males even when not half-grown from immature females. As is known, the males have two processes close together on the seventh thoracic sternite; these processes, which are tubes containing the terminal portion of the ducts from the genital organs, are sometimes rather short (Tecticeps), sometimes rather long (Dynamene), very long (Cymodoce pilosa), or even exceedingly long (Dynamenella bermudensis); they are found in all genera. Of Cymodoce pilosa (M.-Edw.) I collected at Siracusa a rich material consisting of both sexes in very different size and age; an unusually small adult male measures 10·7 mm., the largest male 15 mm. in length, but in numerous immature males measuring from 9·7 to 13·7 mm. no vestige of the appendices on endp. of plp.[2] can be found, while the processes at seventh thoracic sternite are shorter than in the adults, but yet very distinct. The marsupial lamellæ are mentioned above. The length of flagellum of antennulæ and antennæ in the two sexes has not been specially examined, but at least sometimes differences are well marked.

In several genera, viz. Sphæroma (Bosc), Cymodocella (Pfeff.), Scutuloidea (Chilt.), Amphoroidea (M.-Edw.), Cassidinopsis (n. gen.), Cassidinidea (n. gen.), Leptosphæroma (Hilg.), Limnoria (Leach), and Plakarthrium (Chilt.), there are at most rather slight sexual differences in shape of thorax, abdomen, thoracic legs or uropoda; but in some of them the males are larger than the females. In other genera, as Isocladus (Miers), Zuzara (Leach), Cymodoce (Leach), Cilicæa (Leach), Ciliacæopsis (n. gen.), Bregmocerella (Hasw.) Dynamene (Leach), Paracerceis (n. gen.), adult specimens of the two sexes differ exceedingly from each other in various respects; the males are distinguished by processes on sixth or seventh thoracic segments or on the first portion of abdomen, shape of uropoda, frequently shape of the end of abdomen, etc., in Bregmocerella even processes on the head. Leach established some genera on adult males, referring the majority of females and young specimens to Sphæroma or Dynamene, the latter of which was established exclusively on such specimens. Similar confusion is still found in papers published in the last six years. In 1873 Hesse stated the species of Sphæroma are female of Cymodoce, Dynamene females of Næsa. As to the European forms of Dynamene it is quite correct (exotic forms referred to Dynamene cannot remain in this genus), but regarding Sphæroma the case is more complicated; among the European forms referred to the latter genus, those without terminal notch are well-founded species—with males and females—of Sphæroma itself, while those possessing an abdominal notch are females or young males of Cymodoce or other genera. Miers has correctly referred females and males of some exotic species of Cymodoce, but he did not undertake a special study of the family. It is scarcely necessary to give here a detailed account of the sexual differences alluded to in these genera; the notes in the systematic chapters may be sufficient. But one thing must be added. At Sicily I collected a rich material of three species of Cymodoce; while the adult males were not difficult to separate, it was only after a

prolonged examination that I could separate females, and especially half-grown specimens of the three species; the males will be far from easy to describe and figure well, but the specific characters in immature forms and females will be very difficult to describe and figure, so that even a careful student may be able to determine specimens when he has only a small material at his disposal. I should advise carcinologists not to establish species on females or immature specimens belonging to Cymodoce, Cilicæa, Cilicæopsis, Dynamene, Dynamenella, or Paracerceis if males be not at hand from the same locality.

In some genera, as Cerceis (M.-Edw.) and Dynamenella (n. gen.), the shape of the abdominal notch differs generally very considerably in adult males, immature males, and females. In Parasphæroma (Stebb.) there is a marked sexual difference in thickness and equipment with hairs and spines of some of the joints of second and third thoracic legs (a more detailed description is found in Chapter VII). In Monolistra (Gerst.), Vireia (Dollf.), and probably the other genera of the section Monolistrini the second thoracic legs are simple in the females, while in the males they terminate in a prehensile hand. In the section Ancinini (Ancinus) (M.-Edw.), Ancinella (n. gen.), Tecticeps (Richardson), the second legs are simple in the female, and terminate in a prehensile hand in the male, but in one of these genera, Tecticeps, we find besides a remarkable sexual difference in length and shape of sixth joint in seventh thoracic legs, and differences in the end of abdomen, length of exp. of urp., etc.

V.—Remarks on Structural Features and Characters.

The head.—While in Cirolana and many other genera of Cymothoidæ (sens. lat.) a frontal plate is very distinct and well marked off from clypeus, we find in Sphæromidæ only one plate, which has been named "epistome." This epistome is always broad behind, its posterior margin at least conspicuously and frequently so strongly concave that a deep rounded incision is formed; in such cases the posterior part

of the epistome encompasses the anterior half of labrum. In several genera the anterior part of the epistome protrudes as a plate or a process in front of the margin of the head. The epistome is generally well marked off from the front mesial triangular end of the upper surface of the head, but in Ancinus (M.-Edw.) both are completely fused.

The peduncle of the antennulæ is always three-jointed; the two proximal joints afford sometimes generic differences. Flagellum of antennulæ and antennæ show differences of minor importance. The mouth-parts are rather reduced in Plakarthriinæ (see the diagnosis of this sub-family); in all other forms they are well developed. In the small section Ancinini the mandibles are without molar process; in Limnoria besides lacinia mobilis is at most rudimentary; in all other genera both lacinia mobilis and the molar process are well developed, but lesser differences are observed. Maxillulæ and maxillæ are uniform; the maxillipeds vary much in relative length and breadth of second and following joints, and in length of the lobes frequently proceeding from fourth, fifth and sixth joints. But excepting the few genera mentioned the mouth-parts in this rich family are so uniform that descriptions of their shape in various genera are nearly worthless if not accompanied with numerous figures. The most important features are mentioned below in the diagnosis of the family, the sub-families, the section Ancinini, and the genus Hemisphæroma. The metamorphosis of the mouth-parts in the females of several genera is treated in Chapter III.

The thorax.—It is a feature probably unique among Isopoda that in Plakarthrium the so-called epimera are developed as movable plates not only on the six posterior segments—which also is the case in Limnoria—but even on the first segment. The fusion of these plate-shaped joints of the legs with their segment in the Sphærominæ needs no special mention.

In most genera the legs are uniform as to main points; the seven pairs of the same animal and the corresponding

pairs in various forms show numerous minor differences as to relative length and thickness of joints, equipment with hairs, etc., but the differences must be exhibited in figures. Three or four legs from the same side representing the essential deviations found between the pairs of the same animal ought to be selected for illustrations to be done with the same degree of enlargement; the same legs from the same half of different animals must be drawn so that if, for instance, the seventh left leg of one species is seen from below (from in front), this leg of all the other animals ought to be shown from the same side. The most interesting differences in the legs shall be enumerated here. In Amphoroidea typa (M.-Edw.) the three anterior pairs are slender, but especially the three following pairs exceedingly thick and short; in A. falcifer (Thoms), the difference is not so highly developed, but still remarkable. In Sphæroma (Bosc.) and Hemisphæroma (n. gen.) the three anterior pairs are equipped with very long, stiff, plumose notatory setæ on the outer side of some joints; this feature I have not observed in any other genus. In the three genera constituting the section Ancinini the first pair terminates in a robust prehensile hand, the sixth joint being much thickened, and the seventh with its claw folded back along the lower margin of the sixth, quite as in numerous Amphipoda. The sexual difference found in the legs in Parasphæroma (Stebb.) and the genera constituting the sections Monolistrini and Ancinini are mentioned in the preceding chapter.

The abdomen.—In Limnoria all six abdominal segments are free and movable, in Plakarthrium all are fused with each other. But some difficulty is met with as to the Sphærominæ. In all forms of this sub-family (Vireia burgunda (Dollf.) and Cœcosphæroma (Dollf.) excepted) the abdomen consists of two movable parts, and the question arises as to the number of segments constituting each part. But a comparison of the two posterior segments and the articulation between them in Limnoria with the structure in Sphæroma gives the result that in the latter genus the

posterior part of abdomen consists of only one segment, the sixth; the anterior part must consequently correspond with the five anterior segments in Limnoria. In Sphæroma, Cassidinopsis, and numerous other genera, this anterior part has on the upper surface three sutures as rudiments of division into segments; the anterior of these sutures is entire, the two other completely vanished at the middle. Four segments are thus traceable, but as the part corresponds with five segments we must conclude that one segment, perhaps the first, has completely disappeared. In Vireia burgunda (Dollf.) (but not in V. berica (Fabiani)) and in Cœcosphæroma Virei (Dollf.) the two parts of abdomen are immovably fused with each other.

The pleopods are mentioned by various authors in the descriptions of some genera or species; it has been observed that the five pairs of an animal are not similar and that, for instance, fourth and fifth pairs are not uniformly built in all forms. But no author has undertaken a real comparative study of these appendages, which in reality afford characters, not only for genera, but for groups of genera; the omission of this study is a principal reason, not only for the complete want of grouping of the numerous genera, but for a good deal of the confusion as to the reference of species to genera. In the following I use the most important differences in the pleopods as characters in the diagnoses of the sub-families, and especially as the base for dividing the Sphærominæ into groups of genera; other differences are used in establishing sections of genera or in the analytical keys, sometimes even in the diagnoses of genera. In this paper I omit here a more detailed account of these appendages, thinking that a perusal of the diagnoses in the next chapter may convey sufficient knowledge of their structure and the numerous differences observed. It may, however, be added that, for instance, the thickened areas or real protuberances—clothed with spines—on the exp. of plp.[5] in almost all Sphærominæ afford more characters than those mentioned in the following treatment; further elucidation of this and other topics must be postponed to the illustrated paper.

It is well known that the end of abdomen is shaped very differently in the genera. In Sphæroma, Hemisphæroma, and the section Monolistrini the posterior margin of abdomen is broadly rounded, without trace of longitudinal excavation below or of any terminal notch. In other genera the lateral walls of the terminal part of abdomen are bent less or more downwards and sometimes even a little inwards, so that the lower side shows a longitudinal excavation (Isocladus), and when in this case the end of abdomen is cut off we have the dorsal half of a kind of tube (Ancinus). In Cymodocella the distal lateral walls mentioned are so strongly curved that their lower margins touch each other below in the mesial line; the lower distal surface of abdomen is in this case the inner wall of a tube formed by that curvature, and the tube terminates behind in a nearly circular aperture. In other genera the end of abdomen has a real notch; sometimes this notch is very deep, its distal portion narrowed, being only a linear slit, while the proximal part is a rounded or transverse foramen. Such differences have been seen and described by all authors; they have generally been used as specific characters, but they are always of generic value; nobody seems to have noticed that the want of a notch or the essential shape of the notch is of importance as to the biology of the animal. The best instances are the genera Leptosphæroma (Hilg.) and Plakarthrium (Chilt.). In these forms the uropods surround the end of abdomen; the animals are very depressed, with the lower surface concave, the outline continuous, and all parts participating in forming the outline are much expanded. According to Chilton Plakarthrium typicum (Chilt.) lives on the seaweed Eklonia radiata, "to which it closely adheres." Both genera are evidently adapted for clinging closely to firm and flat or regularly rounded surfaces just as is a female Coccus on a Nerium. The end of abdomen terminates in Plakarthrium in a notch; in Leptosphæroma the most distal small portion of abdomen is turned somewhat upwards and has a longitudinal groove below; in both genera a small aperture is thus formed

between the terminal abdominal margin and the uropods, with the result that the animals can live closely clinging to a firm body, and by movement of the pleopoda produce through that aperture a current of water to the rami adapted for respiration.

Næsicopea abyssorum (Bedd.) has a round foramen on the end of a protuberance considerably above the posterior margin of abdomen, and this foramen is the upper part of an exceedingly deep incision or transformed notch, but the major distal and lower part of this incision looks like a suture in the mesial line to the lower margin of abdomen. The result of this structure must be that this rather large animal can walk on very soft muddy bottom with the lower margin of abdomen touching the mud, but yet get pure water through that foramen to the branchiæ; according to Beddard the two specimens known were taken in a depth of 1070 fathoms, and the bottom was "blue mud." In a species of Dynamene from the Mediterranean I find about the same: a foramen on the end of a protuberance above the end of abdomen, but the distance between this end and the foramen is proportionately shorter than in Næsicopea. In some forms (Dynamenella, Cerceis, etc.) there is considerable difference in the shape of the notch in the two sexes, which suggests that some difference in the biology of the sexes may exist. Hemisphæroma pulchrum and all species of Sphæroma have no trace of a notch, and the posterior margin of abdomen is broadly rounded, but in these forms the three anterior pairs of thoracic legs are furnished with very long and stiff natatory setæ not met with in any other genus; their habits are, therefore, probably more natatory than those of other marine genera; they can easily get pure water to the branchiæ from below, which agrees with the total absence both of notch and of groove on the lower distal part of abdomen. According to all these examples (Plakarthrium, Leptosphæroma, Næsicopea, Dynamene, Hæmisphæroma, Sphæroma) we must assume that the shape of the end of abdomen is an important feature, being developed in various ways according

to the normal habits of the forms and the quality of bottom on which they live.

In Chapter III it is shown that, speaking broadly, the mouth-parts of egg-bearing females are almost always metamorphosed in the genera possessing a well-developed notch, while they are generally normal in all other forms. Having now shown the use of the notch, it is possible to understand a part of that curious connection between the mouth-parts and the shape of abdomen in the females with brood. When a notch is deep and especially when it turns much upwards the nature of the habitat offers hindrances to an easy supply of water to the branchiæ and from thence to the brood in the marsupium or the pouches; in this case the proximal half of the maxillipeds is developed as an auxiliary instrument for bringing fresh water to the brood, while in the other forms the current is produced only by the movements of the pleopods. These statements support strongly the assumption set forth above (p. 84) that the current produced by the maxillipeds runs from behind forwards. One remarkable feature remains, viz. that when the proximal half of the maxillipeds is strongly expanded all the other parts of the mouth are reduced, but this I cannot explain.

VI. Classification.

The family Sphæromidæ is more allied to Cymothoidæ (sens. lat.), and especially to Serolidæ than to any other family of Isopoda; in the following characterisation generally only those characters are inserted by which it is distinguished from the two other families. The diagnoses of the sub-families are as complete as possible. The three "Conspectus" of the sub-families are analytical only to a certain extent, because it has been the intention to give all essential characters for subdivisions of every degree and to avoid unessential particulars.

Characterisation of the Family Sphæromidæ.

Head with a well-developed epistome, not divided into frontal plate and clypeus, and rarely fused with the upper surface

of the head. Peduncle of antennulæ three-jointed, of antennæ five-jointed. Mouth-parts, biting or gnawing, never really suctorial; second joint of maxillipeds at least in males and immature specimens without external expansion; mouth-parts in females with brood rather frequently strongly metamorphosed and useless for nutrition. Thoracic segments seven, all free; marsupial lamellæ only on second, third, and fourth "epimera," rarely wanting (section Cassidinidi). All pleopods lamellar; all endopods, and at least the exopods of first and second pairs unjointed; at least both rami of plp.[1] and plp.[2] fringed with long plumose setæ, and at least in all two rami of the posterior pairs (both rami of plp.[5] or the endp. of plp.[4] and plp.[5]) without such setæ, and specially adapted for breathing. Sixth segment large. Uropods with the rami unjointed, these, at least in the females, generally depressed, sometimes one of them wanting; in Vireia the uropods are wanting. The body can be rolled more or less completely into a ball or can be folded.

The family is divided into three sub-families:

1. Limnoriinæ.—Mandibles stout; lacinia mobilis at most rudimentary, without plate on the left mandible; molar process wanting; palp three-jointed. Maxillulæ with the inner lobe well developed; maxillæ with the three distal lobes very short, but yet well developed. Maxillipeds with a single hook on the lobe from second joint; epipod large, longer than broad. Epimera not marked off from first thoracic segment; second to seventh epimera a little movable. Abdomen consists of six movable segments. Plp.[3] and plp.[4] have both rami furnished with long plumose marginal setæ, as have also plp.[1] and plp.[2]; rami of plp.[5] without marginal setæ, respiratory; exp. of plp.[5] without squamiferous areas or tubercles. Endopod of urp. movable. (The brood in the marsupium itself; no sexual difference in the mouth-parts.)

2. Sphærominæ.—Mandibles, at least their basal half, stout; lacinia mobilis well developed, with plate on left mandible; molar process generally well developed (wanting in the section Ancinini); palp three-jointed. Maxillulæ with

the inner lobe moderately or, generally, very well developed; maxillæ with the three distal lobes moderately long. Maxillipeds with a single hook on the lobe from second joint; epipod very small, broader than long, or not discernible. Epimera not marked off from first thoracic segment; second to seventh epimera immovably fused with their segments, but generally some of them marked off by very fine, or nearly inconspicuous, furrows or lines. Five anterior abdominal segments completely fused with each other, but, on the upper surface, transverse furrows—at most three and the two posterior broadly interrupted at the middle—are generally seen as traces of divisions into segments. Last segment generally movable (immovably fused with the preceding part in Vireia burgunda and Cœcosphæroma Virei). Rami of plp.^{5} without plumose marginal setæ; endp. of plp.^{4} generally without setæ, in rare cases with a few short plumose setæ, at least endp. of plp.^{4} and plp.^{5} respiratory; exp. of plp.^{5}, generally with some—at least three—thickened areas or protuberances densely clothed with minute scale-like spines (in Ancinella without spines, in Tecticeps wanting). Endp. of urp. fused with the sympod, or wanting. (The brood most frequently develops in pouches; mouth-parts in ovigerous females often strongly metamorphosed.)

3. Plakarthriinæ. — Mandibles very slender; lacinia mobilis well developed, with plate on left mandible; molar process wanting; palp rudimentary, one-jointed. Maxillulæ with the inner lobe rudimentary; maxillæ reduced, showing only a narrow oblong plate terminating in three spines and some setæ. Maxillipeds without any hook on the lobe from second joint; epipod not discernible. All seven pairs of thoracic epimera movable, large. Abdomen has all segments fused together, on the surface two interrupted furrows as rudiments of division. Exp. of plp.^{3}, plp.^{4}, and plp.^{5} pellucid, scarcely respiratory, with numerous plumose setæ along their distal margin; endp. of the same three pairs opaque, respiratory, without marginal setæ; exp. of plp.^{5} without squamiferous areas or protuberances. Both rami of urp. movable.

(The brood in the marsupium itself; no sexual difference in the mouth-parts.)

It may be preferred first to deal with the genera of the two very small sub-families before proceeding to the rich sub-family, the Sphærominæ.

Sub-family Limnoriinæ.

Only one genus is known, the diagnosis of which may be as follows: Antennulæ and antennæ very short, freely protruding, their proximal joints not fitting in excavations on the head. Endp. of plp.[1] more than three times longer than broad; exopods of all pleopods unjointed. Last abdominal segment with the posterior margin equally rounded, without terminal notch. Urp. with exp. much shorter than endp.

Limnoria (Leach).

Sub-family Plakarthriinæ.

This sub-family is established on a single genus, the diagnosis of which is given here. Two proximal joints of each antennula, and third and fourth joints of the antennæ exceedingly expanded in front, with their anterior margin cut off. All thoracic legs simple. Endp. of plp.[1] nearly four times longer than broad; exopods of all pleopods unjointed. Abdomen terminates in a nearly semicircular notch. Head and abdomen quite excluded from partaking in forming the outline of the animal; this outline is continuous, regularly oval, formed exclusively by the front margin of first and second joint of the antennulæ, third and fourth joints of the antennæ, the outer margin of the thoracic epimera, and the distal margin of the uropods. Animals very depressed, the lower surface concave.

Plakarthrium (Chilton) (Chelonidium (Pfeffer)).

Sub-family Sphærominæ.

This rich sub-family is divided into three sharply defined groups.

(A) Sph. hemibranchiatæ: Plp.[4] and plp.[5] have the

endopods thick, of fleshy aspect, with deep, essentially transverse folds, the exopods submembranaceous and rather pellucid, two-jointed; both rami of both pairs without plumose marginal setæ; exp. of plp.[5] has the subapical squamiferous protuberance on the lower surface very high. Plp.[3] have both rami closely set with long plumose setæ, at least on the distal margin. Endp. of plp.[1] at least rather broad, scarcely ever half again as long as broad.

(B) Sph. eubranchiatæ: Plp.[4] and plp.[5] have both rami subsimilar, with deep, essentially transverse folds, often of fleshy aspect, without plumose marginal setæ; exp. of plp.[5] generally distinctly two-jointed, with the subapical squamiferous protuberance on the lower surface very high. Plp.[3] have both rami closely set with long plumose setæ at least on their distal margin. Endp. of plp.[1] at least rather broad, scarcely ever half as long again as broad. (End of abdomen at least emarginate, generally with a notch or with a slit terminating in a foramen.)

(C) Sph. platybranchiatæ: Plp.[4] and plp.[5] have both rami completely without transverse folds, and their exopods are unjointed; endp. of plp.[4] at most with a few short terminal plumose setæ, exp. of same pair rarely with numerous long marginal plumose setæ (Tecticeps), in most genera both rami without plumose setæ; both rami of plp.[5] without plumose marginal setæ, and the exp. has the squamiferous protuberances slightly in relief, and in rare cases without spines or even wanting. Plp.[3] have sometimes plumose marginal setæ on both rami as plp.[2], sometimes with endp. nearly naked or with both both rami naked. Endp. of plp.[1] rarely broad, most frequently narrow. (End of abdomen sometimes with a rounded notch, often truncate, rounded, or acute.)

Group A. Sphærominæ hemibranchiatæ.

This group comprises a very large number of forms, but in spite of much difference in aspect great uniformity is met with in the large majority of more important features. The

proximal joints of the antennæ never protrude with free expansions in front of the head; they are fitted in oblique excavations. In the mouth-parts only the development of the incisive process of the mandibles and the "palp" of the maxillipeds show noteworthy generic differences, excepting the metamorphosis in the females in half of the forms. The thoracic legs are all simple, without sexual difference. The pleopods in the different genera are so uniform that scarcely more than the exopods of plp.^{3} and plp.^{5} present generic differences. The exopod of the uropods is always present, but sometimes exceedingly small. The brood is developed in internal pouches. The body is never strongly depressed, the faculty of rolling excellently developed, the lateral margin of thorax not continuous.

The group is divided into two sections about equal in number of genera.

(*a*) Sphæromini.—End of abdomen in the female without notch, rounded or somewhat produced and more or less acute; in the male generally as in the female, in some forms the end much produced with a pair of lateral notches, so that the mesial part is shaped as a process narrowed at the base.[1] Mouth-parts similar in both sexes.

(*a*) Maxillipeds with the lobes from fourth, fifth, and sixth joints low or rudimentary. Three anterior pairs of thoracic legs closely set with exceedingly long stiff plumose setæ on the outer margin of third and fourth joints. Exp. of plp.^{3} unjointed. Marsupial lamellæ overlap each other at the mesial line (they are unknown in Hemisphæroma, which probably does not differ from Sphæroma in this respect).

† Mandibles normal, the cutting process not elongate, its

[1] In a species from Simon's Bay, at Cape, closely allied to or identical with Sphæroma scabriculum (Hell.), the end of abdomen in the female is as in Exosphæroma, while in the male a notch, as in the male Dynamenella (compare the diagnosis below) is observed; the specimen described by Heller is evidently a male. The female of the species seen by me cannot be separated from Exosphæroma, while the structure in the male alluded to is very curious. For various reasons I omit this form from the conspectus, hoping to obtain more material of allied species.

end obtuse or with some small teeth. Side of abdomen not expanded below the lateral margin of thorax. Tip of abdomen rounded.

(1) Sphæroma (Bosc).

(††) Mandibles aberrant, having the cutting process very elongate (fig. 5 *a*), its distal part widened and divided by a deep triangular incision into two oblong, plate-shaped, distally acute processes. Lateral wall of abdomen considerably expanded, directed downwards, and extending a good deal below the lateral margin of thorax. Tip of abdomen triangular, acute.

(2) Hemisphæroma (n. gen).

(β) Maxillipeds with the lobes from fourth, fifth, and sixth joints at least rather long. Three anterior parts of thoracic legs without stiff natatory setæ. Exp. of plp.[3] two-jointed. Marsupial lamellæ small, far from reaching each other at the mesial line.

(†) [1]Last thoracic segment unarmed in both sexes. End of abdomen at most somewhat produced, but not acute.

(3) Exosphæroma (Stebb.).

(††) Last thoracic segment in the male with a slender mesial process. End of abdomen somewhat or very considerably produced, subacute. (In the male both rami of the uropods are exceedingly large plates.)

(§) End of abdomen subsimilar in both sexes, very considerably produced, with a real groove on the lower side of the produced part.

(4) Isocladus (Miers).

(§§) End of abdomen in the female somewhat produced, in the male strongly produced with a pair of lateral notches, so

[1] The genera Exosphæroma, Isocladus, and Zuzara (with Cycloidura as a synonym) are so closely allied that the females can scarcely be separated, while it is easy to refer the adult males to their respective genera. When more species are known it will probably be necessary to unite them, preserving the name Zuzara for the genus. If that be not done it will be necessary to establish a new genus for Sphæroma scabriculum (Hell.), and perhaps some other species.

that the mesial part is shaped as a process narrowed at the base; an oblong groove is scarcely developed.

5. Zuzara (Leach) (incl. Cycloidura (Stebb.)).

(*b*) Cymodocini.—End of abomen in both sexes with a notch, which sometimes is semicircular, most frequently bilobed, being divided by a mesial process; in rare cases (especially in Bregmocerella) this process is so large that it overlaps the lateral teeth limiting the notch, so that these teeth are only visible from the side. Mouth-parts strongly metamorphosed in the females. Maxillipeds with long lobes on fourth, fifth, and sixth joint. Exp. of plp.^{3} always two-jointed. Marsupial lamellæ always overlap each other at the mesial line.

(*a*) Epistome without any free process in front (exp. of urp. generally well developed).

(†) [1]Abdominal notch at least with a vestige of mesial lobe; generally this lobe is well-developed, frequently large or even very large.

(§) In the male the anterior part of abdomen is without mesial process, and the endp. of urp. is generally moderately developed.

(6) Cymodoce (Leach).

(§§) In the male the anterior part of abdomen has a large mesial process, and the endp. of urp. is very short or quite rudimentary.

(7) Cilicæa (Leach).

(††) Abdominal notch semicircular, without any vestige of mesial lobe. Endp. of urp. rudimentary in the male.

(8) Cilicæopsis (n. gen.).

(β) Epistome produced into a process which, in the female, reaches somewhat beyond the front margin of the antennulæ while it is exceedingly long in the male (Exp. of urp. rudimentary in

[1] In Chapter VII the slight value of Cilicæa (Leach) and Cilicæopsis (n. gen.) as separated from Cymodoce (Leach) is discussed in the treatment of the last-named genus.

both sexes. End of abdomen produced, with a deep groove below; the mesial lobe large, sub-triangular, its lateral walls bent downwards, so that the longitudinal groove is continued on the lower side of the process, while the two teeth—in Cymodoce constituting the lateral limits of the notch—are situated on the lower margins of abdomen at the base of the process, and quite invisible from above.)

(9) Bregmocerella (Hasw.).

The genus Cassidinella (Whitelegge), which is unknown to me, belongs probably to this section; in the following chapter it is mentioned in the treatment of the genus Cymodoce, and discussed as the tenth genus of the hemibranchiate Sphærominæ.

Group B. Sphærominæ eubranchiatæ.

This group comprises as many genera as group A, but the number of species is much less. Mouth-parts—excepting the metamorphosis in the females of several genera—and pleopods are very uniform in all points of importance; the end of abdomen is at least a little emarginate (Cassidinopsis (n. gen.)), but otherwise with a real notch or a tube or foramen connected by a slit with the end itself. The basal joints of the antennulæ afford sometimes a fine generic character. The thoracic legs are always simple, the two anterior pairs without prehensile hands, and in no case has any special equipment with natatory setæ or any sexual difference been observed; the strong thickening of some pairs in Amphoroidea is the most noteworthy feature discovered. In some genera, containing animals of moderate or considerable size, the rami of plp.[4] and plp.[5] are thick, of fleshy aspect, while they are thinner in small forms, but in all species the folds are well developed. An articulation on exp. of plp.[4] could generally not be perceived, but it is very distinct in Scutuloidea; exp. of plp.[5] is generally divided rather near the end, but this articulation is not always easily observed; this exp. has three bosses, two of which on the

second joint, but while the largest of them, which is a high protuberance, is situated on the lower surface of second joint, the others vary as to place. The arrangements for the brood differ greatly in various genera.

The character used for dividing all the genera of the group into two portions, viz. the absence or existence of an articulation of exp. of plp.^{3}, is certainly practical, but scarcely very important; the two portions arising from this division can scarcely be considered natural sections. But on the other hand, it is impossible to give a better division, because at least three genera—Cymodocella (Pfeff.), Amphoroidea (M.-Edw.), and Cassidinopsis (n. gen.)—are not very closely allied either to each other or to the other genera. For these reasons I do not attempt to sub-divide this group into sections with names, while such division is most natural in the two other groups of the sub-family.

a. Exp. of plp.^{3} unjointed. (Not seen in Næsicopea, but this genus is closely allied to Dynamene.)

(*a*) Basal joint of antennulæ of usual shape, not expanded in a free plate.

(†) Urp. always with an exp. at least half as long as endp. and sometimes (in males) very elongate.

(§) Male with a pair of processes from sixth thoracic segment, its abdomen with a circular foramen (sometimes situated on a low cone) connected with the end by a short narrow slit; uropods have exp. much longer than endp.; no appendix masculina on endp. of plp.^{2} Female without processes, abdomen with a foramen connected with the end either by a slit which is only somewhat narrower than, and not marked off from, the foramen, or by a quite linear slit; rami of urp. lamellar, subsimilar in length; mouth-parts exceedingly metamorphosed, marsupial lamellæ exceedingly large and the brood in the marsupium itself.

(1) Dynamene (Leach) (Næsa (Leach)).

(§§) Both sexes without processes on thorax, but the last abdominal segment with two blunt "processes situated one

behind the other"; the end of the posterior one, situated considerably above and a little beyond the end of abdomen, bears the respiratory circular foramen which is connected with the end by a long linear slit; urp. has exp. in both sexes styliform, narrowing towards the acute end, in the male more than twice as long as endp., curved, in the sub-adult female a little longer than endp., straight.[1]

(2) Næsicopea (Stebb.).

(§§§) Both sexes rather similar in aspect, without real processes; abdomen with a notch which is semicircular or oblong in the female, in the male narrow in the distal part, while the proximal part constitutes a transverse foramen; urp. subsimilar in both sexes, with the rami lamellar. Mouth-parts similar in both sexes; male with appendix masculina on endp. of plp.[2]; marsupial lamellæ overlap each other somewhat, but the propagation is unknown.

(3) Dynamenella (n. gen.).

(§§§§) Both sexes similar, without processes. Distal part of abdomen somewhat produced, with the lateral walls bent strongly downwards and inwards, constituting a rather long tube open at both ends and with a slit on the lower surface; urp. similar in both sexes, rami lamellar, exp. considerably shorter than endp. Mouth-parts similar in both sexes; male with appendix masculina on endp. of plp.[2]; marsupial lamellæ overlap each other somewhat; the brood in an exceedingly large external pouch and in the marsupium.

(4) Cymodocella (Pfeff.).

(††) Urp. without exp., but endp. large, lamellar. Both sexes similar, without processes; end of abdomen with a semi-circular notch. Mouth-parts similar in both sexes; marsupial lamellæ overlap each other considerably, and the brood is developed in internal pouches.

(5) Scutuloidea (Chilt.).

[1] The diagnosis is deficient, because mouth-parts and pleopods had been removed before my examination from the two specimens hitherto known, a female with rudimentary marsupial lamellæ and a male.

(β) Basal joint of antennulæ expanded, protruding as an exceedingly large, free, horizontal, angular plate in front of the head. Both sexes similar, without processes; end of abdomen with a semicircular or triangular notch; urp. with the rami well developed, lamellar. Especially fourth, fifth and sixth pairs of thoracic legs short and very thick, much thicker than the anterior pairs. Mouth-parts similar in both sexes; marsupial lamellæ as in Scutuloidea, but the propagation unknown.

(6) Amphoroidea (M.-Edw.).

(*b*) Exp. of plp.^{3} with an articulation rather near the end.

(*a*) Head of normal size. Basal joint of antennulæ has its distal posterior angle produced into an acute process lying close to the hind margin of second joint. Abdomen with a well-developed notch. Exp. of urp. about as large as or much larger than endp.

(†) Male without any mesial process on sixth thoracic segment. Female with the abdominal notch semicircular, the mouth-parts strongly metamorphosed (the mandibles coalesced with the head).

(§) Male has paired denticles in the abdominal notch, urp. strongly altered, with exp. very elongate, curved. Female has the brood in internal pouches.

(7) Paracerceis (n. gen.).

(§§) Male has a mesial lobe, but no paired denticles in the abdominal notch; urp. not much altered, their exp. straight. Female carries the brood in the marsupium itself.

(8) Cerceis (M.-Edw.).

(††) Male with a large mesial process on sixth thoracic segment. (Female unknown.)

(9) Haswellia (Miers).

β. Head small, narrow in proportion to largest breadth of thorax. Basal joint of antennulæ without process from the distal posterior angle. End of abdomen feebly emarginate.

Uropoda similar in both sexes; endp. laterally expanded, very much broader and a little longer than exp. Both sexes similar, without processes; female with normal mouth-parts and the brood in internal pouches.

(10) Cassidinopsis (n. gen.).

Group C. Sphærominæ platybranchiatæ.

This group is sharply defined from the two preceding ones, but its twelve genera show much variation, not only in general aspect, but in several structural features. It is, however, not necessary to produce here a more detailed account of the differences, because the group is divided into four sections which are natural and sharply limited by a set of characters, and a perusal of the diagnoses of these groups may convey a sufficient idea on the points essential. It may be added that the arrangements for the development of the brood differ strongly in the sections, but the mouth-parts seem never to be metamorphosed in the female.

It is an interesting fact that some of the genera of the eubranchiate Sphærominæ comprise three or four and some of the hemibranchiate genera a large number of species, but each of the platybranchiate genera comprises at most two and the majority only one species hitherto described. Most of the genera are, besides, very rare in collections.

The characterisations of the four sections are given before the diagnoses of the genera in order to facilitate comparison.

(*a*) Section Campecopeini.—Body rather vaulted; thorax and abdomen not expanded laterally, without any row of short hairs on the lateral margin. Eyes well developed. The two proximal joints of the antennulæ fitted in excavations on the head and not expanded plate-like in front. Mandibles with the masticatory process well developed. Anterior pairs of legs without prehensile hands. Endp. of plp.[1] at most somewhat longer than broad. Both rami of plp.[3] with long plumose setæ on their distal margin; exp. two-jointed. Plp.[4] and plp.[5] subsimilar in aspect, with their rami respiratory;

rami of plp.[4] naked or with a few very short terminal setæ. Abdomen terminates in a notch (sometimes visible only from below). Marsupial lamellæ overlap each other somewhat at the mesial line.

(*b*) Section Monolistrini.—Body rather vaulted; thorax and abdomen not expanded laterally, without any row of short hairs on the lateral margin. Eyes wanting. The two proximal joints of the antennulæ fitted in excavations on the head, not expanded plate-like in front. Mandibles with the masticatory process well developed. First pair of legs simple; second pair in the male terminating in a prehensile hand. Endp. of plp.[1] very narrow, more than three times longer than broad. Both rami of plp.[3] and of the following pairs without marginal setæ; exp. of plp.[3] unjointed; endp. of all three pairs opaque, respiratory, while exp. is vitreous and at least not so well adapted for respiration. Abdomen without notch, posteriorly broadly rounded. Marsupial lamellæ very large; the brood in the marsupium itself.

(*c*) Section Cassidinini.—Body much or exceedingly depressed; thorax considerably or strongly expanded; margin of thorax, anterior part of abdomen, uropods and sometimes the two proximal joints of antennulæ constituting a nearly continuous border ciliated with a less or more conspicuous rim of short protruding hairs. Eyes well developed. The two proximal joints of the antennulæ with the anterior part protruding, visible from above in at least almost their whole length, frequently much expanded in front, depressed. Mandibles with masticatory process well developed. Anterior pairs of legs without prehensile band. Endp. of plp.[1] at least somewhat longer than broad, sometimes very narrow. Both rami of plp.[3] with several plumose setæ on the terminal margin; exp. unjointed or two-jointed. Both rami of plp.[4] and plp.[5] without setæ, subsimilar in aspect, respiratory. Posterior margin of abdomen short; a real notch always wanting. Marsupial lamellæ wanting; the brood in a chamber formed by two external pouches (see p. 80).

(*d*) Section Ancinini.—Body depressed; thorax some-

what or considerably expanded, but a rim of marginal hairs feebly developed or wanting. Eyes at least discoverable. Antennulæ vary as to shape and insertion, but never fitted in excavations on the head. Mandibles without masticatory process.[1] First thoracic legs with a robust prehensile hand in both sexes; second legs in the female ambulatory, in the male terminating in a prehensile hand differing much in shape or size from that of first pair. Endp. of plp.[1] broader than long. Endp. of plp.[3] with a few short terminal setæ, exp. with numerous long setæ. Exp. of plp.[4] with or without marginal setæ; both rami of plp.[5] without setæ. End of abdomen truncate or less or more triangular. Marsupial lamellæ overlap each other very considerably (at least in one of the genera, Ancinella, the brood is developed in an enormous external pouch and in the marsupium).

The section Campecopeini comprises two genera.

(*a*) Epistome considerably longer than broad, protruding in front as a rounded process visible from above. Second and third thoracic legs show peculiar sexual differences (see the notes below). Endp. of plp.[1] broader than long; endp. of plp.[3] nearly as broad as long; exp. of plp.[3] with the articulation rather near the end; endp. of plp.[4] with a few very short terminal setæ. Urp. with both rami well developed. Marginal portion of abdomen visible from above. Last thoracic segment unarmed in both sexes. (Brood in internal pouches.)

(1) Parasphæroma (Stebb.).

(β) Epistome much broader than long, without any free frontal process, not visible from above. Second and third thoracic legs not showing sexual differences. Endp. of plp.[1] somewhat longer than broad; endp. of plp.[3] much longer than broad; exp. of plp.[3] with the articulation near the middle; endp. of plp.[4] naked at the end. Urp. with exp. elongate and endp. wanting. Marginal portion of the wall of abdomen bent not only downwards but much inwards, not visible from above.

[1] Of the genus Ancinus, (M.-Edw.). I have only examined a dried specimen from the outside, but having dissected specimens of the two other genera, I think it allowable to draw up this diagnosis of the section.

Last thoracic segment unarmed in the female, in the male wit a mesial process.

(2) Campecopea (Leach).

The section Monolistrini comprises three (probably four[1]) genera closely allied to each other, but distinguishable by at least one practical character.

(α) Urp. consists of the sympod and an elongate movable exp.

(3) Monolistra (Gerst.).

(β) Urp. consists only of a very small oblong-triangular joint.

(4) Cæcosphæroma (Dollf.).

(γ) Urp. wanting.

(5) Vireia (Dollf.).

The section Cassidinini comprises four genera, three of which are exceedingly characteristic, while the fourth, Chitinopsis (Whitel.), has only sub-generic value.

(α) Seen from above, the epistome protrudes as a rather or very long process separating the antennulæ. Two proximal joints of the antennulæ considerably or exceedingly expanded in front of the head. Lobe of fifth joint of the maxillipeds rather long, proceeding only from the proximal part of its inner (front) margin (fig. 6 α). Endp. of plp.[1] oblong, but not fully twice as long as broad. Exp. of plp.[3] two-jointed. Terminal margin of abdomen freely exposed, rounded without notch.

(†) Body rather broad. Two proximal joints of the antennulæ considerably expanded. Endp. of plp.[1] only somewhat longer than broad.

(6) Cassidina (M.-Edw.).

(††) Body oblong-oval. Two proximal joints of the antennulæ exceedingly expanded. Endp. of plp.[1] almost twice as long as broad.

(7) Chitinopsis (Whitel.).

[1] The genus Spelæosphæroma belongs probably to this section, but is omitted, as not only the animal, but the recently published description is unknown to me.

(β) Seen from above, the epistome protrudes as a broad but very short plate separating the antennulæ. Two proximal joints of the antennulæ protrude as a narrow rim in front of the head. Fifth joint of maxillipeds short and broad, with a low lobe occupying nearly the whole interior (front) margin and reaching its distal end. Endp. of plp[1]. about three times as long as broad at the base. Exp. of plp.[3] unjointed. Terminal margin of abdomen freely exposed, sub-truncate.

(8) Cassidinidea (n. gen.).

(γ) Epistome very short, invisible from above. Two proximal joints of the antennulæ strongly expanded, forming a broad rim in front of the head; the inner margin of the expansion of one antennula touches that of the opposite antennula in the mesial line. Fifth joint of maxillipeds short, with a moderately developed lobe proceeding from the whole interior (front) margin. Endp. of plp.[1] exceedingly narrow, four times as long as broad. Exp. of plp.[3] two-jointed. Terminal margin of abdomen completely surrounded by the very long endopods of the uropods; the end turned somewhat upwards, vaulted, with a longitudinal groove below, so that an aperture, visible from behind, is found between the margin of abdomen and the uropods. Body exceedingly flattened; the two proximal joints of the antennulæ, both rami of urp. and the lateral parts of the thoracic segments and of the anterior section of abdomen are strongly expanded, and their margin constitutes a continuous outline with the fringe of ciliæ very dense, regular, and conspicuous; head and last abdominal segment totally excluded from partaking in forming the outline of the animal.

(9) Leptosphæroma (Hilgendorf).

The section Ancinini comprises three genera, one of which is founded on a new form described in the notes below. The diagnosis of the genus Ancinus (M.-Edw.) is incomplete from want of material.

(α) Eyes conspicuous, dark. Antennulæ inserted on the front end of the head, their two proximal joints rather broad,

entirely visible from above. Epistome produced, reaching to the front margin of first joint of the antennulæ, separating these as a sub-quadrangular plate. Last segment of abdomen has the lateral part of the wall bent downwards and somewhat inwards, constituting near the end the sides of a groove; the end itself truncate. Urp. without endp., exp. long, slender.

(10) Ancinus (M.-Edw.).

(β) Eyes colourless; feebly developed. Antennulæ inserted on the front end of the head; their basal joint much produced; about as broad as long, depressed; entirely visible from above. Epistome produced into a triangular process, reaching about to the middle of the inner margin of the first antennular joint. Lobes on fourth, fifth, and sixth joints of maxillipeds very low. Exp. of plp.^{3} two-jointed. Plp.^{4} and plp.^{5} have their rami sub-similar; endp. of plp.^{4} with a single terminal seta; exp. of plp.^{4} without setæ; exp. of plp.^{5} with the bosses feebly developed, without spines. Last abdominal segment with the distal lateral part of the wall not bent inwards, the end narrowly rounded or nearly acute. Urp. has the sympod directed outwards and somewhat forwards, without endp.; exp. long, narrow. Male with the inner margin of endp. of plp.^{2} much thickened, longitudinally canaliculated on the inner side, and appendix masculina is wanting.

(11) Ancinella (n. gen.).

(γ) Eyes well developed, black. Antennulæ inserted on the lower side of the head, their basal joint longer than broad, and quite concealed by the protruding front border of the head. Epistome reaches scarcely the middle of the inner margin of the basal joint of the antennulæ; its end is broadly rounded. Lobes of fourth, fifth, and sixth joints of maxillipeds long. Exp. of plp.^{3} unjointed. Exp. of plp.^{5} and plp.^{4} closely set with plumose setæ along their distal and outer margin. Endp. of plp.^{4} with a few plumose terminal setæ. Exp. of plp.^{5} without bosses. Last abdominal segment has not the distal lateral part of the wall bent inwards; the end

acute in the male, rather obtuse in the female. Urp. with both rami long, in the female sub-equal in length, in the male exp. is elongate, considerably longer than endp. Male with appendix masculina on endp. of plp.[2]

(12) Tecticeps (Richardson).

VII. Notes on the Genera and their Species.

The genera are here dealt with in the same consecutive order as in the preceding chapter. As to the enumeration of the species I refer to the "Introductory Remarks."

Sub-family Limnoriinæ.

Limnoria (Leach).—The type is L. lignorum (Rathke). On the three other species known see Stebbing in 'Fauna Maldive and Laccadive Archip.,' vol. ii, p. 714.

Sub-family Plakarthriinæ.

Plakarthrium (Chilt.)—The type is P. typicum (Chilt.) Whether P. (Chelonidium) punctatissimum (Pfeff.) be a closely allied species or only a synonym I am unable to decide. Pfeffer published (1887) a very detailed, and as a whole useful account of his form, but some of his anatomical statements and morphological interpretations are incorrect, and his opinion on the systematic position of the genus is without foundation.[1]

Sub-family Sphærominæ.

A. Sphærominæ hemibranchiatæ.

(1) Sphæroma (Bosc).—To the characters given above it may be added that in all species the end of abdomen, even if moderately narrow, is really rounded, not triangular or subacute; its marginal part, seen from below, shows scarcely any trace of a longitudinal mesial excavation, not to speak of a longitudinal groove as in Isocladus. According to my own examination the following species belong to this genus:

[1] Pfeffer establishes it as the type for a new family, and adds "Die Fa mili scheint mit den Onisciden am nächsten verwandt."

S. serratum (Fabr.), S. rugicauda (Leach), S. Hooker (Leach), S. sicilience (Leach), S. trigonum (Risso), S. verrucauda (White), S. quadridentatum (Say) (types or co-types of the six latter species in the British Museum), S. Bolivari (de Buen) (co-types from Canon A. M. Norman), S. destructor (Richardson) (co-types from U.S. Nat. Mus.), the latter, according to Stebbing, a synonym, as is also S. vastator (Bate), to S. terebrans (Bate); finally S. marginatum (M.-Edw.) (Copenhagen Museum). Of the other species described in the literature S. chilense (Dana) and S. pentodon (Richardson) seem to belong to this genus.

Most of the remaining very numerous species established in the literature as members of the genus Sphæroma belong to other genera. Some of them have been or must be referred to Exosphæroma (Stebb.), viz. S. gigas (Leach), S. lanceolatum (White), S. leucura (White) (types of these three species were seen in the British Museum), S. Stimpsonii (Hell.) (Copenhagen Museum), and probably S. calcareum (Dana); S. scabriculum (Hell.) is mentioned in the footnote on p. 102. S. armatum (M.-Edw.) has been established as type for the genus Isocladus (Miers), to which besides S. spinigerum (Dana) has been referred. S. dicanthum (Péron, M.-Edw.) must be a Zuzara (Leach); S. integrum (Hell.) is probably a species of Zuzara (Leach), or perhaps of Isocladus (Miers). Many species referred to Sphæroma are in reality females or immature specimens of the genus Cymodoce (Leach); according to typical specimens in the British Museum, S. spongiosum (White) is the female of an Australian Cymodoce, while S. Prideauxianum (Leach), S. Dumerilii (Leach), S. Griffithsii (Leach), S. curtum (Leach), and S. spinosum (Risso) belong to Cymodoce truncata (Leach), and the specimens of S. Ritchianum (Leach) to two species of Cymodoce; judging from descriptions or figures in the literature, S. Lesueuri (Risso), S. granulatum (M.-Edw.), S. pubescens (M.-Edw.), S. Gaimardii (M.-Edw.), and S. yucatanum (Richardson) have been established on females or young

males of animals belonging to Cymodoce. Sphæroma gibbosum (M.-Edw.) and S. micracanthum (Tristan, M.-Edw.) are young males of Dynamene (Leach), probably of D. bidentata (Mont.); Sphæroma? egregium (Chilt.) and S. algoense (Stebb.) must be referred to the genus Cymodocella (Pfeff.); S. orientale (Dana) is a young specimen of the genus Cerceis (M.-Edw.). Sphæroma perforatum (M.-Edw.) and S. globicauda (Dana) are probably species of Dynamenella (n. gen.); if not so, one of them is or both are to be referred to the same genus as S. scabriculum (Hell.) (see the footnote on p. 102). Sphæroma Jurinii (Sav.), S. Savignii (M.-Edw.), S. tristense (Leach), S. anomalum (Hasw.), S. asperum (Hasw.), S. amplicauda (Stimps.), S. rhombura (Richardson), S. octoncum (Richardson), S. plumosum (Whitelegge), and S. latifrons (Whitelegge) do not belong to Sphæroma, but I cannot refer them to genera, because the descriptions and figures are too defective in some respects. On S. Quoyanum (M.-Edw.), S. oregonense (Dana), S. obtusum (Dana), S. læviusculum (Hell.), S. triste (Hell.), S. læve (Hasw.), S. crenulatum (Richardson), and S. australe (Whitelegge) I have no opinion.

(2) Hemisphæroma (n. gen.).—The type is H. pulchrum (n. sp.), of which I have seen an adult male and an immature female. To the diagnosis of the genus on p. 103 a short description of the species may be added. The epistome has a rather deep longitudinal groove and two pairs of marginal processes; first pair, placed near the middle of the margin, is low; second pair, situated near the proximal end, is rather long, vertical. Antennulæ essentially as in Sphæroma. The three anterior pairs of thoracic legs are moderately slender and furnished with a large number of exceedingly long stiff setæ along the whole outer margin of the long third joint and along the distal two thirds of the same margin of fourth joints, besides some rather long setæ on the most distal part of the outer margin of fifth joint. The three following pairs of legs are much shorter and more robust, seventh pair as

long as the second, rather slender and strongly compressed; all four pairs along the margins and on a portion of the sides very densely set with fine hairs; most of the marginal hairs long or exceedingly long. Last abdominal segment is broad behind, the posterior margin as a whole rather flatly convex with a very obtuse angle, but the tip of this angle is feebly produced, acute; the posterior margin is very broad on the lower surface, constituting a rather broad rim, which has a longitudinal mesial carina. The rami of the uropods similar in shape, reaching in the male completely, in the female scarcely, to the apex of abdomen. Length of the male 13·5 mm., of the female without marsupium 8·5 mm. Locality: Sourabaya, Java. Collected by Captain Andréa (Copenhagen Museum).

(3) Exosphæroma (Stebb.).—The genus has been established on Sphæroma gigas (Leach) and S. lanceolatum (White). In these species the end of abdomen is either rather convex, subangular, or constitutes an angle with the tip rounded; the terminal margin is, seen from below, rather sharp and the excavation containing the pleopods produced a little backwards, but no real longitudinal groove is formed. According to an examination of dried typical specimens of Sphær. leucura (White) in the British Museum this species must be referred to Exosphæroma; Sphæroma Stimpsonii (Hell.) (specimens in the Copenhagen Museum) belongs also to the present genus. Several of the nearly twenty species enumerated above as referred to Sphæroma by earlier authors, but whose systematic position I am unable to settle, will certainly prove themselves to belong to Exosphæroma. On the other hand, of the three species established in 1902 by Stebbing as species of Exosphæroma, E. validum (Stebb.) is the immature male and E. setulosum (Stebb.) the female of the same species of Cymodoce, while E. amplifrons (Stebb.) is the adult male of an aberrant species of Cymodoce (see below under this genus).

(4) Isocladus (Miers).—The genus comprises two closely allied species, I. armatus (M.-Edw.) and I. spiniger

(Dana), both originally referred to Sphæroma. Sphæroma integrum (Hell.) may perhaps be an Isocladus, but more probably it is a species of Zuzara (Leach).

(5) Zuzara (Leach).—According to my examination of types in the British Museum and animals received from Dr. Chilton, Zuzara semipunctata (Leach), Z. diadema (Leach), Z. integra (Hasw.), and Cycloidura venosa (Stebb.) belong to this genus, while Zuzara emarginata (Hasw.) must be referred to the genus Haswellia (Miers). Sphæroma integrum (Hell.) is probably a species of Zuzara, perhaps an Isocladus; Cymodoce armata (M.-Edw.) has been transferred to Zuzara by Haswell, but this reference seems to me to be rather dubious.

(6) Cymodoce (Leach).—This genus, Cilicæa (Leach) and Cilicæopsis (n. gen.) are very closely allied; Cassidinella (Whitelegge), which is imperfectly described as to one of the most important features and unknown to me, belongs probably to the Cymodocini, and if so it is scarcely distinguishable from certain forms of Cymodoce. The male of Cymodoce, Cilicæa, and Cilicæopsis are easy to separate, but the females of Cymodoce cannot be distinguished from those of Cilicæa; in adult females of certain species of Cymodoce the mesial lobe of the notch is scarcely distinguishable, and the notch therefore rather similar to that in Cilicæopsis, but the females of the latter genus differ in aspect from those of Cymodoce and have the end of the exopod of urp. produced and very acute, a feature not observed in Cymodoce. It might perhaps have been advisable to cancel Cilicæa and not to establish Cilicæopsis, thus including all species of hemibranchiate Sphærominæ possessing an abdominal notch—Bregmocerella excepted—in the genus Cymodoce. But, on the other hand, it is always difficult to suppress a genus as a mere synonym, when it comprises a certain number of species, and is allied to another very rich genus: if Cilicæa be suppressed the genus Cymodoce will be extremely large. When Cilicæa is maintained it is necessary to establish Cilicæopsis, and in the future two or three new genera of similar quality must be erected.

But after the removal of Cilicæa and Cilicæopsis the genus Cymodoce comprises still a very good number of species described in the literature, and, according to my experience, numerous undescribed species from the Indian Ocean and the Pacific (from Japan to Australia) are found in various European collections. I propose, therefore, to accept Cilicæa, and consequently to establish Cilicæopsis, but to consider both these genera—and probably Cassidinella —as having only sub-generic value.

The genus Cymodoce and its sub-genera are exceedingly difficult to deal with. The difference between adult species of the two sexes is generally very large; the adult males are adorned with tubercles, bosses, or processes, which are wanting or low in the females; when a mesial lobe is present the abdominal notch differs considerably in shape in the two sexes; finally, the uropods show nearly always striking sexual differences. In the females the rami of the uropods are plate-shaped, often nearly similar in size and shape, but sometimes the exp. is rather small, in rare cases even very small; in the male the exp. is frequently elongate, sometimes very long, while the endp. either has preserved the same size as in the female and immature specimens, or has been reduced in size, or is even quite rudimentary. Several females or immature specimens have been established as species of Sphæroma, while the males were described as forms of Cymodoce or Cilicæa.

From the coasts of England, France, Italy, and Tripoli I found in the British Museum animals belonging to the genus Cymodoce labelled with the following names: C. truncata (Leach), C. Lamarchii (Leach), C. emarginata (Leach), Sphæroma Dumerilii (Leach), Sph. Ritchianum (Leach), Sph. Prideauxianum (Leach), Sph. curtum (Leach), Sph. Griffithsii (Leach), Sph. tridens (Spinola), Sph. spinosum (Risso), Cymodoce spinosa (White); furthermore, H. Milne-Edwards establishes C. pilosa from the Mediterranean. But at least C. truncata (Leach), S. Dumerilii, S. Prideauxianum, S. curtum, S. Griffithsii, S. tridens, and S. spinosum belong to the same species,

for which I—at least provisionally—apply the name C. truncata (Leach); some specimens of S. Ritchianum and one of the specimens of C. Lamarchii belong besides to C. truncata, while other specimens referred to the two last-named forms are identical with C. pilosa (M.-Edw.); on C. emarginata (Leach) I shall not express an opinion. Sphæroma Lesueuri (Risso) has been transferred to Cymodoce by M.-Edwards, and I suppose it to be correct; it is probably an immature specimen of one of the Mediterranean species. Gourret has established two species from the Mediterranean of Dynamene, D. corallana, and D. setosa, but according to the shape of maxillipeds and abdominal notch, they are females of Cymodoce. I am acquainted with three European species, but the sum of these statements shows that it will be a most difficult task to name them correctly, and an attempt must be postponed.

In the British Museum I saw besides typical specimens (or co-types) of the following species correctly established as forms of Cymodoce, viz. C. bifida (Leach), C. trilobata (Miers), C. longistylis (Miers), C. convexa (Miers), C. aculeata (Hasw.), C. coronata (Hasw.), and C. granulata (Miers). (The last-named form is similar to Cerceis trispinosa (Hasw.) in the shape of first joint of the antennulæ, surface of thorax and abdomen, shape of seventh thoracic epimera, which are produced and curved as a hook with the apex turning upwards, shape of the abdominal notch and uropoda, but it differs sharply from Cerceis trispinosa in the structure of plp.,[4] and certainly of plp.[5]: according to kind communication from Dr. W. T. Calman—who at my request examined several details of a male from Flinders Isl.—the exp. of plp.[4] is sub-membranaceous, not plicated as in the named species of Cerceis, of which I have examined specimens from Port Victoria forwarded me by Dr. Chilton.) In the same Museum I saw the type of Sphæroma spongiosum (White) and specimens of Sphæroma Gaimardii (M.-Edw.), both referred correctly to Cymodoce by Miers. Cymodoce abyssorum (Bedd.) has with good reason been

established by Stebbing of the type for a new genus, Næsicopea, which belongs to the eubranchiate Sphærominæ. Among the species not seen by me, Cymodoce tuberculosa (Stebb.), C. uncinata (Stebb.), and C. bicarinata (Stebb.) have been correctly referred. Cym. armata (M.-Edw.) has been transferred to Zuzara by Haswell, but this reference is, in my opinion, rather dubious, though I cannot offer any better interpretation. Above it is mentioned that Exosphæroma validum (Stebb.) and E. setulosum (Stebb.) are respectively the young male and the female of a species of Cymodoce. Exosphæroma amplifrons (Stebb.), of which I have inspected a fine typical specimen kindly forwarded me by Mr. Stebbing, is the male of an interesting species of Cymodoce; in the shape of the terminal part of abdomen it is much alike to Bregmocerella, but it differs from this genus and agrees with Cymodoce as to the number of spiniferous protuberances on exp. of plp.[5], and the exp. of urp. is as large as the endp. Judging from the descriptions in the literature Haswell has correctly referred Sphæroma pubescens (M.-Edw.) to Cymodoce, and above it is mentioned that Sphæroma granulatum (M.-Edw.) and S. yucatanum (Richardson) must be transferred to the same genus. Of the other forms established in the literature as species of Cymodoce, C. bidentata (Hasw.), C. tuberculata (Hasw.), and C. inornata (Whitelegge) belong probably to this genus, while C. bermudensis (Ives), according to my examination of specimens from the U. S. National Museum, is the female (and immature male) of a species of Paracerceis (n. gen.) (belonging to the eubranchiate Sphærominæ). Cilicæa linguicauda (Richardson) is probably, Cil. granulosa (Richardson) perhaps, a species of Cymodoce; both differ from the other species of the last-named genus in having the endp. of urp. very short. The description of Cymodoce cordiforaminalis (Chilton) I have not seen, but judging from the name the species can scarcely belong to the present genus.

(7) Cilicæa (Leach).—The type is C. Latreillei (Leach).

Specimens in the British Museum of Cil. crassa (Hasw.) and Cil. tenuicaudata (Hasw.) show that these species have been correctly referred; according to Haswell's descriptions of the abdominal notch, the same is the case with Cil. crassicaudata (Hasw.), Cil. hystrix (Hasw.) and Cil. curtispina (Hasw.), while I am unable to decide whether Cil. spinulosa (Hasw.) belongs to Cilicæa, or to the following sub-genus Cilicæopsis. The three species established by Whitelegge as belonging to Cilicæa are dealt with under Cilicæopsis. According to the examination of specimens forwarded me by Dr. Chilton, Næsa canaliculata (Thoms.) belongs to Cilicæa. On the other hand, Cilicæa caudata (Say) (originally established as a Næsa by Say, but referred to Cilicæa by Harriet Richardson) and Cilicæa caudata (Moore) are species of Paracerceis (n. gen.); Cilicæa caudata Gilliana (Richardson), and C. cordata (Richardson) are certainly also species of Paracerceis.

(8) Cilicæopsis (n. gen.).—As the type I take Cilicæa granulata (Whitelegge); from the East Indian and Australian regions I have seen some unnamed species more or less allied to that form. Whitelegge describes and figures two aberrant species established on males, Cilicæa stylifera (Whitel.), and C. ornata (Whitel.), which differ strongly from C. granulata (Whitel.) as to the shape of the upper side of abdomen, but agree with it in possessing a semicircular abdominal notch and rudimentary endp. of urp., while exp. of urp. is extremely elongate; I think that these two species can be referred to Cilicæopsis, but without an examination of any of them, or, at least, of closely-allied species, I cannot decide the question.

(9) Bregmocerella (Hasw.).—Only one species, B. Grayana (Woodw.), is known; it has been described by Woodward, Haswell, Beddard, and Whitelegge, and figured by the two first-named of these authors. It is in reality, in spite of its aberrant aspect, closely allied to Cymodoce. To the characters pointed out on pp. 104, 105, may be added that exp. of plp.^{5} has not only the three usual protuberances, but besides a protuberance at the inner margin somewhat before

the end of the first joint; this protuberance is wanting in even very large species of Cymodoce examined for comparison. The shape and number of the entrances to pouches with brood are mentioned on p. 76.

(10) Cassidinella (Whitelegge).—This genus has been established on a single male specimen. In the diagnosis the author writes: "Pleopoda foliate; all except the last pair densely ciliate." If that be correct, the genus must belong to the platybranchiate Sphærominæ, and besides disagree with these as to plp.[4]; according to the sentence quoted plp.[4] and plp.[5] would even agree with those in Limnoria and differ from all Sphærominæ. But his eight figures of the typical species, C. insisa (Whitel.), show an animal which is rather alike to two unnamed forms seen by me and belonging to Cymodoce (sens. lat.); in reality, antennulæ, mandibles, maxillipeds, thoracic legs, and end of abdomen do not show any difference; exp. of urp. is several times smaller than endp., but in one of the species alluded to the exp. is still smaller; the upper surface of abdomen has no processes, but this character is of slight value, and processes are, besides, not found in males of all species of Cymodoce. Judging from these facts, I insert Cassidinella, at least provisionally, on this place.

B. Sphærominæ eubranchiatæ.

(1) Dynamene (Leach) (Næsa (Leach[1])).—The type is D. bidentata (Mont.). Leach established the genus Næsa on the adult male of this species, while D. viridis (Leach), D. Montagui (Leach), and D. ruber (Mont.) are the female and immature specimens of the same species; above it is mentioned that Sphæroma gibbosum (M.-Edw.) and Sphær. micracanthum (Tristan) are young males of Dynamene, probably even of D. bidentata. Hesse established (1873) nine new species of Næsa from the western coast of France, but they are probably all unrecognisable and are

[1] As to the synonymical question on the use of either Dynamene or Næsa for the present genus, I refer to the footnote on p. 77.

omitted here. Gourret established (1891) on females two new species of Dynamene, viz. D. corallana and D. setosa from the southern coast of France, but according to kind informations from the zoological authorities at the Museums in Marseille his typical specimens could not be found; judging from the shape of the maxillipeds and the abdominal notch, the animals are females of the genus Cymodoce, and the descriptions and figures given by him will scarcely allow recognition of the species. It may be added that I am acquainted with males and females of two fine species from the Mediterranean; one of these constitutes as to the situation of the respiratory foramen to a certain degree a transition stage to the genus Næsicopea (Stebb).

Of exotic species referred to Næsa no one belongs to the present genus. Næsa caudata (Say) I take as the type for the genus Paracerceis (n. gen.); Næsa ovalis (Say) is my type for Cassidinidea (n. gen.) belonging to the platybranchiate Sphærominæ; Næsa canaliculata (Thoms.) is, as mentioned above, a species of Cilicæa (Leach); Næsa depressa (Say) is the type for the genus Ancinus (M.-Edw.). Of exotic species referred to Dynamene scarcely any one can remain in this genus. D. Eatoni (Miers), established on immature animals, seems to be a species of Dynamenella. According to kind information from Dr. Calman, D. Darwinii (Cunningham) has exp. of plp.[3] divided by an articulation; the species must, in my opinion, be established as a new genus near Paracerceis. Dynamene perforata (Moore) I establish as the type for Dynamenella (n. gen.); Dynamene bermudensis (Ives) is, according to my examination of specimens from the U. S. National Museum, females of a species of Paracerceis closely allied to P. caudata (Say); Dynamene angulata (Richardson), D. Benedictii (Richardson), and D. glabra (Richardson) are probably females and immature specimens either of Dynamenella or Paracerceis, but as the structure of the pleopods, etc., is unknown it is, of course, impossible to refer them to genera with certainty. On D. tuberculosa (Richardson) I have no

opinion, and D. dilatata (Richardson) must, judging from the aberrant shape of the antennulæ, probably be established as the type for a new genus.

(2) Næsicopea (Stebb.).—The type is Cymodoce abyssorum (Bedd.).

(3) Dynamenella (n. gen.).—As the type I take Dynamene perforata (Moore), of which I have examined an adult male and an immature male from the U. S. National Museum. Besides, I have seen specimens of two undescribed species, respectively from St. Thomas and Valparaiso. On other forms perhaps belonging to this genus see my notes on Dynamene. Above (p. 117) it is stated that Sphæroma perforatum (M.-Edw.) and Sph. globicauda (Dana) are not improbably species of Dynamenella.

(4) Cymodocella (Pfeff.).—The genus has been established on C. tubicauda (Pfeff.). I have examined specimens from Dr. Chilton of his Sphæroma? egregium. The two species are identical, and the type must, therefore, be named C. egregia (Chilt.). According to description and figures Sphæroma algoense (Stebb.) belongs to the same genus.

(5) Scutuloidea (Chilt.).—Only the typical species, S. maculata (Chilt.), is known.

(6) Amphoroidea (M. Edw.).—This beautiful and easily recognisable genus was established on A. typa (M. Edw.). Two other species have been described, viz. A. australiensis (Dana) and A. falcifer (Thoms.).

(7) Paracerceis (n. gen.).—The type is P. caudata (Say), referred by Say, Milne-Edwards, and White to the genus Næsa (Leach), by Ives to Cymodoce (Leach), by Moore and Harriet Richardson to Cilicæa (Leach). According to examination of a typical specimen from Say in the British Museum, material from Cuba in the Copenhagen Museum, and specimens from Florida sent me by U. S. National Museum, at least some of the specimens referred by American authors to P. caudata (Say) belong to an unnamed and closely allied species. Dynamene bermudensis (Ives) is (see my notes on Dynamene) a female of that new species.

Cilicæa cordata (Richardson) and Cil. caudata Gilliana (Rich.) are certainly males of species of Paracerceis; on some forms established by H. Richardson as species of Dynamene I refer to the notes on this genus.

(8) Cerceis (M.-Edw.).—C. tridentata (M.-Edw.) is the type; according to my study of a rich material of this genus the species named is a male, while C. bidentata (M.-Edw.) is the female, either of the same or of a closely allied species. To this genus belong besides C. trispinosa (Hasw.) (I have examined specimens from Dr. Chilton) and C. acuticauda (Hasw.), but the reference of C. nasuta (Whitelegge) is doubtful, the basal joint of the antennulæ, the abdominal notch, and the pleopoda being imperfectly known. Sphæroma orientale (Dana) is a young specimen of Cerceis. This genus is closely allied to Paracerceis and Haswellia; at least at present I am not able to point out reliable difference in the structure between the females of Paracerceis and Cerceis, but, as already mentioned, Paracerceis has the brood in internal pouches, Cerceis in the marsupium itself; the females of Haswellia are unknown. It may be mentioned that the outer margin of exp. of plp.^{2} is coarsely serrate in Paracerceis caudata (Say) and at least in some species of Cerceis, but in C. trispinosa (Hasw.)—which besides in the shape of the abdominal notch occupies a rather isolated position—serration is visible only at the end of the margin and even feebly developed.

(9) Haswellia (Miers) (Calyptura (Hasw.)).—The type is H. carnea (Hasw.), of which only the male is known. According to the examination of a male (forwarded me by Dr. Chilton) of Zuzara emarginata (Hasw.), this species must be transferred to Haswellia; in H. carnea the long plate from seventh thoracic segment is broad in the whole length, while in H. emarginata only the proximal third of the process is plate-shaped, the long distal part narrow. Of H. carnea I have examined two adult and two immature males (all from Dr. Chilton); the adult males measure respectively 10·4 and 8·5 mm., the immature speci-

mens 8·0 and 6·4 mm. in length ; in these two young specimens the processes at seventh thoracic sternite are very short, but yet distinct, while no trace of appendix masculina on endp. of plp.^{2} could be perceived in any of them. These immature specimens resemble Cymodoce in general aspect; in both seventh thoracic tergite has a broad but very short protuberance as a rudiment of the plate in the adult male; in the larger of these two specimens the mesial process of the abdominal notch is broadly triangular and reaches beyond the lateral angles of the notch, but in the smaller specimen the notch is almost rectangular, with the basal margin a little convex, the mesial lobe being very low. Judging from these features, I suppose that the notch in the adult female be rounded as in the two preceding genera.

10. Cassidinopsis (n. gen.).—The type is Cassidina emarginata (Guér.), which in many important points—structure of plp.^{4} and plp.^{5}, shape of epistome, mandibles, fifth joint of maxillipeds, end of abdomen—differs strongly from the type for the genus Cassidina, C. typa (M.-Edw.). C. latistylis (Dana) has generally been referred to C. emarginata as a synonym, but Dana's figure of the end of abdomen does not agree well with the shape observed in C. emarginata; a detailed account of this species is given by Pfeffer (1887). No other species referred to Cassidina belongs to Cassidinopsis.

C. Sphærominæ platybranchiatæ.

1. Parasphæroma (Stebb.).—The type is P. prominens (Stebb.). I have examined two females with brood in internal pouches; they are co-types kindly forwarded me by Mr. Stebbing, who describes and figures a male specimen. The two abdominal protuberances are scarcely as high in the female as in the male; the exp. of urp. has the outer margin convex in almost more than three quarters of its length, but its distal fourth is more concave than on Mr. Stebbing's figure, so that the apex of the ramus is less produced, but directed

more outwards than in the male. In the female third thoracic legs are about as slender as fourth legs, and without brushes on any joint, while in the male third to sixth, and especially third to fifth, joints are conspicuously thicker than in the female, and third to fifth densely clothed with brushes of short hairs on their lower surface. Second legs are in the female only a little shorter and thicker than third, and their fifth and sixth joints have a few scattered spines; in the male (according to Stebbing's figure) fourth to sixth joints are much thicker, fifth and sixth with a peculiar armament. First legs are similar in both sexes. No other species is known.

(2) Campecopea (Leach).—The type is C. hirsuta (Mont.); C. Cranchii (Leach) is the female of the same species. In certain features, viz., the shape of epistome and uropods, the marginal part of abdomen being bent inwards, etc., it constitutes to a certain degree a transition to Monolistra. White referred Næsa ovalis (Say) to this genus, but I take N. ovalis as the type for Cassidinidea (n. gen.). Camp. bicolor (Rathke) (referred incorrectly by Milne-Edwards to Næsa) and C. versicolor (Rathke) (referred by Milne-Edwards to Cymodoce) cannot remain in Campecopea, but I have no opinion on their real relationship.

(3) Monolistra (Gerst.).—The type is M. cæca (Gerst.). From the Berlin Museum I received an adult male of this species and besides an immature male of an undescribed form. According to kind information from Dr. Joh. Thiele the female of M. cæca has second thoracic legs simple, without prehensile hand.

(4) Cæcosphæroma (Dollf.).—The type is C. Virei (Dollf.), of which I have seen a single specimen, kindly presented me by Mr. A. Viré. As to this form and the two species of the following genus the reader is referred to a future paper by Mr. A. Dollfus.

(5) Vireia (Dollf.).—To this genus Mr. Dollfus refers two species, V. burgunda (Dollf.) and V. berica (Fabiani). (See the future paper by Mr. Dollfus).

(6) Cassidina (M.-Edw.).—The type is C. typa (M.-Edw.),

apparently not recognised by any zoologist since it was established in 1840. Among numerous marine animals from Akaroa Harbour, New Zealand, I found several specimens of a species of Cassidina, determined it as C. neo-zealanica (Thoms.), examined its structure, and worked out a set of analytical figures. When I, more than a year after, studied the literature on Sphæromidæ and looked on the figures given by Milne-Edwards in 1840, I was struck by the similarity as to certain points between these and my own drawings. I was speedily convinced that C. neo-zealanica must be either a species closely allied to C. typa or only a synonym. Professor E. L. Bouvier kindly lent me a specimen of C. typa marked "Type, Ouoy and Gaimard, Nouv. Zélande"; it agrees completely with my specimens of C. neo-zealanica, and the latter name must therefore be considered a synonym. It may be added that the mandibles are unusually short and peculiarly bent. Besides C. typa and C. neo-zealanica five other species have been referred to Cassidina. C. emarginata (Guér.) differs strongly from C. typa in epistome, antennulæ, mandibles, maxillipeds, and pleopods; it is established above as the type for Cassidinopsis (n. gen.) belonging to the eubranchiate Sphærominæ. C. latistylis (Dana) is with a little doubt considered a synonym to C. emarginata. C. maculata (Studer) cannot remain in Cassidina if Studer's figure, showing the proximal joints of the antennulæ as invisible from above, be tolerably correct, but as to the real relationship of this species I have no opinion. C. lunifrons (Richardson) must probably be referred to Cassidinidea (n. gen.) (see below). C. laticauda (Whitelegge) differs, according to description and figures published by that author, in shape of epistome, palps of maxillipeds, and rami of plp.[1] strongly from C. typa; it must therefore be removed from Cassidina and is very remote from Cassidinidea (n. gen.), but in spite of the lengthy description with five figures—occupying three pages—it is impossible for me to refer this species, not only to any genus, but to any section or group of the Sphærominæ.

The result is that of seven species referred to the genus Cassidina (M.-Edw.) at least one, and probably two, must be cancelled as synonyms, while the five others, having only a broad body and a reduced exopod of the uropods in common, must be referred to at least four genera, and two of these, established respectively in 1887 and 1901, have been so imperfectly described that reference to genus or to group of genera is impossible. This state of things illustrates excellently the confusion arising from extreme want of guiding principles of investigation, etc., in the study of the family.

(7) Chitinopsis (Whitelegge).—The type is C. spatulifrons (Whitel.), of which I have not seen any specimen. No other species has been established, but I have inspected a new form allied to the type. The two species have a curious aspect, but the genus is in reality so closely allied to Cassidina that it can only be considered a sub-genus, and ought perhaps to be cancelled. It seems to be impossible to find any character of some importance; the characters used in the conspectus are, I hope, tolerably practical.

(8) Cassidinidea (n. gen.).—This genus is established on Næsa ovalis (Say). In the British Museum I saw three specimens presented by Thomas Say; the Copenhagen Museum possesses a few specimens from Grenada, and one specimen from Cincinnati. Cassidina lunifrons (Richardson) belongs probably to the same genus; according to a figure published by Miss Richardson, it is very closely allied to N. ovalis, if it may be assumed that a portion of the basal joint of both antennulæ in reality is the front end of the broad epistome. It may be useful to add a few notes on C. ovalis, as nothing has been published on this species since the description of Say in 1878. An adult female measures 6 mm. in length and 3·3 mm. in breadth; judging from Miss Richardson's figure, C. lunifrons is proportionately a little broader, and its head somewhat broader than in our species. The body is very depressed, its upper surface grey-mottled with brown and dark brown. The epistome is a little more than twice as broad as long at the mesial line,

its anterior part is broad, cut off transversely, and protrudes as a narrow transverse band in front of the head. The two proximal joints of the antennulæ somewhat depressed, oblong, with the margins sub-parallel ; third joint slender, as long as the first; flagellum five-jointed. Uropods about as in C. lunifrons (compare Miss Richardson's figure). The single male seen is adult, a little smaller than the female, but the uropods are proportionately a little broader. In both sexes the end of abdomen is cut off transversely.

(9) Leptosphæroma (Hilgendorf).—The type is L. Gottschei (Hilg.), of which I received three typical specimens from the Berlin Museum. No other species referable to this interesting genus has been described, but I have seen specimens of a new, very small form from Singapore. The genus shows in aspect a certain similarity to Plakarthrium (Chilt.), but the agreement is, however, only superficial, which is easily seen by a perusal of the diagnoses of the sub-families Sphærominæ and Plakarthriinæ.

(10) Ancinus (M.-Edw.).—The type is A. depressus (Say), referred by Say to the genus Næsa (Leach). The British Museum possesses a single exsiccated specimen presented by Thomas Say; in 1902 I examined its external structure. The figures (Pl. XXXII, figs. 17–20) in H. Milne-Edwards' 'Hist. Nat. Crust.' convey a rather good idea of the outline of the animal and of the shape of the hands of first and second legs of the male. The specimen named seems to be the only one existing in any zoological Museum ; at least, I have asked for material of this form in Paris and in American Museums, but with negative result. No other species is known.

(11) Ancinella (n. gen.).—This interesting genus I establish on a new species, A. profunda (n. sp.), of which a large number of specimens were found in bottom material secured by Dr. Joh. Schmidt in 1904 during the cruise of "Thor," the Danish ship in the service of the International Commission for marine investigations. The locality is lat. 61° 15′ N., long. 9° 35′ W., 900 meter. As supplement to the diagnosis of the genus a short description of the species may be inserted here.

The body is much depressed, similar in both sexes; an adult female measures 4·5 mm. in length and 2·8 mm. in breadth, broadest at the end of thorax; the largest females are a little larger than the males. Third joint of antennulæ is very slender, as long as the sum of the two proximal joints. Prehensile hand of first thoracic legs large, oblong-oval, in the male a little longer and narrower than in the female; prehensile hand of second legs in the male very much smaller than that of first pair, but rather similar in shape; the "claw" as long as the hand. Last abdominal segment triangular, at the base less broad than the broad but very short anterior part of abdomen; the upper side of the last segment with a raised semicircular ridge sub-parallel with the lateral margin and at the mesial line a little longer from the end than from the anterior margin; parallel with and rather near the whole lateral and posterior margin a sublinear impression is found; the margin itself is finely serrate. Exp. of urp. reaches slightly beyond the abdomen; it is rather narrow, flat, especially on its outer margin finely serrate; the end is bifid, its inner process several times smaller than the outer.

(12) Tecticeps (Richardson).—The type is T. alaskensis (Richardson); a few years after the same author established a second species, T. convexus. The U. S. National Museum has favoured me with several specimens from the same locality ("Albatross," Stat. 3307), labelled T. alaskensis. At a closer examination the majority turned out to be males, but two of them adult females. In the male second thoracic legs terminate in a rather small prehensile hand; the sixth joint ("propodus") is oblong, with a short process on the lower side at the broader base; seventh joint, which is adduced along the lower margin of the sixth, is rather considerably longer than the latter and besides sinuate; sixth joint of seventh thoracic legs is considerably longer and thicker than the corresponding joint of sixth legs, and its proximal half is somewhat curved; the end of abdomen is acute and exp. of urp. considerably longer than endp. In the female second legs are simple as third pair; sixth joint of seventh legs is

slightly shorter and a little more slender than that of sixth pair; the end of abdomen is rather obtuse, and exp. of urp. not longer than endp. But while these remarks on seventh thoracic legs, end of abdomen, and urp. agree well with Miss Richardson's description of T. alaskensis, my remarks on the female agree with description and figures of T. convexus; the latter species must, therefore, be cancelled as established on females of T. alaskensis. The male is, besides, larger than the female; in Miss Richardson's paper some differences between length of antennulæ and antennæ in the two "species" are noted; a difference in the place of the eyes is also mentioned. The eyes are nearly equal in size in the two sexes and they occupy exactly the same place, but the area between the front end of the eyes and the anterior margin of the head is broader in the male than in the female.

EXPLANATION OF PLATE 7.

(1) Cymodoce pilosa (M.-Edw.).

Mouth-parts of an adult male (small specimen); all × 18.

Fig. 1 *a*.—Left mandible, from below.

Fig. 1 *b*.—Distal half of same mandible, obliquely from below and from the inner side.

Fig. 1 *c*.—Left maxillula, from below.

Fig. 1 *d*.—Left maxilla, from below.

Fig. 1 *e*.—Left maxilliped, from below.

Fig. 1 *f*.—Hypopharynx (paragnatha), from below.

(2) Cymodoce pilosa (M.-Edw.).

Mouth-parts of an ovigerous female (specimen of about the same size as the male shown in figs. 1 *a*–1 *f*); all × 18: same enlargement as in the male.

Fig. 2 *a*.—Left mandible, from below.

Fig. 2 *b*.—Left maxillula, from below.

Fig. 2 *c*.—Left maxilla, from below.

Fig. 2 *d*.—Left maxilliped, from below.

Fig. 2 *e*.—Hypopharynx, from below.

(3) Dynamene bidentata (Mont.).

Mouth-parts of an adult male, all seen from below; × 35.

Fig. 3 *a*.—Left mandible.

Fig. 3 *b*.—Left maxillula.

Fig. 3 *c*.—Left maxilla.

Fig. 3 *d*.—Left maxilliped.

(4) Dynamene bidentata (Mont.).

Mouth-parts of an ovigerous female, seen from below; all × 38, thus nearly the same enlargement as in the male.

Fig. 4 *a*.—Left mandible; the dotted line indicates the outer margin of the mandible situated beneath the firm skin by which the mandible is united with the head.

Fig. 4 *b*.—Right mandible.

Fig. 4 *c*.—Left maxillula.

Fig. 4 *d*.—Left maxilla.

Fig. 4 *e*.—Left maxilliped.

(5) Hemisphæroma pulchrum (n. gen., n. sp.).

Fig. 5 *a*.—Left mandible of an immature female, seen obliquely from the inner side and from below; × 24.

(6) Cassidina typa (M.-Edw.).

Fig. 6 *a*.—Left maxilliped, from below; × $\frac{25}{2}$.

The Descriptive Anatomy of the Brain and Cranial Nerves of Bdellostoma Dombeyi.

By

Julia Worthington,
Walnut Hills, Cincinnati, Ohio.

With Plates 8, 9, 10 and 11.

Table of Contents.

Introduction.

The species principally used for this investigation was Bdellostoma Dombeyi, the ordinary hag-fish of the Pacific coast; some points of the gross anatomy, however, were worked out from specimens of B. Forsteri, from the Cape of Good Hope. There is no difference in the gross anatomy of the two species but that of size, and the B. Forsteri, being slightly larger, made some of the fine dissection easier.

The investigation of the brain and cranial nerves of Bdellostoma was suggested to me by Dr. Howard Ayers, who kindly furnished me with a great deal of his own material; and I am glad to have this opportunity of expressing my thanks for the interest with which he has assisted and directed every stage of the work. I also wish to express my thanks to Mr. John G. Koch for his kindness in drawing for me figs. 1, 2, and 3, and in assisting me with fig. 14.

From the standpoint of the comparative anatomist there is interest attaching to a study of the brain of Bdellostoma. The Myxinoid fishes are the most primitive craniates known, and, to judge from the constitution of their central nervous system, they are several degrees lower in the scale than their nearest cousins, the Petromyzonts. For this reason we have in them a simpler pattern, a more primitive or ancestral arrangement of parts than in the higher brains previously studied. Moreover, the embryological work of Price, 1896, and Dean, 1899, shows that this simplicity is

primitive, and not degenerative, as has sometimes been stated. The older naturalists, deceived by the curious jaw formation of the Myxinoids, ranked them as a side branch, decidedly separated from other vertebrate forms, but Dr. Ayers and Mr. C. M. Jackson, in their paper published in 1900, solved this puzzle, proving the homology of the Myxinoid jaws with those of other gnathostomes, and so placing these fishes in the main line of development of the gnathostomes. For all these reasons a thorough working out of the Myxinoid brain promised to throw much light upon that most interesting of problems, the origin and development of the vertebrate brain.

The present paper is a general survey of the ground covered by my studies. The following detailed account of the gross anatomy of the brain and the distribution of the cranial nerves prepares the way for a detailed description of the finer anatomy of the several parts of the brain of Bdellostoma. The work includes the dissection of Bdellostoma heads preserved in formalin, and the microscopic study of sections both of the brain and of the entire head stained in hæmatoxylin or in alum carmine. A discussion of the methods used is reserved for a later paper.

The Cranium.

The fishes, from which the brains studied were taken, measured from 62 to 64 cm. from the tip of the nose to the end of the tail.

The brain and spinal cord of Bdellostoma lie in a tough but flexible capsule of dense fibrous connective tissue. The fibres are very thick and tough, some of them being 6·7 μ in diameter, and may be either straight or wavy. The capsule varies very much in thickness in different parts of its walls, these walls being much thinner at the cephalic than at the caudal end.

The general shape of the cranium is that of a flattened

cylindrical flask, with its closed base at the anterior end, and with its open mouth in the form of a tube passing into the sheath of the spinal cord behind. At the cephalic face, in the centre of the area perforated by the olfactory fibres, the wall is ·192 mm. thick. In the median dorsal line it is ·168 mm. thick over the fore part of the olfactory lobes, and increases gradually in thickness as it proceeds caudad, measuring ·296 mm. over the habenular ganglia, and ·5 mm. over the cerebellum and fore part of the medulla. It then decreases gradually until, passing into the capsule of the spinal cord, it is only ·364 mm. thick. On the ventral surface the thickness is distributed a little differently. The cranium is ·167 mm. thick under the anterior end of the olfactory lobes, and ·224 mm. under the cephalic border of the cerebrum. As it passes under the 'tween brain, however, it becomes thinner, being only ·168 mm. thick immediately anterior to the hypophysis. Caudad to the infundibular process it becomes thicker again, measuring here ·28 mm., and under the central part of the medulla it measured ·296 mm. The capsule becomes very much thicker as it approaches the notochord, and finally receives that structure within itself, surrounding it with a thin sheath, cf. Ayers and Jackson, 1900. The lateral walls of the cranium measure ·168 mm. in thickness by the side of the olfactory lobes, and then thin out to only ·085 mm. beside the fore brain. At the side of the mid brain, just anterior to the foramen of exit of the trigeminus nerve, it is ·207 mm. thick, and just cephalad of the ear it measures ·267 mm. At the side of the spinal cord the thickness is ·28 mm.

The density of the cranial structure varies inversely with the thickness. Where the wall is thinnest the fibres are straight and very closely packed. As it increases in thickness they separate more and more, some of them becoming wavy; and where it is thickest, as in the dorsal surface over the cerebellum and medulla, and just in front of the notochord, the fibres are comparatively loosely packed, and almost all of them are more or less wavy. At the hind

end of the medulla, ventral to it, and just anterior to the notochord, lying between the layers of the fibrous capsule, is a thick plate of cartilage, connecting the cartilaginous ear capsules. This is the only cartilage found in the cranium.

The cranial cavity is 11 mm. long and 3 mm. deep. The width varies, measuring 6 mm. across in the region of the olfactory lobes, 5 mm. across at the foramen of the trigeminus, and $3\frac{1}{2}$ mm. at the foramen of the vagus. The lateral and anterior walls of the cranium are pierced with numerous openings for the exit and entrance of the cranial nerves and the blood vessels that supply the brain. Owing probably to the fibrous composition of the cranium, the nerve foramina are not definite openings in the wall, but rather places where the fibres have been pushed apart, and the meshes between them consequently enlarged. Through these meshes pass the bundles of fibres composing the nerve trunks as threads through a sieve (fig. 10). The inner surface of the capsule is lined throughout with a layer of endothelial cells.

The Cranial Membranes.—The brain of Bdellostoma has but one enveloping membrane—the dura mater. This is composed, as usual, of areolar tissue, and consists of two layers—an outer one lying close to the inner surface of the cranial capsule, but separated from its lining membrane by the extra-dural lymph space, and an inner one following the contour of the brain, leaving, however, a small subdural space between it and the brain (fig. 7). Both ventrad and dorsad of the 'tween-brain, and ventrad of the anterior end of the olfactory lobes, one or both of these layers delaminate into several finer ones. The space between the layers is filled with coagulated lymph, which is infiltrated with connective-tissue cells, and shot through with rather fine wavy fibres, some running singly, others in small bundles, connecting the two layers. These fibres are comparatively few in number around the fore part of the brain, but increase greatly dorsad of the cerebellum, and dorsad and ventrad of the medulla.

There are also large blood sinuses in this intra-dural space. The fibres of both inner and outer layers, throughout the greater part of the dura, are rather larger as a whole than those connecting the layers, and hold in their meshes the usual connective-tissue cells. Toward the caudal end of the medulla, at the level of the anterior end of the plate of cartilage described above, the entire composition of the dura changes. The finer fibres and most of the small cells disappear, and are replaced by heavy, coarse, stiffly-curled fibres, packed closely in the two layers, and fairly closely in the intra-dural space, particularly on the ventral side. In the dorsal intra-dural space, they are farther apart, and enclose in their meshes numbers of large ovoid cells, not found elsewhere. These cells have a fine protoplasmic network, staining lightly in hæmatoxylin and alum carmine, and a small, deeper-staining nucleus containing a very distinct nucleolus, and several granules.

The Brain.

General Description.—The brain of Bdellostoma is about 10 mm. long. It is from 5 to 7 mm. wide through the fore end, and from 4 to 4·5 mm. wide through the hind end. The following table gives the external measurements of four brains. Of these, brain A is that of B. Dombeyi, and B, C, and D belong to B. Forsteri.

Length.	A mm.	B mm.	C mm.	D mm.
Total length . . .	10·0	10·5	9·5	10·0
Olfactory lobes . .	1·5	2·0	1·5	1·5
Fore brain . . .	2·0	2·0	2·0	2·2
'Tween brain (dorsal view) .	1·5	1·5	1·6	1·5
'Tween brain (ventral view) .	2·5	3·0	2·4	
Mid brain . . .	2·0	2·0	2·0	2·0
Cerebellum . . .	1·5	1·5	1·5	1·6
Medulla . . .	5·2	5·0	5·0	5·5

Width.	A mm.	B mm.	C mm.	D mm.
Olfactory lobes (middle) .	5·0	7·3	6·0	6·2
Fore brain (cephalic end) .	4·5	6·5	5·0	5·3
Fore brain (middle) . .	5·0	6·5	5·5	5·6
Mid brain (cephalic end) .	4·5	5·5	4·5	4·6
Cerebellum „ „ .	3·5	3 5	2·6	3·0
Medulla (through *N.* V.) .	5·2	5·5	5·0	5·0
Medulla (through *N.* X.) .	4·0	4·5	4·0	4·5
Spinal cord . . .	2·2	2·5	2·0	2·5

The brain is bilaterally symmetrical in every respect, and when viewed dorsally appears to be divided into main parts —a fore part, consisting of four sets of paired lobes, with a smaller median lobe, situated between the second and third pairs ; and a hind part, an unpaired, shield-shaped division, with a V-shaped cleft cut into its cephalo-dorsal surface, in which cleft the posterior pair of lobes belonging to the fore part rest (fig. 1). The four sets of paired lobes proceeding cephalo-caudad are the olfactory lobes, fore brain, mid brain, and cerebellum, and the small median lobe lying between them consists of the habenular ganglia. The large, unpaired hind portion is the medulla.

In a view of the ventral surface these same divisions appear, but are of somewhat different form (fig. 2). The olfactory lobes alone appear in full size here. Slightly caudad of them the 'tween brain is to be seen, forming the most ventral prolongation of the brain. This important lobe extends caudad as far as the hind end of the mid brain, and laterad about half way from the median line to the lateral face of the brain, thus hiding from view the mesial portions of fore brain and mid brain. Close behind the caudal border of the 'tween brain, lies the cephalic border of the medulla. The medulla is ventral to almost the whole of the cerebellum, only the extreme cephalo-lateral parts of that section showing above it (fig. 2).

The Olfactory Lobes.—The two olfactory lobes are,

next to the medulla, the largest divisions of the brain, and thus give a line at once as to the relative importance of the several sections of the brain in the physiology of that organ. The lobes are of equal size and symmetrical, and almost entirely separated from each other, being united only at their ventro-mesial angles. Their mesial faces are flat and are apposed to each other, with a sufficient space between for a double fold of the inner layer of the dura to penetrate (fig. 7). Dorsally, the two lobes, taken together, are somewhat convex, the lateral faces rounding off from the dorsal to the ventral surface. Ventrally they are flat across the caudal end, and slightly concave across the cephalic end, their greatest thickness, dorso-ventrally, being not near the mesial face, but about half-way between that and the lateral edge. Examined superficially each lobe is seen to have three divisions—an inner, middle, and outer or lateral one (figs. 1, 2). On the dorsal surface the inner and middle divisions are nearly equal in width, and cover the crown of the lobe, the outer border of the middle division being about where the arch begins to fall away, so that the lateral division does not extend as far dorsally as the other two. On the ventral surface they are more plainly marked than on the dorsal, and it is the inner lobe that does not extend as far ventral as the other two, thus making the ventral surface concave. These three divisions have definite relations to the ending of the olfactory nerves.

The olfactory lobes, viewed in section, are highly vascular, and are seen to be very loose in structure. The cephalic half of each lobe is composed mainly of the olfactory glomeruli. On the ventral and lateral surfaces this glomerular layer extends almost as far caudad as the end of the lobes; on the dorsal surface it does not spread as far. It is the divisions of this layer that are indicated on the surface of the lobes, and the absence of the layer from the inner ventral division that causes the concavity of the ventral surface. The glomeruli themselves are quite large, many of them measuring about $80\,\mu \times 120\,\mu$. They are packed close

together, and lie with their axes arranged more or less longitudinally. Aside from the glomerular layer, the olfactory lobes consist of a loose mass of cells and fibres. The cells are, roughly speaking, of two kinds :—comparatively large multipolar cells, staining lightly in hæmatoxylin, and having large, round or ovoid nuclei, and smaller cells, spindle or multipolar, that stain an intense blue black. They are not arranged in any such definite way as the cells in other parts of the brain. The fibres are the secondary olfactory fibres. They come from all parts of the lobe, gathering into tracts as they leave it. The two chief tracts are the tractus olfacto-habenularis, and the tractus olfacto-cerebellaris. The tractus olfacto-habenularis leaves the olfactory lobe at the median dorsal angle and goes directly to the habenular ganglion, without any way station in the fore brain as is usual in the higher forms. The tractus olfacto-cerebellaris leaves each lobe at about the middle of the caudo-ventral border, and courses back, through the floor of the 'tween brain, ascending finally to the roof of the cerebellum.

The fibres of the olfactory nerves enter each olfactory lobe in the dorsal and lateral parts of its cephalic face. Owing to the fact that the nasal organ lies immediately adjacent to the cranium, and covers its anterior end wall, its nerves have neither the need nor the opportunity of uniting into one great nerve-trunk, as in other forms, but pass through the cranium and enter the olfactory lobes in the same bundles in which they leave the nasal organ.

The Fore Brain.—The fore brain, as mentioned above, consists of two lobes, a right and a left, equal and symmetrical. They are slightly smaller than the olfactory lobes, narrower across the caudal than across the cephalic face, and are separated cephalo-dorsad by a deep cleft, the continuation of the cleft between the olfactory lobes. In the median caudal part they fall away from each other, admitting between them the dorsal part of the 'tween brain, the habenular ganglia. The dorsal cleft between them extends

ventrad as far as the base of the habenular ganglia. Ventral to this they are united. There are no superficial divisions of the lobes of the fore brain as there are of the olfactory lobes.

On examining sections of the fore brain with the microscope the lobes are seen to consist of two parts, an outer shell, which is, however, from one to two thirds the thickness of the lobe, and extends over all but the ventro-caudal part, and a small inner core (fig. 8). The cells are of the same two kinds occurring in the olfactory lobes, but they are very differently arranged in the two parts. Those of the core are scattered through it without definite order. The shell, on the contrary, shows a very marked arrangement of parts (fig. 8). First of all comes an outer layer, about ·22 mm. thick, composed, probably, mainly of glia fibres and the dendrites of cells, and having cells scattered sparsely through it. Next comes the main cell layer, about the same thickness, for the most part, as the outer layer, and consisting of cells, crowded close together, with their long axes radially arranged. Inside of this is a third layer, similar to the first, and about the same thickness, only probably without so many glia fibres, and within this a thinner secondary cell layer, similar to the primary one. The space between the secondary cell layer and the core is largely filled with fibre tracts. Of these, the most conspicuous one leaves the dorsal part of the primary cell layer, and runs caudo-ventrad, laterad of the core. As it nears the ventral surface it turns mesad, crossing to the other side in the post-optic decussation, and distributes itself in the floor of the 'tween brain.

In the adult the lobes of the fore brain are solid, the ventricles having been obliterated by the encroachment of their walls on the ventricular cavities. In an embryo some little time before hatching the ventricles are very large, lying within the core, which is here much larger in proportion to the rest of the fore brain than in the adult. In this stage the shell lies cephalad and laterad of the core, but not dorsal to it, the dorsal wall over the ventricle being very thin. It

will be necessary for the later stages in the development of the brain of Bdellostoma to be more thoroughly worked out before it will be possible to homologise the parts of the adult fore brain with those found in higher forms.

The 'Tween Brain.—This important section of the brain is wedge-shaped, its broad flat base covering almost the whole under surface of the fore part of the brain, and its sides sloping inward as they rise, until they meet in a ridge in the habenular ganglia. The wedge is bilaterally symmetrical, and also leans somewhat cephalad, so that a sagittal median section is somewhat trapezoidal in shape, /‾‾\. The habenular ganglia are comparatively large, appearing externally as a separate division of the brain (see above). They lie, as before mentioned, close to the median line,—about midway between the lobes of the mid brain and those of the fore brain (fig. 1), but do not project as high as either of these; consequently they cannot be seen in a side view of the brain. They form the most anterior portion of the 'tween brain, projecting decidedly cephalad of the rest of this segment of the brain. In the specimen measured the right ganglion was 1·2 mm. long, ·5 mm. wide, and ·93 mm. deep; the left ganglion was ·78 mm. long, ·39 mm. wide, and ·74 mm. deep, and was so placed that it extended ·27 mm. cephalad of the right ganglion. Bdellostoma possesses no trace of an epiphysis.

The base of the 'tween brain extends nearly 1 mm. further ventrad than the surrounding parts of the brain, is shield-shaped in appearance, and slightly convex (fig. 2). It is marked by five small rounded hillocks. Through the first two of these, lying one on either side of the median line and about one third of the way back from the anterior edge of the 'tween brain, the optic nerves enter the brain. About half way between these and the hind end of the 'tween brain, situated in the median line, is the stalk of the infundibular process, and immediately behind it, one on each side, are two smaller projections, whose significance I have not been able to discover. The infundibular process is a small

round body, embedded in the brain capsule, and very apt to be torn away in dissecting out the brain. It will be described later.

The habenular ganglia.—These ganglia, though asymmetrical in form, are alike in structure. Their cells are smaller than those of any other part of the brain, but are similar to the two kinds already described. They are pressed close together throughout the ganglia, without definite grouping, and with their long axes running in nearly every direction. There are a good many more of the light staining cells in the ventral parts of the ganglia than in the dorsal. In addition to the cells there are great strands of fibres, the beginnings and ends respectively of the great fibre tracts that centre here. Most of these fibres belong to the tractus olfacto-habenularis. The fibres of this tract come from all over the dorsal half of the olfactory lobes, and gather into thick strands—one for each lobe,—at the caudo-mesial angle of the lobes. Running in this way through the cephalo-mesial parts of the fore brain lobes, they enter the habenular ganglia and cross in their ventral part, just caudad of the fore end of the right ganglion, in angles of from 45° to 90°, thus forming the habenular commissure. Across the caudal part of the ganglia run the fine fibres of the posterior commissure.

The tracts of the habenular ganglia, in addition to the tractus olfacto-habenularis, are the tractus habenulo-tectalis, and the bundles of Meynert. The fibres of the tractus habenulo-tectalis arise in the dorsal part of the ganglia. Some of them cross immediately to the opposite ganglion, others remain with the tract leaving the ganglion of their own side. The tracts are unsymmetrically placed in the ganglia, but are symmetrically placed in regard to the rest of the brain, the left tract leaving the left ganglion just before its caudal end, and the right tract being directly opposite to it. The tracts run ventrally in a slight caudo-laterad direction for about $1\frac{1}{4}$ mm., then turn sharply laterad, though still keeping a slight caudal direction, and

penetrate among the cells of the mid brain tectum, at about the centre of its lateral surface. The bundles of Meynert connect the habenular ganglia with the medulla. The bundles are symmetrically placed, but unequally developed, the right bundle measuring about ·28 mm. by ·185 mm., the left ·196 mm. by ·12 mm. Their fibres arise mainly in the dorsal part of the ganglia, and gather in one common median strand at the cephalic end of the right ganglion. They run ventrad until they reach the ventral level of the ganglia, then, turning sharply, they run caudad, still as one common strand, in the median line, directly ventral to the ganglia. While on this course the strand is increased by fibres coming from the more ventral part of the right ganglion. At the posterior end of the right ganglion the bundles separate and turn caudo-ventrad, running to the base of the mid brain. Here they decussate for the first time, and pass on into the medulla, decussating again at the level of the entrance into the medulla of the fibres of ramus ophthalmicus V, the decussation stopping about ·12 mm. cephalad of the exit of the motor trigeminus fibres from the medulla.

The Thalamus.—The thalamus of Bdellostoma possesses no features of marked interest from the standpoint of gross anatomy.

The Hypothalamus.—The base of the 'tween brain is rather a passage way for tracts than a place of origin and ending. The principal tract ending there is the one described above coming from the fore brain, and the crossing of whose fibres forms the great post-optic decussation. This tract and the tractus olfacto-cerebellaris occupy most of the floor of the 'tween brain.

Anterior to the optic nerves there are, in the base of the 'tween brain, two small groups of cells, one on each side. These groups consist both of the dark and of the light cells, similar to those of the habenular ganglia, only larger. The cells in the caudal end of the 'tween brain floor, also of the same two kinds already described, are arranged somewhat in longitudinal strands, their long axes running athwart the

brain. Bundles of fibres of the fore brain tracts run between these strands of cells.

The optic nerve enters the 'tween brain, as has already been mentioned, through a protuberance on its ventral surface. It is a small nerve, with fine, straight, deeply staining fibres. Upon entering the 'tween brain its fibres take a medio-dorsal direction, at an angle of about 45° to the floor of the brain. They are easily followed as far as the chiasma, which lies at the ventro-cephalic angle of the post-optic decussation. So closely applied is the chiasma to the decussation, that it is only by the different direction of the fibres,—those of the decussation running parallel to the base of the brain, and at right angles to its long axis,—that it can be distinguished from it. After the crossing the fibres no longer remain in the compact bundle in which they entered the brain, but separate into a loose strand, and are directed caudo-dorso-laterad through the post-optic decussation. Beyond this they separate still more from each other, running singly or in small groups, and consequently are quickly lost among the numerous other fibres of this part of the brain.

The Infundibular Process, Hypophysis, and Saccus Vasculosus. The infundibular process is, as has already been mentioned, attached to a stalk in the median line, situated about two thirds of the way from the cephalic to the caudal end of the base of the 'tween brain. The process itself is about 1·35 mm. long, 1·12 mm. wide, where the stalk enters it, and ·22 mm. thick. Its cavity is continuous with that of the infundibulum, and is lined with ependyma cells. These cells are of the cylindrical variety, and have long, stout processes, generally continuous with the long axis of the cell, but which may be deflected slightly from it. The cavity is lined with a single layer of the cells, set very close together, their long axes at right angles to the plane of the floor, and their processes coming from the end farthest from the cavity and penetrating through the wall to its other side. Under a magnification of forty diameters a section of the

floor resembles very closely a section of columnar epithelium. The roof of the cavity is from two to three times as thick as the floor, and is composed of two layers, (1) an inner lining layer, similar to the floor, except that the cells are not placed so close together, and, in consequence, some lie over on their sides, and the columnar effect is not so marked; and (2) an outer (dorsal) layer, containing a great many ependyma cells lying separate from each other, with their long axes in all planes. This dorsal layer also contains occasional large round cells, with round nuclei. Near the caudal end of the roof there is a small evagination, directed forward (fig. 13); the dorsal layer just referred to appears to be a prolongation of this evagination. In places there is a space between these two layers, in other places they are held firmly together by transfixing processes of ependyma cells. Immediately caudal to the stalk the two layers are fused together and merged completely into one. The canal of the stalk, instead of being placed in the centre, lies just behind the anterior surface, so that the cephalic wall of the stalk is the continuation of the thin floor of the process, and the caudal wall the continuation of the thick roof. The infundibular process lies embedded among the thick fibres of the membranous cranium, and directly beneath it, though separated from it by a layer of fibrous tissue, is the hypophysis. This consists of a much coiled tube, probably glandular (Retzius, 1895), but I have not been able to find any outlet for it, nor to determine the cell structure. The cells themselves have not stained at all, or only with the faintest tinge, in my preparations, so that I cannot tell their shape or size. The nuclei, however, which are fairly large, have stained intensely black or deep blue, so that the hypophysis in section looks like a collection of more or less rounded bodies filled with black granules (fig. 13).

A saccus vasculosus does not exist in Bdellostoma, but the elements out of which it develops in higher forms are there, and Bdellostoma consequently marks an interesting stage in its evolution. The foundation of the nervous element lies in

the thickening of the dorsal wall of the infundibular process and of the caudal side of its stalk, already described (fig. 13). From such a thickening the saccus arises in higher forms (Edinger and Hall, 1899), and the slight evagination photographed in fig. 13 is probably its rudiment. The vascular element is also found. The vertebralis impar artery that runs cephalad ventral to the notochord divides at the level at which the fibres of the vagus leave the brain into the right and left intra-cranial arteries. These arteries penetrate into the membranous cranium, and run cephalad in its ventral wall (figs. 10 and 11). At the level of exit of the motor fibres of the trigeminus each of these arteries gives off a large branch that divides immediately into three. The first of these branches, entering the dura, is directed backward, ventral to the medulla, and supplies the caudal end of that section of the brain. The second runs dorsad, entering the medulla at once, and running on into the cerebellum. The third runs forward, first in the membranous capsule, then in the dura, until the anterior end of the medulla is reached. It then turns sharply mediad, and forms, with its fellow of the opposite side, a vascular plexus that lies in the cleft between the anterior end of the medulla and the posterior end of the 'tween brain (fig. 13). This plexus sends arteries into the medulla, mid brain, and 'tween brain. Anterior to the foramen of the trigeminus the intra-cranial arteries give off a large branch that leaves the cranium, but they themselves continue forward in the cranial wall, supplying the fore part of the brain. Referring again to fig. 13, and noting again the evagination and thickening of the dorsal wall of the infundibular process, it is very easy to imagine an increase of this evagination that would eventually find its way into the cranial cavity through the foramen of the stalk. If it continued to grow it would be forced caudad after entering the cranium, the stalk blocking its passage cephalad, and would come into connection with, and finally envelop, the vascular plexus. This

would bring the saccus to the position it actually does occupy in higher forms.

The Ventricles of the 'Tween Brain.—These are of the usual type in Bdellostoma, but differ somewhat from those of higher forms in some of the details. The iter, running directly ventral to the habenular ganglia, is always small, its diameter varying in different specimens. In some the lumen, though contracted, can be traced unbroken into the third ventricle, in others the lumen is entirely closed shortly after the entrance of the iter into the 'tween brain, if not, indeed, before, but its course may still be traced by an irregular column of ependyma cells. In other brains still even the ependyma cells are scattered or absent. The third ventricle, though small, is always open; it is situated a little caudad of the anterior end of the right habenular ganglion, and is bounded as usual by the lamina terminalis. It is very much smaller in the adult than it is in the embryo. The infundibulum arises from what appears in the adult to be the cephalic end of the iter, but what the embryo shows to be in reality the posterior part of the third ventricle. In the adult the dorsal part of the infundibulum is always completely closed, and its presence indicated only by ependyma cells. In some specimens these cells are arranged in separated groups, and are hard to find; in others they are grouped compactly together, and their line may be followed with comparative ease. A little more than half-way between the third ventricle and the ventral surface of the 'tween brain, the infundibulum takes definite form. It is a long, narrow slit, lying in the median plane, showing longer in horizontal sections through its ventral end, than in those through its dorsal end, and directed caudo-ventrad (figs. 5, 13). In sagittal sections it is seen to be somewhat boot-shaped. The lumen becomes wider as the ventral surface of the 'tween brain is approached. At the anterior end of the most ventral level of this slit-shaped lumen is given off a small, unpaired, median recess directed forward. The hind end of the infundibular cavity is inserted into the middle point of a semi-

circular canal (fig. 5). This canal lies in the horizontal plane, its ends directed cephalad. A longitudinal section of the brain running through this canal and the infundibulum makes the two together look like the head of an anchor. There is a bulb-like dilatation of each end of the semi-circular canal, these dilatations lying in the two hillocks seen on the ventral surface of the 'tween brain just caudad of the infundibular stalk. This same relation of parts is found in Myxine (Retzius, 1895), but Johnston (1902) makes no mention of it in describing the infundibulum of Petromyzon.

The Mid Brain.—The mid brain consists, dorsally, of two equal and symmetrical lobes, divided by a median longitudinal cleft that becomes shallower as it proceeds caudad. Anteriorly the cleft widens out, the mid brain lobes separate from each other on the dorsal surface, and admit between them the hind part of the habenular ganglia (fig. 1). The lobes are about the same in length as those of the fore brain, but are narrower behind (fig. 1). Ventrally they taper to a comparatively small point that can be seen in section (fig. 6), but is obscured superficially by the medulla and 'tween brain.

Structurally the mid brain is somewhat similar to the fore brain, and merges into it gradually, so that in examining a series of cross-sections it is difficult to tell definitely just where the mid brain begins and the fore brain ends. Like the fore brain it may be divided into two parts—an outer shell, the tectum opticum, and a central mass. The relative size of the two parts, however, is more nearly equal (fig. 9), nor are they as sharply marked out one from the other. The tectum has but two layers, as against the four in the outer division of the fore brain, an outer glia layer, and an inner layer of cells. These layers are in every respect similar to the corresponding layers of the fore brain. The central mass consists of cells densely grouped together, except where separated by the fibre-tracts passing through this part of the brain.

The mid brain, though not of the same importance in Bdellostoma as in higher forms possessing better developed

eyes, and consequently larger optic nerves, is still an important section of the brain, and its fibre-tracts are more conspicuous than those of any other section except the medulla. The most striking of these is the dorsal decussation, whose fibres connect not only the roofs of the two lobes, but their sides and floors as well. Unlike Petromyzon (Johnston, 1902) the decussation does not extend through the whole mid brain roof, but is confined to its caudal part. Numerous small bundles of fine fibres leave the cell layer of the tectum and proceed towards the central mass, but they are so very fine that it is impossible to trace them in ordinary hæmatoxylin sections, or to separate from among them the fibres of the optic nerve. Conspicuous in the base of the mid brain, filling entirely its basal cone (fig. 6), are the bundles of Meynert, already described, on their way to the medulla; and, dorsal to their decussation, the ansulate commissure, comparatively small in Bdellostoma. This commissure is caudad of the bundles themselves, and lies so close to them that a small bundle of fibres of the right bundle is deflected by it and pierces down through the commissure instead of passing in front of it, rejoining its fellows later on. Leaving the central mass on each side is the tractus tecto-bulbaris et spinalis, running to the medulla and spinal cord. These, with the tractus habenulo-tectalis, already described, are the principal tracts found in the mid brain of Bdellostoma.

The iter enters the dorsal part of the mid brain as a straight open tube, whose lumen is about ·09 mm. in diameter. But before the habenular ganglia are reached the tendency to close asserts itself, the ependyma cells crowd in, the tube narrows, and the lumen becomes very small. In some specimens the lumen, in the fore part of the mid brain, is, in places, entirely closed.

The Cerebellum.—The cerebellum is the smallest division of the brain of Bdellostoma, though very much larger and more conspicuous than the same section in the brain of Petromyzon. It consists, in Bdellostoma, of two small lobes,

equal and symmetrical (fig. 1). Its roof is continuous with that of the mid brain, although superficially a slight depression marks the boundary between the two sections. The two lobes are divided in the dorso-median line by a continuation of the dorsal cleft that divides the mid brain lobes. The cerebellum rests solidly upon the dorsal surface of the forward end of the medulla (figs. 1, 6), covering the anterior end of the fourth ventricle, and, in consequence, has no peduncles. In structure it consists of an outer glia layer, similar to and a continuation of the outer glia layer of the mid brain, and an inner mass of cells. In the base of the cerebellum, on each side of the median line, lying partly in the cerebellum, and partly in the mid brain, is a group of from ten to fifteen multipolar giant-cells. Most of these cells send their axones back into the medulla, though whether all do or not I am not yet prepared to say. These cells are apparently like the Mauthner cells of the medulla. Lying close under the dorsal surface of the cerebellum is a decussation of fibres connecting its two lobes, a continuation backward of the dorsal decussation of the mid brain.

The cerebellum has also two other very prominent sets of fibres. Coming up from the floor of the 'tween brain, running dorso-caudad in the lateral walls of the cerebellum to end among the cells of its roof, are the fibres of the great tractus olfacto-cerebellaris, described above. Mesial to these, lying between them and the giant cells, are the fibres of another tract, equally important, the tractus cerebello-spinalis. These fibres, arising in the roof of the cerebellum, run ventro-caudad, and gather into two strong bundles, one on each side, at the caudal end of the cerebellum, just lateral to the groups of giant cells. On leaving the cerebellum, these bundles turn sharply latero-caudad. When the brain is dissected out, and its fore part is deflected downward, away from the medulla, as in fig. 4, these tracts are seen for a little distance in relief upon the dorsal surface of the medulla (fig. 4, *tr. c. s.*). Not for long, however, for they quickly plunge beneath the surface, penetrating between the

fibres of the sensory trigeminus, still running caudo-laterad, until they near the sides of the medulla. Here they turn again, and run caudad on their way to the spinal cord. The tractus tecto-bulbaris et spinalis, after leaving the mid brain, runs through the floor of the cerebellum, where it is fused with the roof of the medulla. These tracts, after leaving the cerebellum, are also raised above the surrounding dorsal surface of the medulla, and, when the fore part of the brain is deflected, may be seen in relief as two longitudinal strands, one on either side of the median line (fig. 4, *tr. t. b. et s.*). With the cerebellum in place, both these tracts and the tractus cerebello-spinalis are hidden.

The cerebellar ventricle leaves the fourth ventricle in the median line at its anterior dorsal angle, just before the beginning of the iter. The ventricle runs dorsad and dilates into an elliptical cavity that lies immediately ventrad of the glia layer of the cerebellum, and has its distal end projecting caudad (fig. 5).

The Medulla.—The medulla is the most intricate and complex of all the divisions of the brain of Bdellostoma. Although conformiug in the main to the usual vertebrate type, it is, nevertheless, owing to causes as yet undiscovered, in some ways decidedly different, and is well worth careful study. It is the largest single division of the Bdellostoma brain, and is, in fact, very nearly as large as the cerebellum, mid brain, 'tween brain, and fore brain put together (figs. 1—3). It is bilaterally symmetrical, a slight depression marking its median line on both dorsal and ventral surfaces. It is about as broad as the two lobes of the mid brain, and about as long as mid brain and fore brain combined—for its actual measurements see the table on pages 142–3. On its ventral surface it is shield shaped, the top of the shield resting against the 'tween brain, the point at the base cut off by the spinal cord. At the upper angles of the shield the trigeminus leaves the medulla, at the lower angles the vagus. The facialis, acusticus, except acusticus *a*, and glosso-pharyngeus leave along the sides. Directly

caudad of the apex there is a semi-circular band, whose cut ends point forward, sculptured in slight relief (fig. 2). This ring consists of the two bundles of Meynert and their second decussation. A longitudinal depression, not as clean cut as the median one, divides each side of the ventral surface of the medulla into approximately equal parts, so that the ventral surface consists of four longitudinal divisions—two lateral and two median. In the caudal end of the median divisions are continued the ventral fibre-tracts of the spinal cord; in the lateral divisions are lodged the lateral motor columns of the medulla.

Turning now to the dorsal surface, we see that the raised dorsal part of the spinal cord expands into a large Y, whose thick arms compose almost the entire dorsal surface of the medulla, not separating until more than half-way to their anterior end, where they admit between them the lobes of the cerebellum (fig. 1). Three distinct divisions are sculptured on the arms of the Y:—(1) The great strands that run from the lateral part of the raised portion of the cord and form the caudo-lateral part of the arms. These strands are the ascending fibres of the sensory trigeminus; (2) two small bands running from the median part of the dorsal swelling of the cord along the median faces of the arms. These are the fasciculus communis of each side, and are visible on the dorsal surface from the caudal end of the medulla to the fork of the Y (fig. 1, *f. c.*). They are much thicker at the caudal end than at the cephalic; (3) the third division lies between the communis and the sensory trigeminus, and consists of the tuberculum acusticum, the "dorso-lateral strands" of Goronowitsch and some other writers. After the arms of the Y have separated, the acustic bundles run along their inner border to the cephalic end of the medulla (fig. 1, *ac.*). Entering the acusticum, ·12 mm. caudad of the hind end of the cerebellum, is the acustico-lateral nerve acusticus *b*—in young forms, where the cerebellum extends farther caudad, relatively, over the medulla, this nerve enters the medulla in front of the hind end of the

cerebellum; this condition is shown in fig. 14. Cephalad of this strands are seen running from the acusticum athwart the arms of the Y to the sides of the medulla. These are composed of fibres of the acustico-lateral nerve acusticus *a*, and of the ear nerves—acusticus *c* and acusticus *d*.

The Fourth Ventricle.—Leaving the external anatomy of the medulla of Bdellostoma, and turning to a study of sections through it, its most striking feature is found to be the smallness of the fourth ventricle, and the entire absence, except for about 11 μ behind the cerebellum, of the exceedingly thin roof found over the fourth ventricle of other vertebrates. The fourth ventricle of Bdellostoma is composed of three distinct parts, an anterior and a posterior dilatation and a connecting canal. The anterior dilatation is 1·07 mm. long, ·036 mm. wide, and ·59 mm. deep. It lies in the cephalic end of the medulla, and is almost entirely covered by the cerebellum. The thin part of the roof lies over the hind end of this dilatation. The iter leaves the fourth ventricle at its cephalo-dorsal angle, and immediately ventral to the iter is a small finger-like diverticulum (figs. 5, 6). The hind end of the dilatation gives off several small diverticula dorsal to the connecting canal. The number of these varies in different specimens, one brain having two, and two others three. Sometimes one of these diverticula may divide into two. The connecting canal leaves the anterior dilatation at its caudo-dorsal angle, and runs straight caudad to the canal of the spinal cord. It is ·065 mm. in diameter. The posterior dilatation is the most curious part of the fourth ventricle. It begins ·57 mm. cephalad of the commissura infima Halleri, and ends ·21 mm. caudad of it, having a total length of ·78 mm. Before it is reached the connecting canal has become narrower, so that it is about half as wide as it is deep. The posterior dilatation is semicircular in cross section, ·168 mm. wide and ·073 mm. deep, and is placed dorsal to the canal, which opens into it. Canal and dilatation together look, in cross section, like a longitudinal section of a toadstool. As

the spinal cord is reached the whole ventricle contracts, preserving, however, its peculiar form, which is continued in the central canal of the spinal cord (fig. 12), the only difference being that in the cord the dorsal part is much smaller compared to the ventral than in the medulla.

Regions of the Medulla.—In analysing the medulla of Bdellostoma into its component regions it is easily seen that the great ascending tract of the sensory trigeminus is the dominant structure. This tract, judging from Johnston, 1902, is relatively much larger in Bdellostoma than it is in Petromyzon. It is the largest and most important tract of the hind brain, just as the olfactory tracts are the largest and most important tracts of the fore part of the brain, and it dominates all the anatomical arrangements in the medulla, displacing other tracts. It springs from the nucleus funiculi, a diffuse group of cells lying near the dorsal surface of the hind end of the medulla. Whether this nucleus is in Bdellostoma a development of the dorsal horn of the spinal cord, as stated by Johnston for Petromyzon, I am not prepared to say, but its position makes it likely. The nucleus extends cephalad for quite a distance, and the fibre tract, constantly growing larger and more pronounced, takes its way in the dorso-lateral part of the medulla to emerge at its cephalo-lateral angle as the greater part of the trigeminus nerve (figs. 1, 10, 11).

The fasciculus communis, the continuation in part of the dorsal columns of the spinal cord, lies in the dorsal part of the medulla, next to the median line (fig. 1). A little cephalad of the commissura infima Halleri, a crescent-shaped line of cells, the nucleus of the fasciculus communis, appears on the ventral surface of the fibre tract, and continues as a semicylindrical layer as far forward as the exit of the facialis from the brain. A little behind this the fibre tract begins to spread farther over the surface of the medulla, gradually shifting its position to one more removed from the median line (fig. 11). While shifting its position it also sends fibres laterad over the sensory trigeminus to the

sides of the medulla, and, shortly after the level of the posterior end of the cerebellum is reached, the fasciculus communis is entirely displaced from the dorsal to the lateral surface. As soon as its fibres reach the lateral surface they turn caudad, running backwards to the facialis, glosso-pharyngeus, and vagus respectively.

The acusticus nucleus makes its appearance as a small clump of cells a little caudad of the level of the facialis, and lies not far from the median line between the fasciculus communis and the sensory trigeminus. It increases in diameter as it proceeds cephalad, and stains more deeply in hæmatoxylin than the surrounding tissue. As the fasciculus communis passes over to the side the acusticus comes to lie on the extreme dorso-mesial surface of the medulla (fig. 10). The fibres of the two sets of nerves arising from it, those of the lateral line and those of the ear, leave it in entirely separate groups. The most cephalic set of fibres is that of Acusticus *a*. These leave the dorsal surface of the acusticus in a well-defined group ·57 mm. from its anterior end, and pass laterad to the cranium above the dorsal surface of the medulla just cephalad of the acusticus ganglion. The fibres of the ear nerves are given off from just behind those of Acusticus *a* to within about ·5 mm. from the caudal end of the nucleus. Most of them pass laterad over the sensory trigeminus, leaving the medulla along its dorso-lateral edge, but some are deflected by the sensory trigeminus, pass ventrad of it, and leave the medulla in separate fibre groups along its mid-lateral surface, entering the ganglion near its ventral border. The fibres of Acusticus *b* arise in the ventral portion of the acusticus about three fifths of its length behind its cephalic end (fig. 11), and pass latero-dorsad to emerge upon the dorsal surface of the medulla. In the specimen from which the photograph of fig. 11 was taken ganglion cells lie among the fibres immediately outside the brain. If fig. 11 be examined with a magnifying glass a few can be seen in the nerve of the left side.

The motor cells of the medulla are gathered in two main groups, a lateral and a ventral motor column. The latter is the continuation in the medulla of the ventral horn of the spinal cord. It is comparatively small, and lies near the median line, ventral to the commissural fibres of the median raphé, and continues cephalad nearly to the anterior end of the medulla. It gives fibres to the vagus, but so far I have not been able to find any fibres going from it to the other nerves. Near its anterior end, about the level of the hind end of the cerebellum, it contains a number of cells of Mauthner, very large and irregular in shape. The lateral motor column begins at the hind end of the medulla and runs along its ventro-lateral angle as far forward as the motor trigeminus. It consists of three divisions, an anterior, closely packed sphere of cells, a middle division, in which the cells lie farther from each other and without definite arrangement, and a posterior division, where the cells are arranged in several longitudinal rows. This last division supplies the vagus and glosso-pharyngeus. The facialis draws its motor fibres mainly from the posterior half of the middle division, though taking a few from the anterior half. Most of the cells of the anterior half of the middle division, and all of those of the anterior division, send their fibres into the trigeminus.

The two sides of the medulla are connected along its entire length by a system of commissural fibres, which seem to come from all the regions of the medulla, and which cross in the median raphé immediately ventral to the fourth ventricle.

The Cranial Nerves.

Bdellostoma has only seven of the ten to twelve cranial nerves found in most vertebrates, viz. the olfactorius, opticus, trigeminus, facialis, acusticus, glosso-pharyngeus, and vagus. Of these the olfactory and the trigeminus are the largest and most strongly developed,

the optic is the smallest, and the acustic may be divided into four parts, two lateral line nerves, acusticus *a* and acusticus *b*, and two ear nerves proper, acusticus *c* and acusticus *d*. There is no trace in the adult of any of the eye muscle nerves.

N. Olfactorius.—Before describing the olfactory nerve it is necessary to give some idea of the organ innervated by it. The nasal organ of Bdellostoma is about 8 mm. long, 7 mm. wide at cephalic end, and 9 mm. wide at its caudal end, and 7 mm. deep. The measurements belong to specimen B of the previous table. The organ is bilaterally symmetrical, and consists of seven trapezoidal folds, hanging by their broader bases to the dorsal wall of the nasal capsule, and tapering to a point at the cephalo-dorsal surface. The median fold expands into a bulb at the cephalic end. Besides these seven free-hanging folds there are two smaller half folds, or flaps, fastened throughout almost their whole extent to the lateral walls of the capsule. Each half of the median fold belongs physiologically to its own side of the organ, and is innervated exclusively by the nerve of that side. These folds are related in a very definite way to the divisions marked on the surface of the olfactory lobes. To make the description simpler I shall call the half of the median fold A, the other folds in order B, C, D, and the lateral flap E. The nerve-fibres of each of these folds gather into bundles as they leave them, one bundle for each half of a fold, and in this form pierce the cranial wall. Once within the cranium the bundles break up, the different sets of fibres separating to seek their respective endings in the olfactory lobes. The nerve-fibres of A and B run exclusively to glomeruli in the dorsal part of the olfactory lobes, those of A to the inner division only, those of B to the inner and middle divisions. The C fibres run to both the dorsal and ventral parts of the lobe, the dorsal fibres being distributed to the middle division, the ventral fibres ending in all three, but principally in the inner and lateral ones. The fibres of D and E run exclusively to the ventral half of the lobes, those of D

going to the ventral surfaces of the middle and lateral divisions, those of E to the lateral division only (figs. 1 and 2).

Interesting questions arise here: (1) What physiological significance has this difference in distribution, and (2) what, if any, are the differences of connection of these different divisions of the glomerular layer with other parts of the brain? I hope, in a later paper, to throw some light upon at least the last of these questions.

N. Opticus.—The optic nerve of Bdellostoma is very small and delicate, owing to the undeveloped condition of the eye, the latter consisting merely of a round, two-layered cup. On leaving the eye the nerve runs medio-caudad, passing ventrad of r. ophthalmicus V and r. maxillaris posterior V, and dorsad of r. maxillaris anterior V and r. mandibularis V (fig. 14). It enters the cranium in the ventral part of its lateral wall, running caudad in the cranial wall for ·36 mm., and, emerging on the floor of the cranial cavity, runs dorso-mediad to enter the 'tween brain on its ventral surface as described above. There is no external chiasma.

N. Trigeminus.—This is the greatest of all the cranial nerves except the olfactory. It has two roots, a dorsal sensory, and a ventral motor. The sensory root is decidedly the larger and stronger of the two, and lies cephalad as well as dorsad of the motor. Most of the sensory fibres are fine, but a few coarser ones are found among them, notably in the ophthalmic branch; the motor fibres are coarse and stiff. All the fibres are medullated outside the brain. From the moment the nerve leaves the brain the ophthalmic fibres can be distinguished from those of the maxillo-mandibular trunk. They emerge cephalo-mediad of the others, and lie mesial to them in the nerve trunk until the ganglion is reached.

Leaving the medulla at its cephalo-lateral angle (figs. 1 and 2) the Trigeminus runs cephalo-laterad as a thick fibre bundle to the cranial wall, where it breaks up into several smaller strands in order to pass through the sieve-like

foramen. Once outside, the sensory fibres enter the Gasserian ganglion, while the motor fibres run, a compact bundle, along the ganglion's ventral surface. The ganglion itself is cylindrical in shape, its long axis directed cephalo-caudad; it lies just outside the cranium, and extends from the anterior end of the cerebellum to about the middle of the olfactory lobes. The ophthalmic and maxillo-mandibular parts of the ganglion overlap each other, the former extending farther cephalad, and not so far caudad as the latter. The ophthalmic cells of the ganglion, like the ophthalmic fibres of the nerve, lie dorso-mediad of the maxillo-mandibular portion.

R. Ophthalmicus.—This first branch of the Trigeminus is purely sensory. P. Fürbringer declares that it has a few motor fibres, but I have found absolutely no trace of them, either in the origin or distribution of the branch, whether working with sections or by dissection. After leaving the ganglion the ophthalmicus runs forward, passing dorsad of the optic nerve, between the eye and the skull. Immediately in front of the eye it gives off a stout supra-orbital branch, r. ophthalmicus cutaneus (r. cutaneus superficialis posterior, P. Fürbringer; r. dorsalis, Bowers, 1900). This branch runs dorsad between the muscles, appearing on the surface of the head muscles just cephalad of the eye. Running cephalo-laterad across the subdermal lymph space, it reaches the skin, runs cephalad immediately under it for some millimetres, and divides and subdivides into numerous fine branches, innervating the skin on the dorsal and lateral surfaces between the levels of the third tentacle and the anterior end of the nasal capsule (fig. 14). About half way from the Gasserian ganglion to the head, the main trunk of the ophthalmicus divides into a superficial and a deep branch. The superficial branch, which is the smaller of the two, runs forward near the nasal tube, and along the dorsal surface of m. palato-ethmoideo-superficialis (the names of the various muscles are those given by Fürbringer). On reaching the anterior third of the nasal tube it divides, sending one, or possibly two small

twigs to innervate the lateral face of the tube, while the rest goes on to the skin, innervating it in the extreme anterior dorsal head region, near the roots of the first and second tentacles. R. ophthalmicus profundus also runs forward, lying between mm. palato-ethmoideo-superficialis and palato-ethmoideo-profundus. On nearing the anterior end of the head it turns dorsad, piercing the former muscle, and dividing into small ramuli, supplying the skin around the nasal opening, and the second tentacle. The innervation of the first three tentacles is practically the same. The nerve enters in one or two large branches and immediately subdivides into several fine ones that fill the entire space between the cartilage core and the skin. These branches lie so close together that in longitudinal sections there seems to be a solid nerve mass surrounding the core; and it is only in cross sections of the tentacle that the nerve mass is seen to consist of fine ramuli, running in converging lines toward the tip.

Truncus Maxillo-Mandibularis.—This great division, containing both sensory and motor fibres, on leaving its own part of the Gasserian ganglion, divides quickly into four branches, two of them belonging to the r. maxillaris, and two to the r. mandibularis. R. maxillaris anterior (Fürbringer's r. anterior maxillaris externus) is a broad, stout trunk, running forward beneath the sub-ocular arch, and through m. palato-coronaris, where it divides into a superficial and a deep branch. The superficial branch runs forward between m. palato-coronaris and m. copulo-quadratus-profundus, dividing at the anterior end of the latter muscle into four branches. Of these the outer and smaller is purely motor, supplying m. copulo-palatinus, the others are sensory, supplying the first, third, and fourth tentacles, and the skin immediately around them. The nerve of the first tentacle is particularly rich in small skin rami. The fourth tentacle is different in shape from the other three. They have narrow bases and taper to a sharp point, while the fourth, which is really a fold of the lip, has a

broad base, and is short, blunt, and flabby. It has, in consequence, much more space inside of it than the other three, the nerve does not pack it closely as it does them, and its fine fibres are lost in the connective tissue. In addition to supplying the tentacle and the skin immediately around it, the nerve to the fourth tentacle has a large cutaneous branch that curves caudad, and supplies the lip at the lower angle of the mouth.

The deep branch is purely motor. It runs cephalad beneath m. palato-ethmoideo-profundus, into which it sends a large branch. This branch, after supplying the muscle, forks, sending a dorsal branch to supply m. nasalis and m. palato-ethmoideo-superficialis, and a ventral branch to m. tentacularis posterior. The main branch turns ventrad and forks, sending one branch running along the lateral face of m. copulo-ethmoidalis, into which it sends twigs, and then into m. copulo-tentaculo-coronaris; the other branch runs straight forward to end in m. ethmoideo-nasalis.

R. maxillaris posterior is sensory, and helps to supply the mucous membranes of nose and mouth. It has a very short trunk, dividing, before it reaches the sub-ocular arch, into r. nasalis and r. palatinus. R. nasalis passes over the sub-ocular arch and under r. ophthalmicus, lying so close to this nerve that Fürbringer thought it arose from it. It moves toward the nasal tube just in front of the olfactory capsule, and runs cephalad along its side, giving off several branches, thus supplying the posterior two-thirds of the tube. R. palatinus passes under the sub-ocular arch, and runs cephalo-ventrad inside the palatine bar, giving off several twigs to the pharynx by the way. At the anterior end of m. velo-quadratus it divides into four small branches and innervates the roof of the mouth. This nerve is Fürbringer's palatine branch of r. medius maxillaris externus.

R. Mandibularis (Fürbringer's r. posterior maxillaris externus V + r. maxillaris internus V).—This nerve is mainly concerned with the innervation of the dental plate

and its accompanying muscles, thus furnishing another proof of the statement made by Ayers and Jackson, 1900, that the tooth-plate represents the lower jaw, and not the tongue, as assumed by Müller and Fürbringer. R. mandibularis, like r. maxillaris, consists of two divisions—an anterior and a posterior,—both of which contain both sensory and motor fibres. The anterior division consists of four branches, the first of which, r. dentalis, is a large, purely sensory nerve, running ventrad m. palato-coronaris and the wall of the pharynx to the tooth-plate, in which it subdivides profusely. The second branch is motor, and is small and slender. It divides almost immediately into a short posterior branch, running into m. copulo-quadratus profundus, and anterior branch running cephalad outside of r. maxillaris anterior, and on the inner face of m. copulo-quadratus profundus, to which it appears to give small twigs. It then runs on to m. copulo-ethmoidalis, and apparently ends there. The third branch is also motor. It sends a tiny twig into m. velo-quadratus, and then runs ventrad between m. palato-coronaris and m. copulo-quadratus-profundus, supplying this latter muscle and m. hypo-copulo-glossus. Last of all is a small branch—motor and sensory combined,—the motor twig going to m. velo-quadratus, the sensory to the roof of the pharynx beneath.

R. mandibularis posterior consists of two divisions. The anterior division, though mainly sensory, carries a couple of motor twigs to m. velo-quadratus. The sensory trunk runs between this muscle and the roof of the pharynx, sending twigs into the latter, and ending in the pharynx behind the first branchial arch. The posterior branch of r. mandibularis posterior is the great nerve supplying the muscles of the lower jaw, the "club muscle" and its accessories. It runs ventrad between m. copulo-quadratus-profundus and the pharynx, supplying the caudal part of this muscle and m. hyo-copulo-glossus (Fürbringer's r. pro. hyo-copulo-glossus), and then divides into two, an anterior branch, supplying m. copulo-

glossus-superficialis, and m. copulo-glossus-profundus, and a posterior branch to the "club muscle," the retractor of the lower jaw. This last branch also divides into two, an outer and an inner. The outer branch runs straight caudad in the fascia of the outer circular muscle, and on reaching the muscle itself divides into dorsal and ventral branches. The ventral branch runs on the upper surface of the circular muscle just beneath the long central muscle, sending off tiny threads all along the way. At the end of the circular muscle it passes diagonally upward, running across m. perpendicularis. The dorsal branch runs in the depths of the dorsal part of the circular muscle just over the central muscle, and it also gives off tiny threads along the way. About half way back it anastomoses with its fellow of the opposite side, and from this anastomosis three or four strands are given off, all running straight backward. Among them are two principal ones, a dorsal strand that passes very obliquely upward and ends at the broad dorsal tendon of the circular muscle, and a ventral one that remains on the deeper level and, running through m. perpendicularis, ends in the long central muscle. The inner branch goes back a little, then turns suddenly mediad and enters the tendon of the long central muscle, running backward in the tendon.

Caudad of r. mandibularis the main trunk of the trigeminus gives off a small motor branch to m. velo-quadratus.

N. Facialis.—The facial nerve is exceedingly small and fragile, very difficult to dissect out, and hard to follow to its termination even in sections. It leaves the medulla caudad of acusticus *a* and acusticus *b*, passes through the acusticus ganglion, and breaks through the cranial wall just cephalad of the ear capsule. Hæmatoxylin sections give no hint as to its containing more than motor and communis fibres. On leaving the cranium the facialis runs caudo-ventrad under the hyoid arch. Its ganglion lies close to the ear capsule, and is always present, though variable in shape,

consisting sometimes of a compact group of cells, forming a cluster about twice the diameter of the nerve, and sometimes having its cells lying singly, embedded in the nerve cord. After leaving the ganglionic region the facial nerve continues caudo-ventrad until it reaches the anterior border of the crani-hyo muscle, into which it sends a small twig. Then turning, the nerve runs caudo-cephalad, curving over the hyoid arch, and courses cephalad on the mesial surfaces of mm. copulo-quadratus-profundus, and hyo-copulo-palatinus. It innervates the second of these muscles, but the innervation is hard to find, for instead of sending in the usual nerve twigs to subdivide in the heart of the muscle, it sends the fibres in as clusters of separate threads, and they are lost almost immediately. I have not found, either in sections or in dissecting, any twig answering to Fürbringer's r. cutaneus. While running on the inner surface of m. hyo-copulo-palatinus, the facialis divides into two or three branches, the dorsal branch carrying the motor fibres. There is nothing fixed as to the level of this division or the number of branches, the nerves of opposite sides differing sometimes in the same head. The branches turn ventro-laterad, passing between m. hyo-copulo-palatinus and m. copulo-palatinus, and forward over the latter muscle to the skin. They are so very small that I have not been able to trace them definitely to their endings in the skin, but I have every reason to believe that they terminate in the region of the fourth tentacle. I have found no communicating branch between the facialis and the trigeminus, or between the facialis and the glossopharyngeus.

The Nerves of the Lateral Line.—These two nerves, acusticus *a* and acusticus *b*, it is more convenient for purposes of comparison to consider together, for they represent in Bdellostoma what Johnston, 1902, calls the "lateral line VII" of Petromyzon, and Strong, 1895, the "dorsal VII" or "VII *b*" of Amphibia. The position of these nerves in Bdellostoma is exceedingly interesting, for here

only, so far as I know, do they arise and remain free from complication with the facialis or trigeminus. They arise from the tuberculum acusticum, and run, free from all entangling alliances, from there to the skin; hence it seems best to cast aside the old names and call them acusticolateralis *a* and *b*, or, more simply, acusticus *a* and acusticus *b*.

Acusticus *a* (Johnston's "ventral root of lateral line VII," Strong's "dorsal half of dorsal VII"), leaves the side of the medulla a little caudad of the trigeminus and immediately cephalad of the acusticus ganglion. Once past the cranium, its fibres run cephalo-laterad into a small ganglion lying caudad of, and in close contact with, the Gasserian ganglion. On leaving the ganglion the nerve runs cephalo-laterad toward the skin, passing caudad of the eyeball. It continues forward, directly beneath the skin, until near the front end of the nasal capsule, where it divides, sending one branch ventro-caudad, one ventrocephalad, and one cephalo-dorsad, supplying the skin at the side of the head, between the mouth opening and the anterior end of the brain.

Acusticus *b* [Johnston's "dorsal root of lateral line VII," Strong's "ventral half of dorsal VII," M. Fürbringer's "first spino-occipital nerve" (!)], leaves the medulla on its dorsal surface near the level of the hind end of the cerebellum, and runs dorso-laterad to the dura, then curving sharply, it runs caudo-ventrad in the dura, entering the cranium posterior to acusticus *c*, the first ear nerve (fig. 1). It generally has a ganglion lying within the cranial wall. Sometimes this ganglion is a very decided one, sometimes it consists only of a few cells. In addition to this it may have a second ganglion lying outside the cranial wall, or a few ganglion cells among its fibres immediately after leaving the brain. After leaving the cranium the nerve runs cephalolaterad without incident, entering the subcutaneous lymph space immediately behind acusticus *a*. It runs boldly out

to the skin, turns sharply dorsad, and then caudad, supplying the skin immediately over the fore part of the brain.

N. Acustici *c* and *d*. — The acusticus ganglion lies inside the cranium and the dura, close to the cephalic half of the lateral surface of the medulla. Most of the fibres from it enter the medulla along the dorso-lateral angle, but small clusters enter the lateral surface farther ventrad. The ganglion is pear-shaped, with its broader end cephalad, and is placed immediately caudad of the fibres of acusticus *a*. The acustic nerve consists of two branches, r. utricularis, acusticus *c*, and r. saccularis, acusticus *d*, entirely separate and distinct, and leaving the ganglion at different places. R. utricularis is the more cephalad of the two; it leaves the ganglion a little in front of its hind end, curves ventro-laterad, and enters the ear at its cephalo-ventral angle. R. saccularis forms the caudal continuation of the ganglion, running caudad between the glosso-pharyngeus and the cranial wall, its fibres passing mediad of the endolymphatic duct. At the hind end of the brain capsule, just cephalad of the foramen of glosso-pharyngeus and vagus, r. saccularis curves ventro-laterad, passing through the membrane into the ear capsule at its caudo-ventral angle.

N. Glosso-pharyngeus.—(Müller's and M. Fürbringer's r. glosso-pharyngeus X.) The glosso-pharyngeus leaves the medulla along the caudal half of its lateral face. It has from four to seven roots of origin lying one behind the other between the acusticus ganglion and the exit of the vagus, and contains both sensory and motor fibres. Its fibres run caudad between acusticus *d* and the vagus, lying so close to the latter that it is difficult to distinguish one from the other. The joint foramen of the glosso-pharyngeus and vagus lies at the caudal end of the cranium, in the angle formed by the junction of the ear capsule and the cranium, and the glosso-pharyngeal fibres pass out dorsal, anterior, and lateral to those of the vagus. The two nerves pass so close together, though, that I cannot say whether or not a few fibres of each stray into the trunk

of the other. Once outside the cranium the glosso-pharyngeus and vagus run together in the same sheath, and a few millimetres beyond the capsule each gives a few fibres to the trunk of the other. From this point on, however, the two nerves may easily be dissected apart. The glosso-pharyngeal ganglion is small, causing no enlargement of the nerve trunk, and lies a little caudad of the brain capsule. The glosso-pharyngeus runs in the same sheath with the vagus as far as the second branchial arch; then separating, it runs ventro-caudad, passes through m. constrictor-pharyngis, into which it sends a couple of branches, and so to the pharynx wall, dividing into several branches, and innervating the side of the pharynx near the end of the backward position of the second branchial arch.

N. Vagus.—The vagus is the most posterior of the cranial nerves of Bdellostoma, arising from the caudal end of the medulla by four or five roots. These roots run caudo-laterad as separate, though close lying, strands, passing between the dorsal and ventral roots of the first and second spinal nerves, to the wall of the brain capsule. After the give and take of fibres with the glosso-pharyngeus the vagus runs straight caudad, near to the notochord, until it nears the first gill sac. It has no ganglion near the cranium, although a few scattered ganglion cells may be found near the brain, and also outside the cranial wall. In the region of the branchial rami, however, these cells occur in great numbers. They are rather smaller than the cells of the Gasserian ganglion, are of a long, oval shape, as though compressed by the nerve, and are arranged in longitudinal strands. These strands are found from cephalad of the first to caudad of the last branchial ramus, and lie all through the thickness of the nerve, but are more plentiful at the periphery than at the centre. All of the gill sacs are innervated in the same way. A ramus branchialis is given off that divides into an interior and exterior branch. The interior branch runs to the duct connecting the gill sac with the œsophagus, and divides again into dorsal and ventral rami.

The dorsal ramus passes above the duct, innervating its dorsal surface; the ventral ramus divides once more, giving off a small pharyngeal branch, that also sends tiny ramuli to the proximal end of the duct, and another branch that penetrates the duct at its distal end near the gill. In one specimen dissected I found on the first gill of the right side another fine branch that entered the anterior edge of the gill itself on the outer side. After the last gill sac the vagus enters m. constrictor cardiæ, where it forms a dense plexus; emerging from this it joins its fellow of the opposite side to form n. intestinalis impar, running caudad on the dorsal surface of the intestine, and sending fibres into it all along its course.

The Spino-occipital and Spinal Nerves.

These are complete nerves, such as are found in the higher forms, with a sensory ganglion lying just outside the sheath of the spinal cord, and sensory and motor roots. There is a difference between the first two nerves emerging immediately caudad of the cranial nerves and those that come after, and Fürbringer's division into spino-occipital nerves and spinal nerves is a good one, though I cannot place my division where he places his. Judging from his paper (M. Fürbringer, 1897), he worked with mature adults only, and imperfect material. This explains in part his confusion in regard to these nerves, for natural growth causes somewhat of a shifting of relative position outside the brain between the young animal and the mature adult. In fig. 14, for example, which was plotted from a specimen the diameter of whose head was about half the diameter of the adult head, acusticus *b* and the first spino-occipital nerve are seen to be separated by a considerable space, but in the mature adult, such as fig. 1 was drawn from, the foramen of acusticus *b* is farther caudad with reference to the medulla—the medulla has apparently grown backward, carrying this nerve with it—and, as a result, when

outside the cranium, acusticus *b* lies for a little while immediately in contact with the first spino-occipital nerve. This is apparently one reason for Fürbringer's calling acusticus *b* the first spino-occipital nerve, in spite of the fact that it arises from the acusticus centre of the medulla. In regard to his possible ventral root to this nerve, which he admits not having been able to trace as far as the skull, I find nothing whatever answering to it, unless, indeed, he has in mind one of the separated sets of acusticus fibres that enter the acusticus ganglion from the ventral part of the lateral surface of the medulla.

The First Spino-occipital Nerve.—The roots of this nerve are seen emerging from and entering the spinal cord immediately caudad of the last root of the vagus, in that transition region where the medulla ends and the cord begins. It has one sensory root and two motor ones, the sensory root a trifle the most anterior of the three. The sensory and dorsal roots curve away from each other to admit of the passage between them of the glosso-pharyngeus and vagus, and then approach each other as they reach the cranial wall. Here a curious thing happens: the sensory root divides, passing the wall in two sets of fibres, through two distinct foramina. The anterior set has a small ganglion, apparently apart from the main ganglion, lying in or inside the wall. After passing the wall both strands unite to enter the main ganglion of the nerve. The two motor roots divide also, passing the wall through three foramina and uniting again on the other side, and motor and sensory roots unite to form one nerve-trunk laterad of the ganglion. It is possible that this division of the roots in passing the wall indicates the fusion in the first spino-occipital of two distinct nerves, and that study of different stages of embryos will throw some light on the question. The main trunk of the nerve runs cephalo-laterad, then, bending ventrad, it divides into two branches, that run ventro-cephalad on the inner face of the lateral longitudinal body muscle, supplying finally the lateral body muscles and the skin.

The Second Spino-occipital Nerve.—This second spino-occipital nerve is similar, in some ways, to the first, in other ways it is more like the purely spinal nerves that follow it. It also arises from the transition region between brain and cord. It has one sensory and four motor roots, which belong, however, to two main stems, of two roots each, corresponding to the two motor roots of the spinal nerves. In this nerve, as in the following ones, the motor roots are cephalad of the sensory ones. The sensory root passes out through its single foramen to the ganglion, the motor roots are variable on the right side of one specimen examined passing through two foramina, and on the left side uniting inside the wall and passing as a single trunk. It must be noted that in all the spinal nerves, however, the motor roots emerge from the wall as a single trunk, the separate foramina having run together and united before the lateral face of the wall is reached. The motor root, as in the case of the first spino-occipital nerve, runs ventrad of the ganglion, joining the sensory root as it emerges therefrom. The nerve then runs cephalo-caudad for a space, like the first spino-occipital, then turns ventrad, running on the inner face of the lateral body muscles. Unlike the first spino-occipital it does not divide into two branches until near the ventral surface.

The First Spinal Nerve.—This nerve is the first to arise from the region of the spinal cord proper beyond the area of transition, and there are other features about it that distinguish it sharply from the two nerves preceding. It has one sensory and two motor roots. As in all the spinal nerves, the motor roots pass the wall by a Y-shaped foramen, and emerge as one trunk. It is outside the wall that a striking difference is seen between this nerve and those that have gone before, for the spinal nerves supply the dorsal as well as the latero-ventral skin and muscles. The ganglion, instead of being a spherical or ovoidal cluster of cells, is elongate, with two arms, a dorsal and a lateral. The dorsal arm, running upwards outside of the sheath of the spinal cord, gives off the dorso-sensory branch of the first

spinal nerve. This branch runs dorso-cephalad until it reaches the top of the sheath, then mediad to the median line, and straight dorsad to the skin. On reaching the skin it runs cephalad, running near the median line, and supplying the skin until its twigs finally disappear near the end of acusticus *b*. The sensory nerve coming from the lateral branch is the one joined by the motor trunk, and the united nerve runs straight laterad beneath the dorsal body muscles. Several strong twigs, representing together Fürbringer's dorso-motor branch, penetrate this muscle and ramify inside it, while the ventral branch, motor and sensory combined, corresponding to the two spino-occipital nerves that have preceded the first spinal nerve, takes its way down the body wall on the inner face of the lateral longitudinal muscle.

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EXPLANATION OF PLATES 8, 9, 10 & 11,

Illustrating Miss Julia Worthington's paper on "The Descriptive Anatomy of the Brain and Cranial Nerves of Bdellostoma Dombeyi."

Abbreviations used in the Plates.

ac. Acusticus region of the medulla. *ac. g., acus. g.* Acusticus ganglion. *a. v.* Semicircular canal at base of infundibulum. *b. M.* Bundles of Meynert. *c. a.* Ansulate commissure. *cb.* Cerebellum. *cb. v.* Cerebellar ventricle. *c. c. s.* Central canal of the spinal cord. *c. M.* Cells of Mauthner. *d''. b. M.* Second decussation of the bundles of Meynert. *d. c. s.* Dorsal commissure of the spinal cord. *d. e.* Dorsal evagination of the roof of the infundibular process. *d. h.* Dorsal horn of the spinal cord. *d. r.* Dorsal root of spinal nerve. *f. b.* Fore brain. *f. b.* 1. Outer shell of the fore brain. *f. b.* 2. Inner core of the fore brain. *f. c.* Fasciculus communis. *Gass. g.* Gasserian ganglion. *g. olf.* Olfactory glomeruli. *h.* Hypophysis. *h. g.* Habenular ganglia. *i.* Iter. *inf.* Infundibulum. *inf. p.* Infundibular process. *int. a.* Intra-cranial artery. *ip. g.* Interpeduncular ganglion. *l. m. c.* Lateral motor column. *m. b.* Mid-brain. *md.* Medulla. *m. V.* Motor Trigeminus. *N. Acus. a.*,

N. Acus. b. Acustico-lateral nerves. *NN. Gl-ph. & Vag.* Glosso-pharyngeus and Vagus. *N. Olf.* Olfactory nerve. *N. Opt.* Optic nerve. *O. ch.* Optic chiasma. *olf. g.* Olfactory glomeruli. *olf. l.* Olfactory lobe. *olf. o.* Olfactory organ. *R. Nas.* Ramus nasalis. *R. Mand. ant.* 2, *R. Mand. ant.* 3, *R. Mand. ant.* 4. Second, third, and fourth branches of ramus mandibularis anterior. *R. Mand. dent.* Ramus mandibularis dentalis. *R. Mand. post.* 1, *R. Mand. post.* 2. First and second branches of ramus mandibularis posterior. *R. Mand. post.* 2 *a.* Anterior branch of ramus mandibularis posterior 2. *R. Mand. post.* 2 *b.* Posterior branch of ramus mandibularis posterior 2, running to the "club muscle." *R. Max. ant.* Ramus maxillaris anterior. *R. Oph. cut. V.* Ramus ophthalmicus cutaneus Trigemini. *R. Oph. sup. V.* Ramus ophthalmicus superficialis Trigemini. *R. Oph. pro. V.* Ramus ophthalmicus profundus Trigemini. *R. Pal.* Ramus palatinus. *R. pro. v. q.* Ramus profundus maxillaris velo-quadratus. *Sp. c.* Spinal cord. *Sp.* 1. First spinal nerve. *Sp. o.* 1. First spino-occipital nerve. *Sp. o.* 2. Second spino-occipital nerve. *s. V.* Sensory Trigeminus. *t. b.* 'Tween brain. *Ten.* 1, *Ten.* 2, *Ten.* 3, *Ten.* 4. First, second, third, and fourth tentacles. *tr. c. s.* Tractus cerebello-spinalis. *tr. f. t.* Tract running from the fore brain to the 'tween brain. *tr. h. t.* Tractus habenulo-tectalis. *tr. o. c.* Tractus olfacto-cerebellaris. *tr. t. b. et s.* Tractus tecto-bulbaris et spinalis. *v. p.* Vascular plexus. *v. h.* Ventral horn of the spinal cord. III *v*, IV *v*. Third and fourth ventricles. I, II, V, VII, VIII *a*, VIII *b*, VIII *c*, VIII *d*, IX, X. The cranial nerves respectively.

PLATE 8.

Fig. 1.—The brain, dorsal view. × 8½.

Fig. 2.—The brain, ventral view. × 7.

Fig. 3.—The brain, lateral view. × 7.

Fig. 4.—The medulla. The fore part of the brain is bent down till it forms with the medulla an angle of 120°. × 7.

Fig. 5.—Diagram of the ventricles of the brain. The dotted parts are always open. × 7.

Fig. 5 a.—The semicircular canal at the base of the infundibulum. × 7.

PLATE 9.

Fig. 6.—Photograph of a sagittal section of the brain, a little removed from the median plane. × 15.

PLATE 10.

FIG. 7.—Photograph of a horizontal section of the brain, about three fifths of the way from the dorsal to the ventral surface. × 15.

FIG. 8.—Photograph of a cross section of the brain through the entrance of the optic nerve. × 15.

FIG. 9.—Photograph of a cross section of the brain through the hypophysis. × 15.

FIG. 10.—Photograph of a cross section of the medulla, through the exit of the Trigeminus. × 15.

FIG. 11.—Photograph of a cross section of the medulla through the entrance of Acusticus a. × 15.

FIG. 12.—Photograph of a cross section of the spinal cord. × 42.

FIG. 13.—Photograph of a sagittal section through the infundibular process, in the median plane. × 40.

PLATE 11.

FIG. 14.—Diagram of the origin and distribution of the cranial, spinooccipital, and first spinal nerves. The outlines of the brain, eye, and ear are shown in dotted lines. × about 9.

The Ontogenetic Stages represented by the Gastropod Protoconch.

By

H. Leighton Kesteven.

Contents.

A. Introduction.

That the so-called "protoconch" may not be the true protoconch has been known ever since Lankester (7) drew attention to the fact that the primitive shell-sac or gland sometimes became filled with a chitinous plug which was subsequently shed, before the inception of the protective shell.

The shell which is formed immediately subsequent to this is that which conchologists generally have termed the protoconch; but this also may be shed, and leave behind it an apex, perhaps of several whorls, which differs materially from the rest of the shell, which apex has in turn been treated as a protoconch.

From a study of the subject extending over several years, I have come to the conclusion that a Gastropod protoconch may have been formed during one or more of four stages of growth, and it is even conceivable that it may in some instances consist of portions formed during all four. These four ontogenetic stages are: The typembryonic, phylembryonic, nepionic, and ananeanic, and the portions of the conch formed during each may be advantageously

termed respectively: Phyloconch, Veloconch, Nepioconch, and Neanoconch.

Before proceeding to define these conchogenetic stages it may be well to explain why I retain, and use in these definitions, the term "protoconch."

The "protoconch," in the accepted and almost universal interpretation of the term, consists of an indefinite number of the apical whorls of a Gastropod shell, irrespective of whether these apical whorls are the first shell or not. Dall (1) speaks of the Scaphella protoconch, well knowing that the structure so designated was formed subsequent to a horny bulb-like shell, which was discarded in the egg capsule. I have, myself, described the "protoconchs" of numerous species of Lotorium (No. 4), being at the time well aware that what I described were but calcareous casts of the horny veliger shells. Since, then, the term has attained to a general acceptance, to change its significance would immediately cause confusion; to attempt to discard it altogether would have the same effect. I say "attempt" advisedly, for it is not to be supposed that such a suggestion would receive immediate and universal approbation. Moreover the term is a useful one; it is here used in the above broad sense, namely, to include those apical whorls, disregarding their age, which by smoothness, distinctiveness, or size are differentiated from the succeeding whorls, and thereby indicate that at their conclusion the mollusc entered upon a new and important ontogenetic phase—such, for example, as the change from veliger to sedentary life, or the emergence from the egg capsule.

B. A Conchogenetic Nomenclature.

Phyloconch.—The name is taken from the fact that it is formed by the first distinctive "Phylum" character to appear. It is the "primitive shell" of Lankester, and is formed by almost every member of the Phylum, but, with rare exceptions, is shed at an early stage, and does not enter into the composition of the protoconch.

Among Prosobranchs it is retained by Neritina.

Veloconch.—The greater portion of this is formed during the veliger stage, though it may have been begun just before that stage; it is, in fact, that portion of the protoconch which is formed subsequently to the flattening out of the primitive shell gland and before the velum commences to be aborted.

It may be either corneous, as in Lotorium and Scaphella, and shed at an early stage; or calcareous, as in Triphora, and retained, or lost in the normal and almost universal decollation of apical whorls.

Nepioconch.—This is formed during the nepionic stage.

I have in Number III of 'Notes on Prosobranchiata' given reasons for regarding the varix sometimes thrown up at the conclusion of the veloconch as the only example of a true nepioconch.

Neanoconch.—The neanoconch, or perhaps more precisely the ananeanoconch, is formed during the ontogenetic stage from which it takes its name.

In Lotorium it is moulded inside the horny veloconch. In Scaphella (*fide* Dall) the veloconch is a round corneous bulb; the neanoconch is not moulded in this, but consists of three or four calcareous whorls formed by the free edge of the mantle, and having a pointed nucleus, which is formed in the veloconch, but not moulded in it. In Megalatractus aruanus (Linn) there are somewhat the same conchogenetic stages; the neanoconch of five whorls is formed by the free edge of the mantle, whilst the corneous veloconch is cast in lime and generally shed before the creature leaves the egg capsule.

c. Application of the Nomenclature.

I now proceed to apply this nomenclature to a few protoconchs of which descriptions and figures have been published.

In Clausilia, according to Gegenbaur, on the authority of Lankester (7), the primitive shell is retained, so that here the protoconch consists of, at least, phyloconch and veloconch. Lankester, also, mentions other instances of the

retention of the phyloconch, which it is unnecessary to detail here. In the following instances it is not known whether or not the phyloconch is retained.

Murex denudatus (Perry) presents us with a protoconch consisting of veloconch and nepioconch, the latter being represented by a varix (**3**).

The protoconch of Triphora is a veloconch; a portion of it in some species exactly resembles the adult shell, or true conch (**2** and **5**).

The protoconch of Lotorium is composed as follows:— In the veliger stage veloconch; in early neanic stages, neanoconch and veloconch, the former inside the latter; in stages later than ananeanic, of neanoconch only, the veloconch having by that time been shed (**4**).

In Megalatractus aruanus (Linn) the protoconch is a neanoconch, but if it be taken from the egg capsule the calcareous cast of the veloconch will often be found attached to the apical whorl. The shedding of the calcareous cast of the veloconch of this species is particularly interesting; it is parallelled by nothing so exactly as the decay of, and deposit of fresh dentine by, the human tooth. As the outer layer of lime is shed, a fresh layer is deposited inside it. I drew attention to this fact when describing the protoconch, in my recent Report on the anatomy of Megalatractus (**6**).

In Number III of 'Notes on Prosobronchiata' (**5**), I proposed the term "pseudoprotoconch" for the neanoconch of Lotorium, but my study of that of Megalatractus has shown me that a protoconch may be a neanic structure and yet not a cast of a corneous veloconch. I therefore wish to withdraw the term, in favour of the more explicit one, "neanoconch."

D. A Deductive Method of Studying the Early Life History of a Gastropod.

Except by a study of the early development of the mollusc, it is unlikely that it can be ascertained whether or not the

phyloconch is a component part of any particular protoconch. This method would, of course, be the most satisfactory way of ascertaining the presence or absence of any of the other portions, but there is also a deductive method which may be employed with some degree of certainty. Let me illustrate this method with the example which gave rise to this essay, Megalatractus aruanus (Linn).

The large size of the protoconch, as taken from the egg capsule, shows at once and conclusively that the veliger stage was passed within the capsule. What I have here termed the calcareous cast of the veloconch (and in my description above referred to "veliger shell") is distinctly marked off from the neanoconch by a deep encircling groove. This groove as certainly indicates a pause between two growth-stages as does the varix on the adult shell. The most important pause in early growth is the nepionic stage; it is therefore justifiable to conclude that the groove is the indication of that stage, and that the preceding structure is veliger and the succeeding is neanic.

If this deductive method is correct, by its use we shall be enabled to give a brief outline of the early developmental history of any particular testaceous Gastropod.

In conclusion, I would draw attention to the fact that the above nomenclature will very likely prove applicable to the Pelecypods equally with the Gastropods.

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The Development of the Corpus Luteum: a Review.

By

Francis H. A. Marshall, M.A., D.Sc.,
Carnegie Fellow, University of Edinburgh.
(From the Physiological Laboratory of the University of Edinburgh.)

The present paper is the result of an attempt to collect together and give an account of the literature of the formation of the corpus luteum, during the last ten years, that is, since the publication of Sobotta's first paper on the corpus luteum of the mouse.

Of the three original hypotheses put forward to explain the mode of formation of the corpus luteum, and the origin of the lutein cells, that of Paterson, who regarded the structure as derived from the blood coagulum left in the cavity of the Graafian follicle after its discharge, gained few or no adherents among subsequent investigators. The other two theories, those of von Baer and Bischoff, on the other hand, have each received a considerable amount of support. Von Baer supposed the corpus luteum to be a connective-tissue structure, in the formation of which the membrana granulosa or follicular epithelium had no share; while Bischoff concluded that the lutein cells were formed by the hypertrophy of the epithelial cells of the undischarged follicle. Among the principal supporters of von Baer's view appear the names of Leuckart, His, Kölliker, Slavjansky, Gegenbaur, Benckiser, Schottländer, and Minot. Those who have adopted the alternative theory of Bischoff include Pflüger, Waldeyer, Call and Exner, Beigel, and Schulin.

The first really systematic effort to deal with the question was made by Sobotta, whose earliest paper on the subject was published in the 'Anatomischer Anzeiger' in 1895. In the following year the complete paper was issued. These papers describe an investigation on the development of the corpus luteum in the mouse, in which a large series of stages was examined, each of them being collected according to a definite plan, the animals being killed at known intervals after the occurrence of coition, in reference to which the period of ovulation had been previously determined. Sobotta's investigation resulted in confirming Bischoff's view that the lutein cells are the much hypertrophied epithelial cells of the undischarged follicle, the connective-tissue element which forms an anastomosis among the lutein cells being derived from the inner layer of the theca. The theca externa is described as taking no share in the ingrowth, while the theca interna is stated to become entirely used up in the formation of the interepithelial network. The hypertrophy of the epithelial cells is described as being of the nature of a simple enlargement, unaccompanied by cell division. The cavity of the follicle is said to become eventually filled up by a central plug of connective tissue.

The conclusions reached by Sobotta regarding the formation of the corpus luteum were subsequently corroborated by him in an investigation on the corpus luteum of the rabbit, the stages of development being also obtained by killing the animals at stated intervals after coition.

In 1898 Stratz published descriptions of stages in the formation of the corpus luteum of Tarsius, Tupaia, and Sorex; and these agree in all essential particulars with the accounts given by Sobotta.

The development of the rabbit's corpus luteum was also studied by Honoré, who adopted the same method as that employed by Sobotta. According to Honoré the interepithelial proliferation of connective tissue is derived in part from the theca externa, and not exclusively from the inner theca, as supposed by Sobotta; while the theca interna is

stated to be not entirely exhausted by the ingrowth, some part remaining to form a layer within the outer theca, after the full formation of the corpus luteum.

Kreis's observations on the young human corpus luteum likewise support the hypothesis put forward by Bischoff.

Belloy, who investigated the formation of the corpus luteum in the rat and guinea-pig, while regarding the lutein cells as being derived from the follicular epithelium, describes an active proliferation of these cells soon after the follicle's rupture. No figures are given by Belloy, and it seems possible that he has confused the ingrowing cells of connective tissue from the theca interna with the membrana granulosa cells. Bouin, who also investigated the corpus luteum of the rat and guinea-pig, reached conclusions similar to those of Belloy.

Heape, without entering into a discussion on the origin of the lutein cells, lays some stress on the absence of division among these cells in the ovaries of certain monkeys, pointing out that the enlargement is the result of a simple hypertrophy.

Rabl, writing especially on the human corpus luteum, concludes that the lutein cells have a double origin, arising both from the membrana granulosa and from the theca interna.

A number of investigators, on the other hand, since the publication of Sobotta's work, have adopted the theory originally put forward by von Baer, that the lutein cells arise from the connective-tissue wall, the follicular epithelium being either completely discharged along with the ovum and the greater part of the liquor folliculi, or else being partially discharged and partially degenerating in situ. Among those holding this view are His, Kölliker, and Paladino, who have lately reiterated their former opinions.

Von Baer's theory has also received considerable support in recent years from Nagel, who has described the corpus luteum in the human subject as an entirely connective-tissue structure. In this he has been followed by Clark, who worked on the formation of the corpus luteum in the sow and in the

human female, and claimed that the result of his investigation had put the matter almost beyond question. Clark's account has been confirmed by Doering, who also worked upon the sow's corpus luteum. Others who have adopted the view that the lutein cells have a connective-tissue origin are Bühler, Wendeler, and Stöckel, who have examined and described developing human corpora lutea.

None of these investigators, however, appear to have given an account of the growing corpus luteum in all stages of development, while in the case of several of the accounts, it is not clear that the structures described were not in reality atretic follicles, that is to say, follicles which had undergone degenerative changes without discharging their ova. On the other hand, the words used in a description by Clark point to the conclusion that this author was dealing with the degenerate epithelial cells of an atretic follicle. It seems not improbable that the young human "corpus luteum" which Doering describes was also an undischarged atretic follicle; while Kölliker's opinion that the corpus luteum is a connective-tissue structure appears to be founded on the assumption that the changes undergone by discharged follicles and retrogressive undischarged or atretic follicles are identical in character. His, and also Bühler, with reference especially to Sobotta's work on the mouse, have remarked that it can scarcely be an accidental circumstance that the accounts given of the development of the corpus luteum in the larger animals and in man are radically different from those described for the smaller species. That the discrepancy between the accounts of various investigators depends upon the size of the animals employed does not seem, on the face of it, a very probable suggestion. It is to be noted further that in the investigations of all these writers who have upheld the connective-tissue theory the ages of the developing corpora lutea were unknown, the material in no case being obtained by Sobotta's method of killing the animals at definite intervals after coition.

In 1901 the present writer published a preliminary account

of an experimental inquiry upon the formation of the corpus luteum in the sheep. In this inquiry the sheep were killed at stated periods either after coition or after the animals had been observed to undergo œstrus. The relation which was found to exist between the condition of development of the corpus luteum and the length of the interval that was allowed to elapse between œstrus and the killing of the animal, was in itself a strong presumption that ovulation in the sheep occurs normally during œstrus. Thus the approximate age of the young corpus luteum or discharged follicle could in every case be determined. The result of this investigation was to confirm in all essential particulars Bischoff's theory, which had been accepted by Sobotta. The sheep, however, was found to present some differences from the mouse in regard to the mode of formation of the corpus luteum, the connective tissue ingrowth being derived partly from the theca externa, and not merely from the theca interna, and the follicular epithelium continuing to undergo division after the rupture of the follicle, but with greatly decreased frequency. The former of these two observations is in agreement with Honoré's statement in regard to the interepithelial connective tissue in the rabbit. The theca interna was said to become entirely used up in the formation of the connective-tissue ingrowth, this statement agreeing with Sobotta's description, but differing from that of Honoré. Two years later the complete account of the development of the corpus luteum in the sheep was published.

The description given in these papers is thus completely opposed to His's suggestion that the mode of formation of the corpus luteum in the larger mammals is different from what it is in small animals like the mouse and rabbit, unless, as Sobotta remarks, it was intended to include only the elephant and the whale in the former category.

Meanwhile, in 1901, the same year in which the preliminary account referred to above was issued, van der Stricht published descriptions of the developing corpus luteum of bats belonging to the genera Vesperugo, Vespertilio, and Placotus. This author's researches also resulted in con-

firming Bischoff's hypothesis, but he differs from others who hold this view in stating that a certain relatively small number of lutein cells arise from interstitial cells existing in the inner theca of the connective-tissue sheath. Van der Stricht differs from Sobotta, while agreeing with the present writer in finding mitotic division among the follicular epithelial cells after the follicle's rupture. A figure is given in one of van der Stricht's papers of a section of a human ovary in which such division is also shown to exist. It would thus appear that the lutein cells, at any rate, in certain mammals, do not arise entirely by simple hypertrophy of the follicular epithelial cells, but by hypertrophy accompanied by a greater or less amount of cell division. The very early appearance of fatty particles in these cells in the bat's discharged follicle is a point of considerable interest to which van der Stricht calls attention.

At the meeting of the "Anatomische Geselischaft" at Bonn, Kopsch exhibited sections of corpora lutea from the sow, representing three-, six-, and ten-day stages of development. These preparations in a general way supported the follicular epithelial theory.[1]

Sobotta's account of the formation of the corpus luteum in the rabbit has been recently further confirmed by Cohn, who also obtained a series of stages by killing the rabbits at stated periods after copulation. Thus the development of the rabbit's corpus luteum has formed the subject of experimental investigations by three separate observers—Sobotta, Honoré, and Cohn,—who have all arrived at the conclusion that the lutein cells are hypertrophied follicular epithelial cells.

An important paper on the corpus luteum of the "Marsupial cat," Dasyurus viverrinus, by Sandes shows that this structure is formed in a similar way in marsupials to what it is in the Eutheria. The theca interna folliculi is shown to be rudimentary in Dasyurus. Owing to this circumstance Sandes points out that it is easier to follow the subsequent changes

[1] Vide Sobotta, Merkel and Bonnet's 'Ergebnisse d. Anat. u. Entwick.,' vol. xi, 1902.

undergone by this layer during the formation of the corpus luteum than in certain of the other mammals. Bühler had suggested that Sobotta might have confused the cells of the theca interna during an early stage of ingrowth with those belonging to the follicular epithelium, which they undoubtedly at one period resemble, saying that the latter author had not properly described the connective tissue sprouting into the cavity of the newly-discharged follicle. Sandes's description is of value as showing that Bühler's criticism loses all force when applied to Dasyurus, with which the membrana granulosa undergoes so considerable a hypertrophy prior to the thecal ingrowth as to sometimes almost fill the cavity of the follicle, and thus all possibility of a confusion between epithelial and connective-tissue cells is precluded.

Sandes describes also the fate of those follicles which do not rupture in Dasyurus. In the case of the smaller follicles both follicular epithelium and ovum frequently degenerate, but the former may persist as a single layer of cuboidal epithelium. Sometimes a metaplasia of epithelial cells into spindle- or star-shaped cells is said to take place, as in other animals. In this way the cavity of the follicle becomes filled up, or it may be obliterated by the ingrowth of connective tissue. Other atretic follicles may for a time remain cystic, with a layer of cuboidal epithelial cells, which eventually disappear. Follicles which have become ripe, or almost ripe, however, are stated to pass through changes precisely similar to those undergone by corpora lutea, except that the ovum, instead of being extruded, degenerates in situ, becoming invaded by leucocytes and by connective tissue.

The corpus luteum in the marmot (Spermophilus citillus) has been shown by Völker to be formed in essentially the same way as that of the mouse, the rabbit, and the sheep. It resembles that of the sheep and (according to Honoré's description) that of the rabbit, in the fact that the thecal ingrowth is not merely confined to strands of tissue arising from the inner layer. It also resembles the rabbit's corpus luteum (according to Honoré) in that the theca interna need

not become entirely spent in the formation of the inter-epithelial connective tissue. Völker finds also in unruptured atretic follicles lutein cells which are similar to those of discharged follicles.

Two authors who have recently written on the formation of the corpus luteum reject Bischoff's theory, on what substantial grounds I find it difficult to understand. Of these, Jankowsky bases his opinions on the study of a miscellaneous collection of material obtained mostly from the sow, but without any attempt at systematic investigation. The few figures which this author gives do not seem to me in any way opposed to the follicular epithelial theory, while the figure of the developing corpus luteum from the guinea-pig appears rather to support the hypothesis that the hypertrophied cells arise from the membrana granulosa, and the anastomosis between those cells from the tissue of the theca. Jankowsky's view is largely based on the appearance of "lutein cells" in the theca interna prior to the rupture of the follicle.

Williams, in a recent work on obstetrics, takes up the same position as Jankowsky, partly on the ground that "the membrana granulosa presents extensive degenerative changes, and is usually cast off in great part at the time of rupture," and partly because the cells of the theca interna undergo marked changes during the follicle's development, and eventually come to resemble lutein cells. The former statement, indeed, is very far from being proved, while the latter appears to me to be singularly inconclusive. Williams argues also that the degenerative changes which have been observed in the epithelium of atretic follicles afford evidence that similar changes occur in discharged follicles. "Observations based upon the study of several hundred human corpora lutea have convinced me that the connective tissue origin of the lutein cells is established beyond all reasonable doubt." Williams, however, does not say that these specimens have been described in any published paper, and, in the absence of the evidence, I am unable to regard his opinion as in any way conclusive.

The changes undergone by the discharged follicle have also been studied in various lower vertebrates. Bühler, who investigated the ovaries of Cyclostomes and certain Teleosteans, was unable to find any hypertrophy of the follicular wall, and Cunningham arrived at a similar conclusion for the spent follicles of Teleosteans. The present writer has examined the discharged follicle of the common fowl without being able to detect any hypertrophy of the follicular epithelium. On the other hand, Mingazzini has discovered such hypertrophy in certain reptiles, structures resembling mammalian corpora lutea being found to occur; while Giacomini, who has investigated the subject in birds, amphibians, and, more particularly, in elasmobranch fishes, also gives an account of the formation of corpora lutea by the hypertrophy of the follicular epithelium. The latter author describes and figures the corpus luteum of Myliobatis as a glandular body in which the follicular epithelium is penetrated by an extensive ingrowth of connective tissue and blood-vessels. This account agrees substantially with what is found to take place in the mouse, the rabbit, and the sheep. A similar description is given by Wallace of the spent follicles in the fishes Zoarces and Spinax. Zoarces, however, presents a comparatively slight resemblance to the mammals in regard to this point, there being merely a slight hypertrophy of the follicular epithelial cells. In Spinax, on the other hand, there is a considerable hypertrophic enlargement of these cells, together with a thecal ingrowth at various points in a radial manner, and an ingrowth of blood-vessels. Lucein has also described corpora lutea in the reptiles Anguis and Seps, with which there is a simple hypertrophy of the cells of the follicular epithelium, unaccompanied by mitotic division.

It thus appears that the follicular epithelial theory of the origin of the corpus luteum of mammals has been found to be true also for various members of the other vertebrate groups.

The chief results obtained by the investigations of Sobotta,

Stratz, Honoré, van der Stricht, Cohn, Sandes, Völker, and the present writer, all of whom agree in adopting this theory, may be summarised as follows:

The lutein cells of the fully-developed corpus luteum represent the epithelial cells of the undischarged Graafian follicle. These cells, after rupture, undergo an enormous hypertrophy, which may be accompanied in the earlier stages by mitotic division, but usually only to a relatively slight extent (Ovis, Vesperugo, etc.). Meanwhile, the thickness of the wall of the developing corpus luteum is further increased by an ingrowth of connective tissue from the side of the follicle, forming eventually an anastomosis of cells, generally fusiform in shape, between the hypertrophying follicular epithelial cells. This connective tissue is derived either from the theca interna alone (Mus, Tarsius, Tupaia, Sorex, Dasyurus, Vesperugo, etc.), or it may arise from both theca interna and externa (Lepus, Ovis, Spermophilus). The formation of the anastomosis is accompanied by an ingrowth of blood-vessels, which gradually increase in number throughout the young corpus luteum. The theca interna may become entirely spent in this process (Mus, Tarsius, Tupaia, Sorex, Ovis, Dasyurus), or certain strands of this layer may remain outside the hypertrophied epithelial cells after the complete formation of the corpus luteum (Lepus, Spermophilus, Vesperugo, etc.). Certain cells in this layer are stated in some cases to become transformed into lutein cells (Vesperugo, etc.). The cavity of the discharged follicle becomes completely filled in eventually by the further growth inward of connective tissue accompanied by blood-vessels.

The corpus luteum may attain to very great dimensions, this structure, when fully formed, in the cow, having a diameter of from two to three centimetres, according to Schmidt.[1] Its large size is all the more remarkable in view

[1] Schmidt's paper, besides containing observations on the corpora lutea, has also an interesting account of the variation noted in the duration of the œstrous cycle, or the interval between two successive "heat" periods, in

of its resulting to a large extent from the simple hypertrophy of certain of its constituent cells, namely, those which comprised the epithelium of the ripe follicle. The wonderful property which these cells possess of enlarging within a very short time of the follicle's rupture, a rapidity which seems to be especially marked in the case of the sheep's corpus luteum, is apparently without parallel in the histology of the Vertebrata. This unique characteristic becomes additionally interesting when considered in relation to Pflüger's hypothesis, since supported by Schäfer and others, that the cells of the follicular epithelium have a totally different origin from those belonging to the thecal tissue, being in fact derived from the same group of cells as that from which the ova arise.

Postscript.

Heape, in a recently published paper, describes the formation of the corpus luteum in the rabbit as follows:—"The corpus luteum is formed by the ingrowth of cells surrounding the follicle together with the follicular epithelium; the ingrowth being at one time apparently a forcible rush before which the loosened epithelium is driven. The ingrowth takes place in the first instance in the region of the base of the follicle."

Miss Lane-Claypon, in a paper lately communicated to the Physiological Society, "On the Post-Natal Formation of Primordial Ova," states that the ovarian interstitial cells, and the follicular epithelial cells, like the primordial ova, are all "derived from the original ingrowths of the germinal epithe-

cattle, as deduced from the study of 500 cases. The most usual length of this period appears to be twenty-one days, but the variation was found to range from six days to one hundred and twenty-one, or even more days. All variations between these periods were noted to occur. Schmidt's observations are in direct opposition to Beard's speculation regarding the "Span of Gestation and the Cause of Birth" (Jena, 1897), according to which the interval between two "heat" periods is assumed to bear a fixed relation to the length of the gestation period.

lium," and not "from the mesoblast, which gives rise to the connective tissue and blood-vessels." If the epithelial and interstitial cells are potentially and by origin identical, this fact helps to elucidate Van der Stricht's discovery that in the bat's ovary both of these elements may take part in the formation of the lutein cells. It is possible also that it provides an explanation of some of the discrepancies between statements by various authors regarding the mode of development of the corpus luteum in different animals. Miss Lane-Claypon says that in the rabbit "the interstitial cells form by far the largest part of the adult ovary," while in the sheep, judging by my own observations, they are relatively scarce. F. H. A. M.

September 28th, 1905.

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The Lime-forming Layer of the Madreporarian Polyp.

By

Maria M. Ogilvie Gordon, D.Sc., Ph.D., F.L.S.

Having just received Mr. Duerden's new and valuable work on 'The Coral Siderastrea Radians and its Post-larval Development,'[1] I wish to draw attention to one or two of the points in which his work covers the same field of investigation as my work on 'Madreporarian Types of Corals,'[2] published in 1896. My work in no sense professed to be a study of the histology of Madreporarian corals; it explicitly dealt with the coral skeleton, and it set forth, for the first time, the exquisitely fine lamellar structure of the skeletal parts. My microscopic observations of skeletal structures have been verified by many students since the work appeared, but zoologists generally have regarded my view that the crystalline deposit took origin within organic tissue as quite wrong. Mr. Duerden, who approaches this subject from the histological standpoint, arrives at results which, in this very important feature, corroborate my view.

Those familiar with the scientific literature of the Madre-

[1] J. E. Duerden, 'The Coral Siderastrea Radians and its Post-larval Development,' Washington, U.S.A. Published by the Carnegie Institution, December, 1904.

[2] M. M. Ogilvie, "Microscopic and Systematic Study of Madreporarian Types of Corals." London, Royal Society 'Transactions,' vol. 187 (1896), B., pp. 83—345.

poraria know that, previous to my work, the ectoderm was said to consist of a single layer, and the calcareous skeleton to derive origin from this ectoderm by secretion. As Koch wrote, "The ectodermal cells actively separate out calcareous matter, and at the same time continue their own existence."

I found that in all variations of form, from the simple dissepiment to the highly decorative row of granulations on many septa, the calcareous skeleton had a quite particular relationship to the polypal wall, and that the skeletal lamellæ were composed primarily of a number of minute, crystalline, calcareous groups, in size closely corresponding to that of the ectodermal cells, but that secondary mineralogical changes tended to obliterate, more and more, this first definite relationship (l. c. aut., p. 113, 115, etc.). Mr. Duerden entirely corroborates this result (Duerden, l. c. pp. 30, 34, 44, etc.).

I also found that the unit-groups of crystalline fibres composing the skeletal lamellæ showed the presence of organic residue, usually as minute granules and specks, and that in transverse sections of thick septa, the organic residue was quite apparent in the series of skeletal lamellæ at the margins next the ectoderm, even after the older lamellæ had undergone considerable calcification changes. In some of the criticisms of my work by zoologists I was told I had mistaken such appearances, and had seen only fragments of algal filaments penetrating the skeleton. I had foreseen this might be said and purposely given drawings of coral skeletal parts penetrated by filamentous algæ to show that I was familiar with this quite different adventitious appearance. What I described was a persistent essential feature in every skeletal part of every species I examined, and now Mr. Duerden entirely corroborates this observation. But this is the observation which overturns the previous conception of the origin of the Madreporarian calcareous skeleton, for, as I pointed out, that skeleton is composed of layers primarily organic, secondarily inorganic, and separated successively from the polypal ectoderm during the growth-periods of the polyp.

So far, then, Mr. Duerden's work confirms mine. But in

any comparative reference to Mr. Duerden's work and mine a difficulty arises from our different use of the term "calicoblast."

Von Heider, from his observations of the coral polyp, advanced the idea that the skeletal matter was laid down within certain cells, and he termed these cells "calicoblasts," or "lime-forming cells." Subsequent zoological writers insisted that the calcareous matter was secreted by the ectoderm, and laid down outside it, nevertheless they adopted von Heider's term "calicoblast," using it for the ectodermal cells in these parts of the polypal body-wall outside which the calcareous skeleton was deposited. This adoption of von Heider's term by zoologists who upheld the principal of deposition of the limy skeleton external to all organic tissues was, in my opinion, inappropriate, and has been very misleading in the literature.

When I succeeded in separating the skeletal unit with its minute group of calcareous crystals and its organic residue, and found its size corresponded on the one hand with that of an ectodermal cell, on the other with the breadth of a skeletal lamella, I considered it to be the true representative of von Heider's "calicoblast," and applied the term to it. I never applied von Heider's term to an ectodermal cell in the ectoderm, but strictly to the unit-component of the skeletal layers, saying that the unit-component was the product of an ectodermal cell, was at first entirely organic, but that afterwards a group of calcareous crystals developed within it, and the "outlines of the individual calicoblasts became vaguer as their calcification was more complete." I showed that "each skeletal lamina (average width ·003 to ·005 mm.) was originally a deposit of calicoblasts," the calicoblastic laminæ in the septum being an exact replica in form of the ectoderm of the polyp (aut., l. c., pp. 115, 117, 124, 127, 137, etc.).

Thus I discriminated between:—

(*a*) The ectodermal cell-layer from which a series of calicoblastic layers takes origin.

(*b*) The layer of "fibre-containing calicoblasts next the skeleton."

(*c*) Older layers of similar fibre-containing calicoblasts in more and more advanced stages of calcification.

At the same time, in my work, I took the broad position that the ectoderm might be regarded as a many-layered structure, the innermost layer being the persistently organic, cellular layer of the body-wall, the next layers being "calicoblastic," i. e. undergoing transformation from organic to inorganic condition, each farther layer being more and more crystalline. "We may look upon the superficial layers of the skeletal elements and of incompletely calcified calicoblasts as the outer layers of a many-layered ectoderm" (aut., l. c., p. 116).

Mr. Duerden's description of the relationship of the skeletal laminæ to the ectoderm is the same as mine, but he uses a different terminology. He follows the precedent of Dr. Bourne and others in using the term "calicoblast" for the part of the polypal ectoderm adjacent to the skeletal tissues, constantly using the term "calicoblast ectoderm." He then applies a new term to the next layer of organic tissue in which the calcareous crystals are deposited (i. e. the outer layer which I called "calicoblastic"),—describing it as a "homogeneous, mesoglœa-like matrix in which the minute calcareous crystals forming the skeleton are laid down" (Duerden, l. c., p. 34). Then he states that the "calicoblast ectoderm" does not lay down the skeleton, but that it probably secretes this matrix or membrane in which the skeleton is laid down.

Thus Mr. Duerden applies the term "calicoblast ectoderm" to that which I called simply "ectoderm," and describes as a homogeneous "matrix," "membrane," or "sheath" the next layer described by me as a layer composed of individual calicoblasts in which the crystalline groups made their appearance. The difference in the use of terms will be evident from the following quotation, where Mr. Duerden describes the appearance of the lime-forming layer after decalcification.

"When of sufficient thickness to have contained skeletal fibres, now dissolved away, this membrane appears fibrous, but immediately bordering the calicoblast layer" (by which he means the ectoderm) "it is homogeneous" (Duerden, l. c., p. 34).

Seeing that the term "calicoblast" means "lime-forming," why not apply it to the membrane which, in Mr. Duerden's own interpretation is a lime-forming layer? Why apply the term "calicoblast" to the persistent ectoderm, which is not directly lime-forming, as was erroneously supposed by those who made the precedent? Moreover, in common with me, Mr. Duerden considers this lime-forming layer to be separated time after time by the ectoderm, and to be originally organic, secondarily inorganic. Had he used the same terminology as I did, designating as "calicoblasts" the skeletal unit-elements (partially organic, partially fibrous) present in the layer, and the whole layer a "layer" or "lamella of calicoblasts," the similarity of our results would have been self-evident, the important feature being that the ectoderm does not separate out calcareous matter, but organic matter, within which the crystals develop.

The point of difference between Mr. Duerden's results and mine is one of histological detail. From the remarkable coincidence in size between the cells of the persistent ectoderm and the calicoblast unit-element in the apposed skeletal or lime-forming layer, I drew the conclusion (aut., pp. 115, 117, 124, 125, 217, etc.) that the change from the organic to the inorganic state went on in individual cellular parts of the lime-forming layer, and that each individual lime-forming part or "calicoblast" of the layer derived its origin from an ectoderm cell in virtue of divisional processes, part of the cell layer continuing as ectoderm, part being shed as the layer of calicoblasts.

Mr. Duerden finds the lime-forming layer homogeneous in character, without cell limitations, and, as I made no further investigation of the subject, I willingly accept Mr. Duerden's

observation of the primarily homogeneous nature of the lime-forming layer. It is no less a layer of organic matter, and Mr. Duerden compares it with the mesoglœa between the endoderm and ectoderm (l. c., pp. 22, 34). Mr. Duerden finds the skeletal unit-elements laid down within this layer as definite in size as I did, and the width of the successively separated lime-forming layers about ·0025 mm. (cf. Duerden, l. c., pp. 44, 113). I determined the height of the calicoblast or skeletal unit-element in Galaxea as ·0025—·0035 mm., which corresponded to the width of the septal lamellæ. Mr. Duerden adopts a term I frequently used for these unit-elements, viz. "calcareous scales," but the other term, "calcified calicoblasts," which I also used, he discards, because he confuses it with what he himself terms "calicoblast," viz. a cell in the ectoderm. My use of the term was for cellular parts separated from the ectoderm and incorporated in the lime-forming layer (Duerden, l. c., p. 113).

The absence of cell limitations in the organic tissues of the coral polyp is not unusual, the middle layer or "mesoglœa" being generally homogeneous. Of Siderastrea radians Mr. Duerden writes, "In sections the combined ectoderm and endoderm vary from ·015 to ·003 mm. in thickness, both layers being about equal. The endoderm is a syncytium showing no signs of cellular divisions." . . . "The actual calicoblast layer (= ectoderm), like the endoderm, is at first very narrow, and in the growing areas of the skeleton shows no evidence of cell limitations. . . . The nuclei are nearly as numerous as in the endoderm, and are large and finely granular" (Duerden, l. c., pp. 30, 31). He notes that at the growing edges of the septa nuclei become more frequent in the ectoderm, and tend to exhibit a definite network. Active fission at these parts is well known to observers, and I took the nuclear fission to be associated with the separation of the organic outer layer. Mr. Duerden, however, thinks the organic outer layer probably originates from the ectoderm by a process of secretion, in some manner wholly external to the polypal tissues. Accordingly, he says the calcareous

fibres which develop in the organic outer layer are "ecto-plastic in origin (l. c., p. 113). But is this quite accurate when they arise in an organic mesoglœa-like membrane?

Whatever its mode of origin, whether by secretion or by fission of cellular tissue, the lime-forming layer is at first organic and continuous. And it appears to me it will not cover the facts simply to say that the skeletal units arise in an organic sheath or matrix, and are in their individuation wholly unaffected by the ectoderm cells or nuclear parts.

On Mr. Duerden's interpretation, if I understand it aright, we are to believe that the exceedingly particulate skeleton arises by a sort of crystallisation in an organic cuticular matrix produced by, but distinct from, the ectoderm. I still uphold my opinion, supported by remarkable correspondences of measurements, which cannot be mere coincidences, that the individual ectoderm cells or nuclear parts exert a determining individual influence on the origin of the lime-forming skeletal units (= "calicoblasts" in my work) in the cuticular product, which is, after all, a composite product from many ectodermal cells, and persists in retaining its originally particulate character.

I should like also to refer briefly to an excerpt which Mr. Duerden makes from my work, and which, away from the context, might easily convey to the reader a somewhat erroneous impression. On p. 43 Mr. Duerden refers to my explanation of the appearances of "dark points" and a "dark line" in the median plane of the septum, and says "Miss Ogilvie considers that the dark appearance of the centres results from the presence of the carbonised residue of the originally unchanged parts of the calicoblasts, within which she considers the Madreporarian skeleton to be formed. It must be stated, however, that the dark appearance is only seen when sections are viewed by transmitted light. With reflected light the middle region appears lighter than the rest of the septum, and thus can scarcely be occupied by black organic matter."

From the excerpt, it might be concluded that I supposed the middle region of the septum was always occupied by black organic matter. On the contrary, I examined my slides both by transmitted and reflected light, and knew very well that in some cases the middle region of the septum appeared lighter. But, as I wished to point out, there were cases where bright coaly specks were undoubtedly present, and would justify the term "dark points" met with so often in the literature of Madreporaria.

In my work I accepted Dr. Bourne's term "centres of calcification" for the "dark points" expressly because it assumed nothing with regard to the actual condition of any deposit that might originally, or by secondary changes, be present at the "centres" of calcification. My aim, in the passage to which Mr. Duerden made reference (aut., p. 127), was to demonstrate the frequent presence of organic matter at the septal axes or centres of calcification, and to identify it with the organic residue in the skeletal units or calcifying calicoblasts which composed the lime-forming skeletal layer, doubled at the septum.

I wrote, "The appearance of the 'dark line' with transmitted light, although generally opaque, is not always so. . . . The 'points' in a transverse section of Galaxea, for example, appear at one place homogeneous, and yellowish or dingy-brown in colour; in another place the 'point' seems a fairly large, circular area, filled with granular, powdery material, and then it is usually dark." Again, "In all cases we have simply to do with centres and axes (ideal) of calcification, around which the calicoblasts are grouped in the living polyp, and from which therefore similarly oriented fibres ultimately radiate when complete calcification has taken place." In explaining lateral ornamentation of the septa, I wrote, "Small pits are present on the ectodermal, skeletal-producing surface. Subsequently the skeletal layer of the septum is an exact cast of the form of the ectodermal flap. . . . The component calcified calicoblasts of the layer have their fibres set at right angles to the sides of the pit, and the

eminence of the growth-lamella assumes a hemispherical, conical, or any other form, according to the shape of the ectodermal pit. . . . In all cases the fibres radiate around what was formerly the axis of the pit. By continuous deposition of lamellæ a fascicle of fibres is determined, whose axis coincides with this axis" (aut., l. c., p. 137, cf. p. 139, etc.).

Mr. Duerden writes, "I conceive that the so-called centre of calcification is really the organic centre or axis around which the skeletal matter is deposited in a radiating or feather-like manner, and that at an early stage in the living, growing skeleton, the centre is occupied by the mesoglœa-like matrix, within which it has been shown that the calcareous fibro-crystals are deposited" (Duerden, l. c., p. 43). This interpretation of the "dark points" and "dark lines" does not differ from mine in so far as relating them to organic residual fragments of an organic layer undergoing changes of calcification.

Further, my explanation (aut., l. c., p. 113) of the cause of the appearance of dark and light bands in the succession of growth-lamellæ, viewed by transmitted light, is the same as given by Mr. Duerden on p. 42 of his work; the margins of "dark, finely-granular particles, similar to those at the centres of calcification," having been detected by me and their significance interpreted as all-important evidence in my demonstration of the separation of successive calicoblast or lime-forming layers, and the gradual transformation of each to build up a skeletal growth-lamella (cf. Duerden, p. 44; Ogilvie, pp. 111, 150, fig. 30B.)

Pseudospora volvocis, Cienkowski.

By

Muriel Robertson,

Zoological Laboratory, University of Glasgow.

With Plate 12.

I have adopted the name "Pseudospora volvocis" for the protozoon here discussed, as the two most obvious phases correspond with the creature described under that name by Cienkowski.

Pseudospora was noticed amongst Volvox, supplied by Mr. T. Bolton to the Cambridge laboratory and, later on, to Glasgow. As the creature showed features of considerable interest and the knowledge of its life history appeared to be very imperfect, it seemed advisable to make it the subject of special investigation.

I wish here to acknowledge my great indebtedness to Professor Graham Kerr. This paper, which was begun at his instigation, would certainly never have been completed but for his kind supervision and guidance.

All the processes described were followed out on the living specimens and checked by stained preparations. The investigations were made for the most part on material mounted on slides under cover-slips supported with wax. It was impossible, however, to keep slides of this description under observation for more than twelve to eighteen hours, as after that period the Pseudospora invariably died. Cultures in watch glasses, even when kept in a damp chamber, were not alto-

gether satisfactory, partly owing to the Volvox not thriving in the small volume of water, partly on account of the inroads of bacteria. I finally found that Pseudospora could be got to live quite normally for a week to a fortnight on slides with a circular moat hollowed out round the part where the object to be observed was laid. Spirogyra was placed in the moat, and the whole covered with a supported coverslip. The edges of the coverslip were then sealed up with vaseline.

The preserved preparations, with very few exceptions, were stained under the coverslip and mounted in balsam. The stains used were: Ehrlich's hæmatoxylin, iron hæmatoxylin (Heidenhain's), borax carmin, picrocarmin, safranin, and Romanowski's stain. Paracarmin was tried, but never gave good results. Orange G., methylene blue, and eosin were also not, as a rule, very successful.

Mitosis was followed out on corrosive material stained with Ehrlich's hæmatoxylin and checked by osmic material stained with Ehrlich's hæmatoxylin and picrocarmin. For the observations on the development of spheres, Romanowski, safranin, and Ehrlich's hæmatoxylin were used, the best results being obtained with Romanowski, which proved, however, to be an exceedingly difficult stain to use.

Pseudospora volvocis was first named by Cienkowski in 1865 ('Archiv f. mikr. Anat.', vol. i, p. 214); he described a flagellate and an amœboid form, and also a definite double-walled cyst, surrounded by a gelatinous veil. As will appear later, I have been unable to find this encysted form. Bütschli in Bronn's 'Klassen und Ordnungen,' 1883, places Pseudospora amongst the Isomastigoda, but does not describe its life history.

Zopf, in his work on 'Die Schleimpilze,' mentions it under the name of Diplophysalis volvocis, and refers to Cienkowski's paper. The next reference is in Klebs' 'Flagellaten Studien,' 1892, where he points out the ambiguous systematic position of the genus. The group to which Pseudospora belongs seems from the literature somewhat neglected since Zopf's work on 'Die Schleimpilze.'

Pseudospora volvocis, Cienkowski.

There are three adult forms: A. an amœboid form; B. a flagellate form; and C. a radial form, with very fine pseudopodia. Cienkowski's paper describes only the first two of these forms.

A. Amœboid form. Size, ·012 mm. to ·03 mm.

Structure.

Ectoplasm.—In the amœboid Pseudospora (fig. 1) there is a narrow band of not very markedly differentiated ectoplasm forming a comparatively firm outer layer which is capable of being prolonged from time to time into pseudopodial processes. The shape of the creature is very changeable and inconstant. The pseudopodia, which are sometimes very long, vary considerably in shape; they can be extended from apparently any part of the animal, and, though seen to branch, do not anastomose. The pseudopodia are frequently prolonged into either very fine processes, in which case they are often arranged in bunches, as in fig. 2, or else are simply broad at the base and pointed at the end. Occasionally, however, blunt pseudopodia are met with. The various forms of pseudopodia merge into one another, and are capable of changing shape with considerable rapidity. The longer and more slender processes are in some specimens occasionally bent backwards and forwards after the fashion of flagella, but the movement is fitful and slow.

Endoplasm.—The endoplasm alters greatly in character according to the exact condition of the animal. In very young specimens, and in individuals which have been in the free-swimming state for a considerable time, the protoplasm presents a very homogeneous and hyaline appearance. If no food has been ingested for some time, the endoplasm is usually somewhat grey in colour, with highly refractive granules, apparently of stored-up food material; the number and size of these are quite inconstant. After feeding, however,

many large green and brown masses are to be seen in the endoplasm. The green particles are the still undigested Volvox individuals, the brown, those which are in process of digestion. Definite food vacuoles do not seem, as a rule, to be formed in the amœboid Pseudospora, that is to say, the outline of the vacuole is so close to the food particle as to be indistinguishable. Sometimes the creatures are so densely filled with food particles as to appear quite green.

Very bright spherical particles, which are, as a rule, to be seen in Pseudospora individuals, which have sojourned for some time in the Volvox colonies, appeared, upon treatment with osmic acid, to be globules of some fatty substance. Two or three contractile vacuoles are present; these are, however, not very conspicuous in either the amœboid or the flagellate forms.

Nucleus.—The resting nucleus (figs. 1 and 2) measures about ·0046 mm. to ·0057 mm. in diameter. It is a single, well-defined body lying in the centre of the creature. It is bounded by a fine membrane staining with chromatin stains. Inside the membrane lies a deeply-staining spherical body or karyosome surrounded by a very definite clear space. In preserved specimens the karysome stains with Romanowski's stain, safranin, Ehrlich's hæmatoxylin, Heidenhain's iron hæmatoxylin, borax carmin, and picrocarmin. The chromatin lies diffused through the karyosome, which presents, in the resting state, an almost homogeneous appearance. The karyosome is produced into fine rays, which pass to the nuclear membrane, these stain somewhat less intensely than the karysome.

B. Flagellate form. Size, ·012 mm. to ·03 mm.

The flagellate Pseudospora is an oval, oblong or pear-shaped creature bearing two flagella at one end (fig. 3); these are usually equal in length, though in many cases one is shorter than the other. The flagella are comparatively thick and do not taper at the end; they are two or three

times the length of the individual. A slight depression is in some cases to be seen at the point of insertion of the flagella. This is, however, not by any means a constant feature. In the pear-shaped individuals there is often a blunt process about one third of the way from the flagellate end. The ectoplasm, endoplasm, and contractile vacuoles show no special features. The nucleus is identical with that described in the amœboid form, and is situated immediately behind the insertion of the flagella.

Method of swimming.—These flagellate forms swim with one flagellum dragged behind—in the cases where the flagella are unequal the longer one—the other is lashed out in front. The movement of the front flagellum varies slightly; usually the flagellum starts from a position in a straight line with the longitudinal axis of the animal, it is rapidly lashed to one side, and then slowly returns to its original position. This may be repeated either first on one side and then on the other or over and over again in the same direction. When the flagellum is used in the latter way the creature tends to swim in a circle, the flagellum which is dragged behind correcting this to a certain extent. The flagellum is sometimes passed round the individual, causing it to revolve round its longitudinal axis. Occasionally both flagella are used with a very rapid vibratile movement.

C. Radial form. Size, ·012 mm. to ·02 mm.

The radial Pseudospora (fig. 4) differs considerably in its external features from the two previously described forms. It is normally a spherical creature with fine radial pseudopodia; these spring most frequently from all parts of the animal, though they are at times confined to certain parts. The pseudopodia are sometimes three or four times the length of the diameter of the creature, and are not always equally fine.

While moving about either in the Volvox colony, or in the free condition, the creature frequently temporarily adopts a spindle shape (fig. 5) with a number of long pseudopodia at

each end, the pseudopodia elsewhere being usually, but not invariably, withdrawn.

Occasionally the radial Pseudospora in the colony assumes a semi-amœboid appearance, the pseudopodia being for the most part withdrawn. In this condition it could not be distinguished from an individual in the amœboid phase, which possesses fine pseudopodia arranged in bunches, were it not that here the amœboid shape is very transitory, the creatures soon again becoming radial.

The protoplasm of the radial form is on the whole more homogeneous and translucent than that of either the flagellate or the amœboid form; the contractile vacuoles are very large and conspicuous; four, and even five, are to be seen in the creature at one time. Occasionally they arise very near the surface as in a heliozoon, causing a temporary protuberance, which disappears when the vacuole bursts.

In the radial phase the food particles are often contained in large vacuoles. The nucleus corresponds with that already described, but is usually somewhat smaller in size; its average diameter is ·0034 mm. The radial form is more passive than either of the other two forms; in the free state it floats much as an Actinosphærium does or creeps in the manner already described. It attacks and leaves the colony without losing the radial character. It ingests Volvox individuals either by engulfing them bodily, or by passing them down a broad pseudopodium, or by drawing them towards itself by two adjacent pseudopodia. The radial form merges into the flagellate and amœboid forms, from which it differs merely in shape and method of moving. It is, however, so far as my experience goes, always the predominant form in a culture where the Volvox are not moving. If the culture continues to be fed on Volvox in this condition the flagellate forms disappear entirely. The radial Pseudospora divides by fission, often without withdrawing the pseudopodia.[1]

[1] I observed at different times a number of Pseudospora individuals—usually but not always of the radial type—which presented a peculiar and very evenly granular appearance. Upon being watched for some time the Pseudospora

Some Pseudospora, usually transparent radial individuals, adopt a peculiar amœboid form which has eruptive lobopod pseudopodia. I have never seen this except in cultures where the radial form predominated, and then only when most of the Volvox had been destroyed. It is quite possible that this is merely a pathological form due, perhaps, to the protoplasm becoming more fluid. This form occurs very rarely, but has been seen too often to be passed over without mention.

Life History and Habits.

The amœboid Pseudospora (A) may conveniently be taken as the starting point of the life history. This form is found in the Volvox colony, it creeps about on the outside, finally boring its way into the interior. The creature feeds upon the Volvox individuals either by surrounding them with broad protoplasmic processes or by engulfing them bodily. Sometimes a long pseudopodium is seen to surround a Volvox cell which is at some distance from the main part of the body; the food particle is then either digested at the end of the pseudopodium or is passed along it into the interior of the animal.

Pseudospora individuals collect in masses round the young

broke up, setting free the granules which now moved rapidly about. In many cases the granules were in very active motion inside the Pseudospora for a considerable time before it disintegrated. When seen with the ordinary powers of the microscope, I took this process to be some form of spore formation. On referring to Dallinger and Drysdale's work on the life history of Monads, I found that the process of spore formation there described appeared to be very similar to what I had observed in Pseudospora. Under the three-millimeter immersion objective, the granules appeared rod-shaped, and were seen to move in straight lines; while progressing they turned slowly on their longitudinal axes.

In view of Schaudinn's recent work on Trypanosoma the bacteria-like appearance of the particles was not in itself sufficient ground for attributing the phenomenon to the agency of parasites. Finally, however, after searching carefully through the cultures where these individuals were seen, I found that the rod-shaped bodies entered from the outside and multiplied so as to form a dense mass absorbing the protoplasm of the Pseudospora. They are, beyond doubt, parasitic bacteria.

daughter colonies ; they are often to be seen lying in groups closely resembling the destroyed daughter colonies. Sometimes the Pseudospora attacks a segment as large as itself, slowly absorbing it or even creeping into its interior if it is very large (fig. 6).

As a rule the Pseudospora begins to feed at once upon arriving in the colony. One individual which I observed ingested no less than fifteen Volvox cells in two and a half hours. Nevertheless, starved or very young specimens will often lie in a colony for some time (six to seven hours) before beginning to feed.

When well established in the colony, Pseudospora divides about once in every twenty-four hours (at temperatures 6°—16° C.). When about to divide the creature withdraws its pseudopodia and becomes spherical in shape, and the food particles become arranged in a band round the centre. A constriction then appears and the animal divides in two, the daughter individuals usually lying in close proximity for some time after they are quite separate. Finally the creatures put out pseudopodia and creep actively away. The time elapsing between the withdrawing of the pseudopodia and the division of the animal varied between three quarters of an hour and an hour and a half in the specimens which I observed.

The first preparations for division of the nucleus (figs. 7–11) occur, just before the animal rounds itself off, in the breaking down of the nuclear membrane. The whole nucleus seems nevertheless to be still quite separate and clearly defined from the general cytoplasm. The chromatin at this stage begins to gather together into irregular masses, giving the karyosome a somewhat mottled appearance. The whole nucleus now increases in size and the rays become indistinct. The achromatic part of the karyosome can still be distinguished, while the chromatin seems to have segregated out from it, forming a number of small masses lying towards the equator of the nucleus. Careful examination of the nucleus at this stage (fig. 7) shows a roughly oval structure with

faintly staining granules towards the periphery; these are possibly derived from the rays. Towards the equator of the oval lies the chromatin separated out from the karyosome.

In the next stage (fig. 8) the nucleus shows the completely developed spindle. There is now no sign of the faintly staining granules described in the prophase. The spindle seems to be formed entirely from the achromatic intra-nuclear elements. The chromatin has now formed separate chromosomes, which appear to be rod-shaped, though from their small size and highly refringent character it is difficult to make certain of this; they are arranged round the equator of the spindle. The chromosomes now move apart (fig. 9 *a*), leaving the central fibres exposed. Finally the space occupied by these fibres is nipped across (fig. 9 *b*), and the two nuclei are completely separated. Each nucleus soon shows the karyosome (fig. 10), somewhat irregular in shape, mainly composed of deeply staining masses of chromatin, which appear to surround the remains of the achromatic spindle. The rays are not, as a rule, visible at this stage. Shortly afterwards the whole animal divides (fig. 11). This frequently occurs before the nuclei have quite reached the resting state.

After a time the Pseudospora leaves the colony (fig. 12) and swims away in the flagellate condition. If, as is often the case, the amœboid form has retained its flagella, it now withdraws its pseudopodia and becomes oval or oblong. In the case of the non-flagellate individuals, the flagella can sometimes be seen to develop gradually from fine elongated pseudopodia; more often they seem to arise directly without any obvious pseudopodial stage. The shape of the animal is often very irregular for some time after the flagella are formed; finally, however, the creature completely withdraws its pseudopodia. The nucleus always comes to lie directly behind the insertion of the flagella.

The method of leaving the Volvox is very constant; the creature approaches the periphery of the colony and pierces through the jelly by means of pseudopodia. The protoplasm then flows into the pseudopodia until the creature is hour-

glass-shaped; finally it slips out, keeping the hour-glass shape until almost quite free. While in the free swimming state the creature is capable of becoming amœboid and of again recovering its original shape without withdrawing the flagella.

Well-fed flagellate individuals can be seen to divide in the free state by transverse fission, but I have never seen this occur twice in succession in a specimen out of the colony, nor have I ever been able to observe it in creatures which had been without food for some time. Cold, or prolonged lack of food, causes the animal to withdraw its flagella, round itself off, and sink to the bottom of the pond, but I have never observed the formation of a very definite cyst.[1]

The flagellate Pseudospora soon attacks another colony, but not as a general rule so long as it contains green food particles. If water containing free-swimming individuals is put into a tube with healthy Volvox the first to attack are those which still contain brown particles. Those specimens, curiously enough, which are quite transparent owing to the absence of food particles, take from twelve to twenty-four hours, or sometimes even longer, before attacking the Volvox colonies. Pseudospora are often seen to attack both Eudorina and Pandorina even when Volvox are present, and on some occasions when starved they ingested small green algæ, but I have never seen Spirogyra or other filamentous algæ attacked. A starved Pseudospora sometimes ingests another Pseudospora which is densely filled with green food particles. This appears to be merely a process of feeding and to have no connection with either conjugation or association.

I have never observed the formation of a true plasmodium, but temporary fusion of the protoplasm only may occur be-

[1] I have been unable to find the double-walled cyst described by Cienkowski. The rounded-off Pseudospora appears to possess a more definite covering than the flagellate or amœboid individuals, but nothing that in my opinion would justify the term cyst.

These rounded-off individuals disintegrate at once upon the drying up of the water, so far as my experience goes. This may, however, be due to the process having taken place too rapidly.

tween two or more individuals. In the cases I observed the creatures separated after about fifteen to thirty minutes. The process is very rarely to be seen, and occurs more frequently within the Volvox than in the free state. This might possibly be a step towards the formation of plasmodia. Protomonas and Protomyxa, nearly allied genera, form plasmodia, but they are not known to occur in either Vampyrella or Pseudospora aculeata. I have, however, not sufficient evidence to draw any conclusions as to the meaning of this phenomenon.

Pseudospora, when attacking the Volvox, attaches itself firmly to the colony by pseudopodia; these are extended apparently indifferently from the non-flagellate end of the creature or else from either side.

When newly arrived in the Volvox or while still on the outside the animal is very sensitive to any change of conditions; for instance, rise of temperature, evaporation of the water, or stoppage of the motion of the Volvox will cause the creature to leave the colony; this is to be seen even in starved individuals.

The processes above described—alternation of the flagellate and amœboid condition and reproduction by fission—continue for some time (fourteen to twenty-one days) and then a different form of reproduction appears.

Gametogenesis and Conjugation.

In the amœboid Pseudospora there are developed spheres of a clear greyish appearance. The number of these to be found in a single individual varies; in one culture one- and two-sphered forms greatly predominated, in another I found individuals with three or four and on one occasion with eight spheres. The individual in which the spheres arise does not form any kind of cyst. The pseudopodia are not withdrawn; in some cases the flagella persist, and movement and feeding may still go on after the spheres are a considerable size. Finally, the protoplasmic body surrounding the sphere disintegrates, but the time at which this occurs varies greatly in relation to the state of development of the sphere. The pro-

cess of sphere formation occurs both in the colony and in the free condition. These spheres are destined to give rise to the gametes.

Development of the Spheres.—In tracing out the development of the spheres it is more convenient to consider first the case of a single-sphered individual and thereafter to note the slight differences that occur in the cases where there are more than one sphere. The sphere arises directly from the nucleus (cf. figs. 13–19). In the very earliest stage the nucleus differs only from the resting nucleus in that the rays have become thicker and the membrane more distinct (fig. 13 *a*). Later the whole nucleus increases in size and the karyosome assumes a somewhat eccentric position. The first signs of the sphere itself now begin to appear. The substance within the nucleus becomes differentiated—showing a different staining reaction, e. g. blue with Romanowski—to form a spherical mass which fills almost the whole nuclear space. The rays now appear as small rounded masses; some of these are within the sphere; the greater number, however, lie on the outside and seem rather to be connected with the membrane which at this stage appears surrounding the sphere with its enclosed karyosome (fig. 13 *b*) than with the sphere itself. The karyosome gradually moves further from the centre until it finally comes to lie quite outside the sphere (fig. 14 *k*). It appears to take no further part in the process. The position of the karyosome at any one moment bears no exact relation to the size of the sphere. It is sometimes to be seen lying within a comparatively large sphere, in other cases it is already on the outside although the sphere occupies little more than the space of the original nucleus. The size of the sphere has, however, no very constant relation to its state of development.

In the stage shown in fig. 14 the small chromatin masses derived from the rays have decreased in number. Those on the outside have as a rule disappeared, though in some cases they can just be distinguished as very minute particles. Those inside the sphere now appear as definite spherical masses; in one case I could only count three of these.

A later stage (fig. 15) shows a very considerable increase in the nuclear material; about eight to thirty nuclei larger in size than the original masses can be seen in the sphere. I am unable to say how the increase in the number of nuclei takes place; this much, however, seems certain that all the nuclear material of the sphere is derived from the thickened rays of the original nucleus. The sphere increases still further in size (fig. 16) and the nuclei break up apparently into minute particles, as for some time before segmentation begins they can no longer be detected in my preparations. When the sphere has reached its full size, ·007 mm.–·011 mm. in diameter, segmentation occurs. A constriction appears which divides the sphere into two equal segments (figs. 17–18). The parts, however, remain closely apposed to one another. Each of these now divides. After this the division of the segments is not quite regular, and the spherical shape is usually lost. Finally a very large number of segments are formed—in one case I counted a hundred and sixteen, and even in very small single-sphered individuals I have never found fewer than sixteen. The process of segmentation occupies as a rule from ten to thirty minutes. After the segmentation is complete (fig. 19) the segments lie motionless for a while, and then move a little apart before actually becoming motile. If the protoplasmic body which surrounded the sphere has not already broken down they pierce through it and escape. The segments set free are small oval or round uniflagellate gametes, varying in size from ·00116–·00186 mm. The flagellum is thick (average length ·0046 mm.) and slightly curled; it arises from a point about half way from the anterior end and propels the animal forwards. Each gamete appears to possess a nucleus, that is to say, in stained specimens a spot that stains more deeply than the rest of the creature can just be discerned with the highest powers of the microscope. This probably is the nucleus, but I can say nothing as to its structure.

Shortly after becoming free the gametes fuse in pairs, forming zygotes with two flagella (fig. 20). Gametes arising

from the same sphere conjugate together, but I have never seen this occur in all the spores from any one sphere; some individuals were always seen to swim away singly. In colonies where the spheres of several individuals had segmented, I observed conjugation of gametes at some distance from the place of segmentation of the spheres, but had no means of making sure that the conjugating individuals had really arisen from different Pseudospora. Conjugation on one occasion certainly took place between individuals from different spheres which had arisen in the same Pseudospora. In this case the conjugating gametes were unequal in size, but that this was of no special significance was shown by later observations, in which the gametes were equal. On account of the small size of the nuclei and the difficulty of observation, I am unable to say anything about the fusion of the gamete nuclei.

In the many sphered individuals the essential processes of the development of the spheres correspond with those already described in the case of the single-sphered Pseudospora. Two or three spheres may arise inside the same nuclear space (fig. 21); this possibly is to be regarded as a precocious segmentation of the single sphere, though I am unable to say what is the cause. In other cases the nucleus appears to divide before the formation of spheres, and from each of these nuclei is formed one or more spheres. Occasionally in these individuals only one of the nuclei becomes converted into a sphere, the other apparently disintegrating.

The cultures in which the process of sphere formation was observed were kept at an almost even temperature of 11°–13° C. and well supplied with food material. In two cultures of uninfected Volvox which were inoculated with sphere-forming individuals it was found that Pseudospora reproduced by fission for exactly fourteen days and then again formed spheres. In one culture this period repeated itself thrice in succession. In other cases the period varied from fourteen days to about twenty-one. The formation of gametes is often nearly synchronous throughout a culture,

almost all the Pseudospora individuals breaking up within twenty-four to forty-eight hours.

The zygote derived from the fusion of the two gametes after a time withdraws the flagella and appears as a round transparent little creature, with a just discernible spot which, on staining, appears as the nucleus. It now becomes amœboid and creeps into a Volvox individual, where it feeds and increases in size; it destroys the Volvox cell, and is to be seen lying in its place surrounded by the brownish-coloured débris of the chromatophore.

The small Pseudospora is usually spherical at this stage, and the protoplasm appears slightly granular. The creature now either becomes amœboid again and invades another individual, or puts out flagella of the type found in the adult. In either case the animal developes directly into the adult, the development of the flagella in the former case being merely postponed for a time. The young flagellate individual becomes very easily amœboid without losing the flagella.

The zygotes appear to have some considerable power of resisting unfavourable circumstances. Thus on one occasion I was able to start a culture from sediment containing them in which there had been no Volvox for about three weeks. The individuals which first appeared were the small flagellate and amœboid creatures just described; these gradually developed into the normal individuals.

If immediately after the formation of gametes in a culture of Pseudospora motionless Volvox be introduced, the zygote developes into the radial form instead of the amœboid or flagellate. Here also, as in the amœboid form, spheres are formed.

General Considerations.

The nucleus of Pseudospora seems to me to indicate a condition intermediate between the centro-nucleus described by Keuten and the metazoa-like nucleus of Actinosphærium. It is a centro-nucleus in so far as the spindle apparatus is intranuclear, but the formation of the spindle and chromosomes shows a marked advance upon such forms as Euglena.

I have made no attempt to reconcile the formation of spheres as I observed it in Pseudospora with the sporocysts described by Zopf in Polysporella, Pseudospora aculeata, Vampyrella, and other forms. The true test of their similarity would lie in the relations of the nucleus in spore formation.

As to the behaviour of the karyosome in sphere formation Hertwig ('Archiv f. Prot.,' vol. i, 1901) refers to a somewhat analogous form of nuclear multiplication where "in einem grossen, oft so gar riesigen Mutterkern zahlreiche Tochterkernanlage entwickelt werden, welche in dass umgebende Protoplasma heraustreten während der Mutterkern zu grunde geht." In sphere formation we have an essentially similar process, only here the cell bodies for the daughter nuclei are not derived directly from the maternal cytoplasm but from protoplasm built up within the original nuclear membrane. Part of the achromatic portion of the maternal nucleus appears indeed to become converted directly into a mass of rapidly growing protoplasm.

In Pseudospora the nucleus is more specialised than in the form described by Hertwig, and a definite part, the karyosome, is ejected when the nuclei of the spheres are formed.

The phenomena of sphere formation in Pseudospora serve to accentuate the close relationship between the achromatic part of the nucleus and the protoplasm of the surrounding cell-body.

The tendency of recent work on the Protozoa appears to be to accentuate the importance of Doflein's main subdivision Plasmadroma, and, on the other hand, to point towards the possibly artificial character of the subdivisions of the Plasmadroma. Pseudospora with its amœboid, heliozooid, and flagellate phases accentuates this. It seems clear that taxonomic distinctions resting on observations of anatomical features during one phase of the life history are so unreliable as to be almost worthless. Such characters are shown by the transformation of "species" of Amœba into one another by slight modification of the external conditions to be of the most superficial kind, being mere morphological reflections of surrounding

conditions, and of no phylogenetic weight. In classifying the Protozoa it is essential to have regard to the whole life history.

Summary.

1. A Pseudospora individual may adopt three forms—an amœboid, a flagellate, and a radial form. This last, at least, appears to be a direct reaction to external conditions.

2. A single nucleus is present. It is bounded by a membrane, which contains the karyosome surrounded by a clear space; fine rays pass from the karyosome to the membrane.

3. The nucleus divides by mitosis. The chromatin forms chromosomes, which are apparently rod-shaped. The spindle appears to be formed from the achromatic intra-nuclear substance.

4. Pseudospora reproduces by fission. After fourteen to twenty-one days gametes are formed.

5. Gametogenesis. The nucleus of the Pseudospora becomes converted into a sphere, the nuclear substance of which appears to be derived from the rays of the original cell nucleus. The karyosome is extruded from the sphere.

6. The sphere segments to form a large number of gametes.

7. The gametes conjugate in pairs, forming zygotes, which develop into the adult Pseudospora.

EXPLANATION OF PLATE 12,

Illustrating Miss M. Robertson's paper, "On Pseudospora volvocis."

The figures are drawn under Zeiss apochromatic homogeneous immersion objective, three-millimetre focus and compensating eye-piece No. 12.

Fig. 1.—Amœboid Pseudospora, with short pseudopodia. *n.* Nucleus. *k.* Karyosome. *c. s.* Clear space round the karyosome. *r.* Rays passing from the karyosome to (*m.*) the Membrane. *f.* Food particles.

Fig. 2.—Amœboid Pseudospora, with fine pseudopodia arranged in bunches.

Fig. 3.—Flagellate Pseudospora.

Fig. 4.—Radial Pseudospora. *c. v.* Contractile vacuole. *f.* Food particle contained in a vacuole.

Fig. 5.—Transitory spindle shape adopted by a radial Pseudospora.

Fig. 6.—Pseudospora attacking an unsegmented Parthenogonidium (*p.*).

Fig. 7.—Early stage of mitosis. *k.* Karyosome. *ch.* Chromatin come together in irregular masses.

Fig. 8.—*sp.* Spindle. *ch.* Chromosomes arranged in an equatorial plate.

Fig. 9 *a.*—Chromosomes at separate ends of the spiudle. *c.f.* Central fibres.

Fig. 9 *b.*—Nuclei reforming. *c.f.* Central fibres being nipped across.

Fig. 10.—Karyosome reforming. *ch.* Masses of chromatin.

Fig. 11.—Pseudospora immediately after division. *n.* Nucleus.

Fig. 12.—Pseudospora leaving the colony.

Fig. 13 *a.*—*k.* Karyosome. *r.* Thickened rays terminating at the nuclear membrane. The protoplasm has shrunk a little away from the nucleus.

Fig. 13 *b.*—*k.* Karyosome. *ch. 1.* Chromatin masses on the outside of the sphere. *ch. 2.* Chromatin masses inside the sphere. *sph.* Sphere. *m.* Membrane.

Fig. 14.—*k.* Karyosome. *n.* Nuclear masses in the sphere: the karyosome is quite outside the sphere: the nuclear material has come together to form definite masses.

Fig. 15.—The karyosome is outside the sphere, which has increased in size, definite nuclei are to be seen in the sphere. *m.* Last vestige of membrane with small masses of chromatin which are greatly reduced in size.

Fig. 16.—Sphere shortly before segmentation.

Fig. 17 *a.*—Segmentation of sphere, two segments formed, protoplasmic body has not yet broken up.

Figs. 17 *b* and 18.—Segmentation becoming irregular.

Fig. 19.—Individual in which the gametes are already formed, although the protoplasmic body has not yet disintegrated.

Fig. 20 *a.*—Gametes.

Fig. 20 *b.*—Zygote with two flagella.

Fig. 21.—Pseudospora with three spheres arising from one necleus. *k.* Karyosome.

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New Series, No. 194 (Vol. 49, Part 2). Price 10s. net.

NOVEMBER, 1905.

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; HONORARY MEMBER OF THE BIOLOGICAL SOCIETY OF PARIS;
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 ENGRAVINGS ON WOOD.

LONDON:
J. & A. CHURCHILL, 7 GREAT MARLBOROUGH STREET.
1905.

Adlard and Son,] [London and Dorking

CONTENTS OF No. 194.—New Series.

MEMOIRS:

Studies in Spicule Formation.

I.—The Development and Structure of the Spicules in Sycons: with Remarks on the Conformation, Modes of Disposition and Evolution of Spicules in Calcareous Sponges generally.

By

W. Woodland,

University College, London.

With Plates 13—15.

Contents.

It being my immediate intention to systematically study the subject of spicule formation in various animal groups, I

hope to publish, from time to time, papers containing the results of my inquiries in this direction. Whilst so engaged in elucidating the facts I may, on occasion, be tempted to accompany them with interpretations, but, however this may be, I propose to more fully deal with the theoretical aspect of the subject at the close of the series of studies contemplated, ensuring by this procedure a sound basis of facts upon which to found my final conclusions.

The present paper on Sycon spicules—forming the first of the series—was concluded long before I had decided to extend my researches to other groups, and in consequence the Theoretical Considerations forming Part II are to be received with caution. I retain these speculations because, crude as they may ultimately turn out to be, I am firmly convinced, judging from my later researches, that they contain a large element of truth.

Part I. Description of Spicule Formation in Sycons.

Introductory.

An account of the development of the spicules in Homocœlous Calcarea (Ascons) having been published in January, 1898,[1] a parallel inquiry into the conditions obtaining in the Heterocœla (Sycons) is desirable in order to establish the mode or modes of formation of these skeletal structures for the Calcarea as a whole. This is more especially necessary since Dr. O. Maas[2] has recently attempted to describe the development of the Sycon spicules, and in so doing has unfortunately presented a very erroneous view of the facts.[3] For these reasons then, and at the suggestion of Prof. Minchin, I under-

[1] E. A. Minchin, "Materials for a Monograph of the Ascons," I, 'Quart. Journ. Micr. Sci.,' vol. xl.

[2] "Die Weiterentwicklung der Syconen," in 'Zeit. für wiss. Zool.,' lxvii, 2, 1900.

[3] Since Maas' statements and figures have already been incorporated in the text-books (e. g. Haller's), it is still more essential that these erroneous views should be combatted.

took to work out the histology of the Sycons, more particularly in reference to the spicules, in order to ascertain whether the very anomalous condition of things described by Maas had any actual existence or not. I say anomalous condition of things, since it was improbable à priori that such a fundamental character as spicule formation should materially differ in the two subdivisions of the Calcarea, and, as I shall show, cautious inquiry entirely disproved the supposition.

Before proceeding further, I should like to here acknowledge my great indebtedness to Prof. Minchin, who has so kindly afforded me his valuable assistance in the practical part of the work and supplied me with information and criticism in connection with the theory. With reference to this latter, however, I had better add that I alone am responsible for the speculations advanced in Part II of the present paper.

Maas' paper is based on the two species Sycon setosa and S. raphanus; the present account applies to Sycon coronata and S. ciliata,[1] both common species on the English south coast. The Sycons examined by me were obtained from Plymouth, and were prepared as follows:—After detachment the specimens were quickly (to ensure full distension of the oscular rim) transferred to 1 per cent. Osmic acid, in which they remained for some few minutes, and then washed with several changes of distilled water; afterwards they were immersed in Ranvier's (Weigert's is as good) picrocarmine for three hours, again washed with changes of distilled water, and graded either into 60 per cent. glycerine for surface-view examination, or into absolute alcohol for section-cutting and surface-view examination.

These two species of Sycon are cylindrical in form, tapering at the two extremities—the basal or affixed, and the apical or free. From the base to near the apical extremity the body wall of the sponge possesses uniformly-distributed short diverticula or chambers projecting from its sides, which diminish in size from below upwards, and are, as is well

[1] The process of spicule formation in Grantia compressa also is identical with that described for S. coronata.

known, lined with the collar cells characteristic of sponges. Immediately above the region of these chambers is a short space also lined with the choanocytes, but distinguished from the rest of the body wall situated below by the absence of evaginations, so retaining the primitive asconoid condition. Succeeding the choanocytes again is a layer of flat epithelial cells which extends to the upper extremity of the sponge, where it is reflexed and continued as the exterior dermal coat. This internal layer of flat epithelial cells marks out the region of the oscular rim, the area which, in these sponges, is alone suitable for surface-view observations of the developing spicules. The substance of the body wall, bounded internally and externally by the gastral and dermal layers respectively, is gelatinous in consistency, and contains numerous free cells, the majority of which are the scleroblasts concerned in spicule formation.

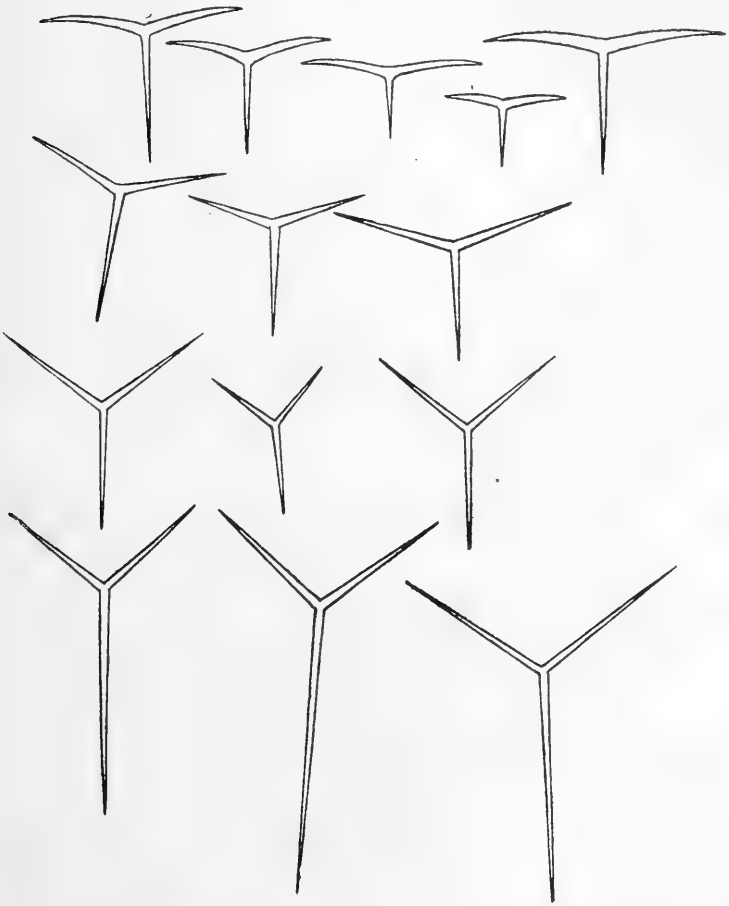
In mounting the oscular rim for examination it is first severed off just below its origin, and the short cylinder thus obtained, after having been slit on one side, is laid out flat in glycerine (or balsam) on the surface of the slide, preferably with its gastral aspect uppermost, and covered.

The Spicules in Sycon coronata and S. ciliata.

Three primary forms of spicules occur—the simple monaxon and the compound triradiate and quadriradiate. Monaxons are present in greatest abundance at the apical extremity of the sponge, where they form the "brush" or circlet of long spines, but they also occur in the sponge wall generally, through which they protrude, and are especially noticeable at the extremities of the chambers where they form projecting tufts. In the region of the osculum, i. e. above the region of the diverticula, the monaxons all assume a more or less vertical disposition, but below this region the vertical disposition disappears with the development of the diverticula, and the arrangement becomes comparatively irregular.

The triradiate spicules are fairly uniformly distributed in the substance of the sponge wall (the three rays of each

Text-fig. 1.



Showing secondary forms of triradiates consequent on difference of localisation in the sponge. The top row represents spicules from the extremity of the oscular rim; the second row from the base of the oscular rim; the third row from the median region of the sponge body, and the bottom row from the base of the sponge. All these figures have been drawn with the camera lucida from the actual objects.

spicule lying in its curved plane, and therefore not protruding at the sides), and, like the monaxons, assume a regular and

symmetrical disposition in the region of the oscular rim, i. e. above the region of the lateral chambers. This regular and symmetrical disposition of the triradiate spicule consists of one ray being vertical in position and situated next the base of the sponge, and the two companion rays in consequence (since the spicule is equiangular) lying towards the apex of the sponge at an inclination of 30° to the horizontal. The reason for this regular disposition of the triradiates will, as also in the case of the monaxons, be supplied later.

Triradiates situated in the "body" of the sponge are approximately both equiangular and equiradiate in form, but, corresponding to the unlike conditions to which they are subject, those situated in the extreme upper and lower regions of the sponge depart somewhat from this type. Triradiates, e. g. of adult Sycons situated in the oscular rim, have their paired rays the more depressed towards the horizontal the nearer they are situated to the upper extremity (text-fig. 1), and (to provide an explanation which will be more fully appreciated when Part II has been read) it seems probable that this depression is associated with the greater tendency of the wall to be invaginated in this region (see p. 265)—just as the upraised arms of a semaphore apparatus, joined horizontally by a spring, would incline the more to the horizontal the greater a weight borne by them. Generally speaking the "structural differentiation of the rays (in sagittal triradiates) is correlated with their position and function in the sponge" (Minchin[1]), and, as is doubtless the case elsewhere, the secondary forms just mentioned are, in all probability, determined in each individual instance by exposure of the formative (apical) cells, during their activity to the incident forces peculiar to the localisation of the spicule in the sponge —formative cells naturally being susceptible to all such influences. Indeed, the conformation of these apically-situated triradiates proves this susceptibility of the formative cell, for, at their central junction, the three rays are strictly equiangular, i. e. contain three equal angles—showing that the spicule would

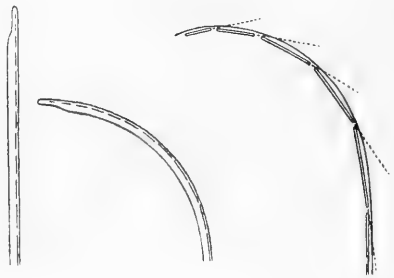
[1] Lankester's "Treatise on Zoology;" Chapter on Porifera.

have assumed the equiangular triradiate form had it remained undisturbed,—and it is only the more distal portions of the three rays that become depressed towards the horizontal—this equally showing that some external cause must have exerted a disturbing influence during the later stages of growth of the spicule. It is true that in other sponges this depression of the paired rays in triradiates—the "alate" form—cannot always be attributed to a tendency to invagination of the sponge-wall, since mere contact with a lining membrane appears to be capable of producing the same effect. Nor is it a fact that in all sponges the triradiates situated at the edge of the osculum have depressed paired arms, since in many Ascons they protrude through the thin body-wall instead, so resembling the monaxons. But despite these exceptions I think it will be admitted (judging from the figure above provided) that the forms of the Sycon spicules (and these same modified forms occur in numerous other vertical and cylindrical sponges, both Ascons and Sycons) bear a relation to the incident forces I have briefly indicated, and, taken in conjunction with the general hypothesis to be elaborated below as to the cause of the symmetrical disposition of the spicules, little room for doubt as to this relationship can remain.

Another example of the modification of the ideal triradiate form due to environmental influence is supplied by the varying length of the vertical "posterior" rays of triradiates situated in different regions of the sponge. If text-fig. 1 above, e. g. be again examined, it will be observed that the posterior rays of triradiates situated at the base of the sponge are longer than is consistent with the equiradiate type of spicule; and that, on the other hand, the posterior rays of triradiates situated at the extremity of the oscular rim are shorter. One possible explanation of this difference of length is provided, as before, by the fact that the sponge is constantly undergoing flexion (see p. 260). In the upper regions of the Sycon flexion attains its maximum, and hence there is, on this account, less room for elongation of the vertical posterior ray (as indi-

cated in text-fig. 2), i. e. the posterior rays are here shorter for the same reason that the terminal segments of a crab's claw are shorter than the more proximal segments, or caudal vertebræ than thoracic. Towards the base of the sponge, however, actual flexion is very small, and hence there is more room for elongation of the posterior rays; moreover, this elongation is here probably aided by the fact that the stresses due to flexion of the sponge are greatest in this region, and are, of course, borne entirely by the longitudinal element of the skeleton; in other words, it is quite possible that this mechanical stimulation exerts an influence on the formative cells, leading to their increased activity. Additional evidence

TEXT-FIG. 2.



of the foregoing explanation as to the cause of elongation of the posterior rays of basal triradiates is afforded in the case of other sponges. In the erect Leucosoleniidæ, and in those forms of Clathrinidæ "characterised by a more erect growth, such as Clathrina blanca and lacunosa, the posterior ray is indicated by its greater length, so that the triradiates become sagittal. In lacunosa this feature is carried to an extreme in the stalk, where a distinct peduncular skeleton is developed, composed partly of sagittal triradiates, partly of dinctinal monaxons," Minchin). The triradiates in this last instance are of the "tuning-fork" type, and afford a good illustration of growth taking place in the direction of least resistance (in the ovoid body of lacunosa supported by the

narrow stalk, on the other hand, the triradiates are regular).[1]

The quadriradiate spicules each consist of a triradiate basis, bearing on its gastral aspect an additional ray, which is apposed at the common junction of the three ray axes (or near it), and at right angles to them. These additional rays, so attached to a certain proportion of the triradiate spicules, are fairly uniformly distributed over the interior surface of the gastral cavity of the sponge, and project into this cavity with a slight upward inclination. It is not improbable that this upward inclination is directly due to the current of water which constantly flows up the gastral cavity and out of the oscular aperture. The gastral actinoblasts (formative cells) which deposit the substance of these gastral rays, as they are termed, must be influenced by such a current, and the inclination of the rays is perhaps the visible expression of this influence; at least there is no other assignable cause. The gastral rays probably possess no function, being, as will hereafter be explained, inevitable results of the architecture of the sponge wall.

The mode of formation of these three forms of spicule will be described first: theoretical problems being reserved for separate consideration in Part II.

As a preliminary to the following description, it is well to mention, especially in view of Maas' misinterpretations, that it is sometimes necessary to use a certain amount of dis-

[1] In connection with the "tuning-fork" triradiates of Clathrina lacunosa developed in the thin swaying stalk of that species, it may be pointed out that (as shown in text-fig. 1) even in Sycons in which the cylindrical body is of much wider diameter, the paired rays of the majority of the triradiates certainly enclose less than 120°. This conformation is doubtless an approach towards the "tuning-fork" type of spicule, a type always produced in connection with the swaying of a narrow cylindrical sponge-body. It will be seen from this (and still better from what follows in Part II) that the movements of the sponge-wall can influence the triradiate spicule in two ways: flexion of the curved wall without invagination tends to render the paired rays more vertical in inclination (see also p. 268 for the cause of the vertical disposition of the monaxons), whereas invagination tends to depress them.

crimination in describing the spicule and the cells in connection with it. Without due care, it is at first possible to err in distinguishing between the various adjacent cells—epithelial cells, collar-cells, pore-cells, wandering-cells, and isolated future spicule-cells (scleroblasts), not to name cells belonging to other spicules than the one under observation—and the formative cells of a particular monaxon or triradiate, but with a little practice such mistakes cannot be made. Criteria of those cells which alone appertain to an individual spicule are as follows :—(*a*) position of cell—the cell always being in close proximity to the ray (especially to be observed in regard to the vertical, i. e. to the focus) and (in viewing triradiates from the gastral surface) with the nucleus lying in the plane of, or below, the spicule if basal in position (i. e. central), and above if apical (i. e. at end of ray); (*b*) form of cell—such, as will be seen from the figures provided, always having a well-marked relationship to the ray, as regards the long axis of the cell and the cell-contour; (*c*) character of cell—scleroblasts *inter alia* possessing greater definiteness of form than epithelial cells, a larger nucleus and less granulation than the choanocytes, difference of outline from pore-cells, and more granulation and absence of refringency as regards amœbocytes.

Unless otherwise stated, the following account refers to *Sycon coronata*; however, a like version holds true for *S. ciliata*, save in certain minor particulars which are duly considered in their place.

The Monaxon Spicule.

In both the species of Sycon examined the monaxons vary considerably in thickness, as the accompanying text-figure shows; nevertheless, the process of deposition is the same for all, though it is possible, and even probable, that the large thick monaxons have a slightly different origin from that of the thin variety (Appendix B). Judging from the few

instances that I have observed, the first indication of the production of the future monaxon from an isolated scleroblast (the "mother-cell" of the spicule, Pl. 13, fig. 1) is the enlargement of the scleroblast nucleus (fig. 2). This enlargement is the precursor to division, and in this manner there is produced at the outset the two-celled, i. e. bi-nucleated, condition of the calcoplasm, which, in Sycons, remains constant throughout the entire growth of the spicule (fig. 3). In this bi-nucleated scleroblast the nuclei next separate from each

Text-fig. 3.

Monaxon spicules drawn with camera lucida from an oscular rim preparation.

other, and a concomitant of this is the appearance in the cytoplasm of a pale streak (fig. 4; not always easily seen in Sycons, though doubtless always present) stretching from nucleus to nucleus; it is in the interior of this mould that the spicule itself is first deposited as a minute refringent needle. As will be seen, the nuclei do not retain this initial position at the extremities of the young spicule, but soon come to lie on one side, and this fact seems to me to indicate that the nucleus is relatively unimportant in the actual secretion of lime, or at least of no immediate importance.

In fig. 3*a* two cells have apparently associated to otherwise produce the bi-nucleated condition of the calcoplasm, and if, as seems likely, a monaxon spicule similarly results from this somewhat differently-constituted bi-nucleated mass, this spicule should exhibit some difference in external appearance from one produced in the manner above described. In Appendix B I have supplied some evidence in favour of the supposition that the thicker variety of monaxons is derived from the association of two mother-cells (whose nuclei do not undergo division), and the thin from the division of the single mother-cell.

In all monaxons, even the thinnest, the two extremities are dissimilar, the proximal end, or end embedded in the sponge substance, tapering very gradually to the point, and the distal end, or end protruded through the sponge-wall to the exterior, being thicker, or, in other words, terminating more abruptly, and, in very young forms, resembling a barb (figs. 5 and 6). This latter feature is doubtless correlated with the prolonged adherence of the apical cell now to be described.

In all early stages of monaxons, the two cells associated with the spicule are situated at its extremities (figs. 5, 6, and 7), but as growth proceeds, the distal cell, after remaining stationary for some time, migrates towards the proximal end (figs. 8—11) until, in the adult structure, it replaces the proximal cell in position, this latter having previously deserted the spicule, after constructing the greater portion of it (fig. 12). The fact that the proximal cell does take considerably the larger share in the formation of the spicule is evidenced by the constant absence of the distal cell on the part of the spicule exposed to the outer world (at least two-thirds of the entire length), this evidently implying that during the protrusion of the spicule through the body-wall, the work of secretion in lengthening the monaxon has been solely performed by a proximal agency. The originally-distal cell, having replaced the proximal cell, adheres to the proximal extremity of the spicule for some

time, and finally also deserts. The function of this distal cell is that of secreting the thick distal extremity of the monaxon before mentioned, and also possibly adding a secondary layer of lime to the body of the spicule in the course of its migration proximally.[1]

It may be added that longitudinal sections of Sycon ciliata (Pl. 15, figs. 45—49) show that monaxons may originate in the bi-nucleated cytoplasm before the mother-cell has separated from the epithelium whence it is derived; however, this is not by any means always the case, since many of the youngest monaxons are to be found embedded in the middle of the wall-substance, and apparently not in any way connected with either the dermal or gastral epithelium (figs. 48 and 49). Still, monaxons are found thus medianly situated, which still retain a connection with the gastral wall by means of fine protoplasmic processes, as shown in fig. 47.

The Triradiate Spicule.

Trios of more or less approximated spicule-cells (fig. 13) are to be found in every sponge-preparation, and these incipient congeries must be regarded as the first observable stages in the development of the triradiate (and quadriradiate) spicules. That the aggregate of three cells (fig. 14)—the "trefoil" (Minchin)—is formed by the association of three cells, and not by the continued division of one, is evidenced (apart from other considerations discussed below, which leave no room for doubt upon the subject) by the fact that aggregates of three cells showing two nuclei of smaller size than the third are never found, whereas aggregates of four cells containing two nuclei and aggregates of five cells containing four nuclei, of less diameter than their companions, occur

[1] Since this was written Prof. Minchin has found that such a secondary thickening distinctly occurs in the monaxons of Leucosolenia variabilis (Ascon). And still more recently I have detected the same in ammonia-carmine preparations of Sycon coronata (Pl. 15, figs. 41 and 42).

pretty frequently (figs. 15, 16), small size of nuclei denoting recent cell-division, each cell of the trefoil subsequently dividing to form the "sextet" soon to be described. It is well to mention that nuclei only in the same individual sponge can be thus compared as regards size, since this character appears to vary slightly in different specimens, and certainly does in the two species, the nuclei of S. ciliata being much smaller than those of S. coronata. Three cells thus approximate to constitute the trefoil, and the more advanced the stage, the more closely associated are the cells. It is difficult to say exactly what this association consists of —whether, as seems probable, the cell-edges actually fuse, or whether they merely come into contact—but, however this may be, the three cells come together in much the same way as three billiard-balls would, and consequently possess the same triradiate formation thus conspicuous from the very beginning. Each of these constituent cells in the mature trefoil then divides centripetally and approximately radially in a direction more or less inclined towards the gastral epithelium, so forming the sextet, or six-cell stage, in the development of the triradiate spicule (Pl. 13, fig. 17, and Pl. 14, fig. 31).

It must here be pointed out that in the figures given depth of coloration of the nuclei is an artifice adopted to indicate relative elevation (high focus), or, in other words, proximity to the gastral surface from which the preparations are viewed (cf. nuclei of apical and basal cells); the depth of coloration actually observable in the preparations denotes, as remarked below, functional activity, and bears no relation whatever to the position of the nuclei. The device must be carefully distinguished from the fact.

The next advance from the sextet stage is the deposition in three centres of small calcareous elongated masses, needle-like, as in the case of the monaxons, and radially disposed, so as to include three equal angles at the centre of the cluster of six cells (fig. 18 for S. coronata, fig. 32 for S. ciliata). These three at-first-separate needles constitute the rudiments

of the future compound spicule, into which they develop by the further activity of the sextet cells, two of these being devoted to each of the three rays. Whether this deposition of three needles is preceded in each case, as in monaxons, by the formation of a vacuole or mould in the substance of the cell (bi-nucleated) is not easy to determine, though its probable occurrence is evidenced by a like condition occasionally to be seen in more advanced stages. A further point of some importance concerning this initial appearance of the triradiate system of deposits is the fact that each of these small needles is formed more in connection with the inner cell (i. e. the cell situated towards the gastral surface and which afterwards becomes the apical cell) than with the outer (cf. monaxons). Though both cells of each of the three pairs composing the sextet are essentially concerned in the production of the monaxon (it being probable, as pointed out below, that the monaxon or elongated form is in all cases determined by the presence of the two cells), yet this, in all probability, is for the greater part actually secreted by the future apical cell. The reasons for this supposition (for actual observation is difficult) are, as Minchin remarks, supplied both by the relations subsequently assumed by the two cells to the spicule-ray, and by the fact (which I have myself observed) that the nucleus of the inner cell stains more deeply at the initial stage of secretion than its companion—a reaction corresponding to functional activity. One more feature worthy of notice is the great preponderance as regards size of one of the three primary deposits in the sextet of S. ciliata (fig. 32)—a preponderance very slight and not at first apparent in the case of S. coronata. This initially-larger needle generally, if not always, becomes the large vertically-disposed or "posterior" ray of the adult triradiate spicule, although the young triradiate may not originally be so placed as to render this particular ray "posterior" in position from the first. Why one pair of sextet cells should thus form a needle so much larger than its two companions is a question at present not easy to answer.

Each of the three separate primary needles being thus deposited in connection with a pair of the sextet cells situated approximately on a radial line, and these three needles collectively having a triradiate disposition corresponding to that of the original trefoil, the next step in the growth of the compound spicule is the junction of the three needles centrally and their individual thickening (figs. 19 and 33). About this time also the two cells connected with each of the three rays become more easily distinguishable into (1) a basal cell, which is outer in position, i. e. nearer the dermal surface, and remains until a late stage of development at the base of the ray, where it is wholly active, with its two companions, in firmly cementing, at their central junction, the proximal portions of the three constituent rays of the spicule, and (2) an apical, or inner cell, which advances in the direction of the ray at its extremity, and is, indeed, chiefly concerned in its construction. As the figures show, the apical cell (more deeply coloured for the purpose) lies well this side of the spicule ray, and therefore next the gastral epithelium, whereas the basal cell is situated pretty well in the plane of the spicule and in one of the interspaces contained by the rays. In figs. 19*c* and 19*d*, and some others, are shown spicules which are very hollow in appearance, but this feature is, as I subsequently ascertained from Prof. Minchin, due to corrosion by traces of acid contained in the glycerine in which the spicule preparations were mounted, and is therefore purely artificial in origin.[1] The three apical cells, having built up the three rays of the spicule to their full length, leave the spicule for the surrounding mesoglœa; also, about the same time, the basal cells, having effected the junction of these three rays at their

[1] The spicules of S. coronata are much more easily attacked by acid than those of S. ciliata, though so similar in appearance, and Prof. Minchin informs me that the same is the case with Leucosolenia variabilis and L. complicata—the spicules of the former species being much more susceptible. Great care must be exercised to ensure that all reagents used in connection with the preparation of calcareous sponge spicules are neutral.

bases, begin to travel up their respective rays, so following in the course of the apical cell (figs. 26—30), and, like this, ultimately deserting the spicule. It is possible that during its migration towards the extremity of the ray, the basal cell may supply a thin secondary coating of lime, but this I have never observed.[1] The destination of the apical and basal cells after leaving the spicule is unknown, and I have not attempted to ascertain it.

It is worth while remarking that after the apical cell has separated from the basal cell, the two cells apparently carry on their work quite independently of each other, and, as we have seen, their respective functions are, after the initial deposits have been formed, essentially distinct in nature. This subsequent independence of activity, or, otherwise speaking, lack of co-operation, is well shown by a curious type of spicule found by Minchin[2] in Leucosolenia complicata which he has termed "derelict." In this type of spicule, whilst the basal cells have been fully active, the apical cells seem to have shirked their duty, and the result is a large nodular mass with three small irregular rays arising from it—a conformation not only clearly exhibiting the independence, but also the respective natures of the activities of the two sets of cells.

The Quadriradiate Spicule.

As before stated, quadriradiate spicules are simply triradiates plus an additional ray, which is attached to the common junction (or near it) of the triradiate basis on its gastral aspect, and at right angles to the plane in which the triradiate lies—the plane of the sponge wall. The derivation of the mother-cell of this additional ray—the gastral ray—I have not been able to discover as yet, owing to the complexity of structure of the body-wall introduced by

[1] The non-cylindrical disposition of the substance of the basal cell (see p. 250) may be worth remarking in this connection.

[2] Prof. Minchin has not yet published these researches, but will shortly do so.

the presence of the diverticula, but certain facts tend to the conclusion that the origin of the gastral actinoblast is the same as that found in the Ascons. In this group, as Minchin has shown in detail, the mother-cell is produced by the division of a pore-cell in the vicinity of the future quadriradiate (when this is situated in the body of the sponge; when the triradiate is situated in the oscular rim, the mother-cell is supplied by one of the unspecialised cells of the epithelium in that region), the resulting scleroblast settling over the point at which the gastral ray is to be developed, and, without further division, secreting a calcareous mass which adheres to, or near to, the centre of the triradiate basis, and gradually assumes the form of the adult ray. Evidence for the assumption that a similar state of things occurs in the Sycons is afforded by the general similarity of spicule formation found in the two subdivisions, and by the fact that pore-cells are generally situated in the neighbourhood of gastral rays.

The youngest stages of development of the gastral rays which I have observed in sections of Sycon coronata are represented in figs. 35 and 36, in which the mother-cell must be distinguished from the basal cell or cells belonging to the triradiate basis. It must be pointed out that the figures given of the succeeding stages of gastral ray development represent the formative cell or cells as being more or less limited in respect to the area of the ray over which the cell-substance extends, and such they appear to be in the ordinary picro-carmine preparations,[1] but if the sections be immersed in Kernschwarz for ten minutes or so, the cell plasma (in which granules are scarce) becomes stained, and is seen to more or less entirely envelop the gastral ray whatever the stage of development may be (fig. 38). In studying the

[1] The numerous figures given of the picro-carmine stained actinoblasts of the monaxon and triradiate spicules are correct, the cell-substance being, as shown, strictly limited in these cases (see p. 250); only the actinoblasts of the gastral rays of quadriradiates are entirely enveloped by the cell-substance (p. 250).

figures of the gastral rays given, this must be borne in mind. The gastral ray attains a considerable size before its mother-cell divides into two (figs. 39 and 40)—at least two thirds of the adult length. Division occurs about midway up the length of the ray, and the two cells so produced apparently tend to separate. The further destiny of the two cells I have been unable to trace, probably because no further developments occur, the two cells persistently adhering to the ray throughout the life of the sponge.

As implied, never more than two cells are associated with the development of the gastral ray in S. coronata and S. ciliata, but, as in Ascons, it is probable, nay certain, that this limit of cell divisibility is merely a specific feature, not necessarily holding for other species and genera in which the spicule attains larger dimensions. In fact, a comparative study of calcareous spicules shows beyond doubt that the number of formative cells produced from the original mother-cell (or mother-cells) is, other things equal, strictly correlated with the maximum size of the spicule attained (from the standpoint of cause and effect, the order should obviously be reversed), and hence in the case of exceptionally large structures the number of actinoblasts is above the average.

One feature in which the formation of the gastral ray differs from the type of development presented by the monaxons and triradiates is the apparent production of the ray in a single cell, the second not being formed until a very advanced stage of growth, and then bearing a very different relation to the spicule compared with that of the basal cell of triradiates or the distal of monaxons; that is, the second gastral actinoblast is concerned with the further growth of the ray, and not with its initial production. As pointed out below, it is probable à priori that one or more of the basal cells of the triradiate base co-operates with the division product of the pore-cell in order to supply the initial stimulus necessary, under normal conditions of growth, to the production of a monaxon structure.

The Relation of the Scleroblast to the Spicule.

If spicule preparations be stained with Kernschwarz for about ten minutes, the limits of the cell-substance become clearly defined, and the relationship of the form of the cell to the spicule is thereby rendered more evident than it is under ordinary conditions (the sheath of the spicule remaining though the calcareous matter becomes destroyed). Such preparations reveal the fact that the cells of monaxons, the apical cells of triradiates, and the cells of gastral rays form cylinders enveloping a portion (in the case of the two former) or the whole (in the case of the gastral ray) of the length of the ray on all sides (Pl. 15, fig. 50, shows monaxon cells thus treated; Pl. 14, fig. 38, shows a gastral actinoblast only slightly stained). On the other hand, the basal cell of triradiates (fig. 51) is not cylindrical in form, but simply adheres as an elongated mass to one side of the ray. The reason for this difference of conformation is one I shall presently point out (p. 276); at present I may again remark that this non-cylindrical disposition of the triradiate basal cell is perhaps responsible for the non-secretion of a secondary layer of lime in the course of its migration up the ray (p. 247). In the case of the cylindrical distal cell of monaxons, a secondary layer of lime is secreted during migration, as also previously mentioned. This simple method of defining the cell-limits just described effectually disposes of the idea that in all cases the spicule is entirely enveloped by the distended cell-substance (e. g. see Maas' figures). If this limited extension of the cell-substance at first seems inadequate to account for the relatively large mass of the spicule secreted by it (as e. g. in the case of the apical cell of triradiates), it must be remembered that the secreted substance is wholly derived from the surrounding medium, and that the cell, like the familiar kettle on the fire, is only the secreting agency and will, if allowed sufficient time, deposit any amount of lime required, though, like the kettle, it becomes worn out in the

end. This truth also renders it more easy to understand the causes of variation in size of calcareous spicules, also to be referred to presently.

As regards the secondary migration of the basal cells of triradiates, and the distal cells of monaxons when their power of excretion is exhausted, there is nothing more remarkable in the phenomenon than in the re-assumption of locomotive powers by an amœba or infusorian after feeding or being otherwise engaged, and the spicule ray evidently serves as a guiding path: the stimulus to movement in the latter case is possibly the same as that in the former. It may also be pointed out as a possibly significant fact that the gastral rays, which are alone directly immersed in the surrounding medium, are alone among spicule rays wholly enveloped by the cell-substance; on calling to mind that the external portions of protruding monaxons never possess a cell on their surface, it seems possible that a connection exists between these two phenomena.

Part II. The Spicules of Calcareous Sponges in general; Theoretical Considerations.

Conditions and Features of Lime Secretion in Calcarea.

Although owing to lack of information with regard to the chemical and physical aspects of lime secretion, it is as yet difficult to definitely account for many minor features of spicule formation, it is yet possible to indicate the main features of the process and such it is now desirable to do if we wish to attain to a true conception of the evolution of the calcareous skeleton of sponges.

The first general and obvious condition essential to the deposition of lime—the first law of spicule formation—is the proximity of the cell-substance to the area over which calcareous matter is being secreted. Illus-

trations of this condition have already been supplied in the above account of Sycon spicule formation, as e. g. by the respective positions of the basal and apical cells of triradiates corresponding to the thickened centre and elongated rays of the spicule, by the thickened distal end and secondary coating of monaxons corresponding respectively to the stationary condition and migration of the distal cell, etc., etc.

Other calcareous sponges afford like evidence. Thus the "derelicts" of Leucosolenia complicata before mentioned, the clubbed extremities of the triradiates of Clathrina clathrus (correlated, as Minchin shows, with the prolonged adherence of the apical cell), the gastral ray spikelets in Clathrina cerebrum corresponding to the fragmentation of the actinoblast nucleus, etc., etc., all illustrate the same law.

It must be observed, however, that although it is necesary for the surface which is receiving fresh deposits of lime to be covered by a layer of "calcoplasm," yet the fact that the mass of protoplasm containing the nucleus is necessarily situated to one side of the growing ray does not affect the symmetry of deposition, as the figures of the Sycon monaxons show, and as is elsewhere abundantly illustrated.[1] The process of spicule growth may, in fact, be compared in this particular with the building of a jetty by a multitude of labourers who, for a given reason, have moored a boat containing the provisions, timber, stores, etc., to one side. The one-sided disposition of the boat and stores relatively to the jetty evidently will not interfere with the bilaterally-symmetrical

[1] The entire lack of influence exerted by the nucleus on the deposition of lime is well shown by the spherical lime deposits so often found in single cells, in which again the nucleus is necessarily placed to one side of the deposit. And if another illustration be required, it is to be found in the case of the bi- or multi-nucleated calcoplasm covering gastral rays and large clathrinid monaxons, in which nuclear-division is not accompanied by a corresponding fission of the entire mass of cytoplasm, as in ordinary cell-division, nor therefore by any interference with the growth of the spicule. In short, the nucleus stimulates the cytoplasm, and the layer of cytoplasm next the spicule deposits the lime, and the conformation of the deposited lime is solely related to that of its immediate producer.

growth of the latter for the sole reason that the ship and stores do not constitute the building agency—are not engaged in the distribution of the added material, but are solely concerned with the nutrition of the building elements and the supply of material for that which is built.

A second essential condition to the deposition of lime in any quantity in Calcarea (i. e. not taking into account the minute granules of lime often found in single scleroblasts) seems to be the co-operation of two dermally-derived cells, the deposition in every case (as may be inferred from the converse of the law just enunciated, viz. that where the bulk of the calcoplasm is situated there lime will be deposited) assuming an elongated form. Thus isolated monaxons are either formed as above described, on the occurrence of nuclear division in a single cell, i. e. on the separation of the substance of the cell into two distinct masses at opposite poles, or, as there is reason to believe (Appendix B), on the association of two cells—in either case two masses of cell-substance with their contained nuclei being distinguishable. As also already described, it is apparently necessary that each of the constituent cells of the trefoil should divide before the three monaxons composing the triradiate can be deposited. And like evidence is perhaps afforded by the divided-off small nuclei (i. e. cells) of the gastral rays of Clathrina cerebrum, each of which doubtless "co-operates" with the mother-nucleus in order to produce a spikelet. At first sight the formation of the gastral ray appears to be an exception to the rule, but, seeing that there is no evidence to the contrary, it is legitimate to suppose that each gastral actinoblast "co-operates" with one or more of the basal cells of the triradiate system to produce the ray, and is thus conformable. That this is the case is evidenced by other considerations about to be discussed.

Assuming the truth of these two laws—the necessity of the proximity of the cell-substance to the site of lime secretion, and, in Calcarea, the necessity of the presence of two masses of dermally-derived cell-substance, between which the young

spicule is deposited—it is possible to consistently explain the existence of the three kinds of spicules characteristic of calcareous sponges, showing not only why the three kinds of spicule occur, but also why other kinds do not.

Immigration of dermal cells into the median gelatinous substance of the sponge wall mostly occurs in those portions of the sponge which are in course of rapid growth, as, e.g., in the oscular rim, and in the diverticula of immature sponges. As already implied these isolated dermal cells or scleroblasts are incapable of depositing lime in appreciable quantity whilst in the uni-nucleated condition. In the majority of Clathrinidæ and some other sponges, and also in the very young stages of many sponges which possess monaxons at a later stage of development, the stimulus to lime secretion, whatever may be its nature, is not even called into existence when the scleroblastic basis of the future spicule is bi-nucleated (and bi-nucleated scleroblasts and two-cell associations are to be found), but in the majority of Leucosoleniidæ and Sycons monaxon spicules are produced either when the nucleus of a single scleroblast divides, or when two scleroblasts associate together, or on both occasions (see p. 273). As already indicated, the bi-nucleated, i.e. two-massed, scleroblastic basis necessarily produces a monaxon structure under such conditions owing to the elongation of the secreting layer of calcoplasm involved in the bi-polar redisposition of the mass of the cell-substance, i.e. the monaxon form is directly related to the conformation of the secreting agency.

When three scleroblasts associate together, the conditions as regards secretion are somewhat more complex. It is evident à priori that a monaxon cannot be formed between any two of the three cells, since the presence of the third (the potency of which is equal to that of either of the other two) must exercise a disturbing influence; in other words, three approximately equal secretory centres being present and grouped about a common centre, the deposition of calcareous matter must be symmetrical with regard to all. Why calcareous matter is not deposited at the centre of the

trefoil, so fulfilling this last obligation, it is impossible as yet to say. Nor is it possible to supply a definite answer to the question as to why it is that a triangular system of three monaxons is not produced; it can only be pointed out that we possess no evidence that a nucleus can, in Calcarea, stimulate secretion in two places at once, and that the trefoil stage tends to show that the nucleus does not possess such a capacity. In actuality, deposition does not occur until each constituent cell of the trefoil has divided, and then the three monaxons produced inevitably tend, from the initial triradiate construction of the trefoil, to form a triradiate system. As in the case of the monaxon, the triradiate form is directly related to the conformation of the secreting agency. I am aware that this interpretation of the form of the triradiate is disputed, but until it is clearly shown how, e.g. surface tension can by itself "lead to the growth of three actines inclined at angles of 120° to each other" (Sollas), or how pore-distribution can effect the same result when the pores are absent (as in sponge larvæ), I must adhere to the explanation I have provided. A simple explanation which, as will be seen, simultaneously explains the conformation of all three types of spicule—monaxon, triradiate, and quadriradiate—has, I think, something to recommend it.[1]

[1] The objections urged against this proposition, viz. that the triradiate form is directly correlated with the conformation of the sextet are, in the words of Prof. Minchin, as follows: "The actinoblasts are never exactly equal, or perfectly regularly placed, nor are the rays formed exactly in the axis of the cell, but almost always a little to one side or the other; hence, if that were the only factor at work, we should rather expect irregularity to be the rule and equality between the angles to be a rare exception." But it is evident from this statement that Prof. Minchin does not really dispute the proposition that the mere triradiate form owes its origin to an association of three cells (in the same way that a monaxon is due to the presence of two, or a spherical spicule results from one), since if, among the triradiates, irregularity were the rule and "equality between the angles . . . a rare exception" he would readily accept it; the real objection of Prof. Minchin is to the minor doctrine, viz. that the mere association of three cells is sufficient to account for the extreme regularity (equirayed and equiangular) of the

If the supposition hitherto made, viz. that the association of scleroblasts in twos and threes is largely, if not entirely, fortuitous, or, at most, only due to those influences which lead to conjugation and syzygy in Protozoa, be legitimate, then it follows that higher associations must also occur, and, if such be the case, there must be an explanation of the fact that four- and five-rayed spicules are rarely, if ever, met with.[1] On mere grounds of probability these higher associations must be few in number, and in observations of the sponge-wall they would probably be passed over as stages in the formation of sextets, but I have no doubt that systematic search on a large scale would reveal their existence.[2] Why these higher associations do not result in multi-rayed spicules can be explained as follows :—Suppose four cells to associate, then, as in the case of the trefoil, and for the same reasons, neither a central concretion nor a square of monaxons would be deposited. But, assuming that the four cells are

triradiate form so often observed in these spicules, as e.g. in Clathrinidæ. And in this minor objection I largely concur, as is proved by the fact that I have on p. 271 named additional factors capable in my opinion of producing the remarkable regularity characterising the spicules of Clathrinidæ, and have further on p. 270 supplied other reasons as to why the spicules of Leucosoleniidæ, Sycons, and sponge larvæ should be more irregular in form. At the same time I believe that, in the very young spicule, the regular triradiate form (conspicuous at the basal insertion of the rays in the most irregular of adult spicules) is solely due to the initial triradiate construction of the trefoil. Prof. Minchin's objection on the score of the one-sidedness of the cell to the ray has little weight, as I have shown in the footnote on p. 252. The cell situated on a monaxon secretes a straight ray despite its one-sidedness, and if this asymmetry of position is of such little account in the production of a monaxon spicule, why should it be of so much importance in the development of a triradiate?

[1] Prof. Minchin has shown me an anomalous equiangular spicule in an Ascon sponge consisting of five rays in one plane. As explained below, it is probable that this rare form of spicule resulted from a chance association of five cells, which happened to be so symmetrically disposed with respect to each other as to prevent a resolution of the cell congeries into triradiate and monaxon groups.

[2] Groups of cells occasionally occur which are not as a whole recognisable as developing or developed sextets.

not quite symmetrically placed about a common centre (a most improbable occurrence), there is no reason why the two nuclei most closely approximated should not, in virtue of their greater proximity, produce a monaxon, since, unlike what occurs in the case of the triradiate, the third nucleus is prevented from exercising a disturbing influence owing to the presence of a fourth nucleus, which is able to "saturate" its "affinity," so to speak. In other words, granted the asymmetry of disposition, the four cells would pair off into two monaxon groups, and the potency, or "affinity" of each cell would be satisfied. And similarly with an association of five scleroblasts, which, if it occurred, would probably resolve itself into a triradiate and a monaxon group. It will thus be seen that all these higher associations of scleroblasts differ from the trefoil in that in the former the "affinity" of each of the constituent cells can be immediately satisfied, whereas in the latter such is impossible, each of the three cells having to undergo division before secretion can occur. This hypothesis of the "saturation" of cell "affinities" thus not only readily explains the existence of the three kinds of spicules, but also shows reason for the sole existence of these three forms.

The production of a gastral ray on an already-formed triradiate basis is a phenomenon of a like order to the above. If in the oscular rim the central portion of a triradiate closely situated to the gastral layer comes into proximity to one of the unspecialised cells composing the actively-growing epithelium of that region, then, two dermally-derived cells (one in the epithelium and one of the basal cells in an interspace of the triradiate—unless all three of the latter co-operate) being brought into apposition, the conditions essential to the production of a monaxon structure are fulfilled, and a monaxon disposed at right angles to the plane of the triradiate will be produced. It is true that the basal cells of the triradiate are already engaged in lime deposition, and hence are not free to co-operate with the future gastral

actinoblast in the same degree that an isolated scleroblast is able to, but, as is shown by the basal cells of all young triradiates, the mere presence of a cell is sufficient to stimulate another to active work (the one in the meantime remaining passive so far as secretion is concerned), and hence the basal cells of the triradiate can well fulfil this condition in the formation of a quadriradiate spicule.

But this last assumption naturally suggests a further question. If an unspecialised cell of the internal oscular epithelium is able, when brought into proximity with the basal cells of a triradiate spicule, to forcibly compel these to co-operate (forcibly, since a change or lapse of function is induced) and share in the production of a monaxon spicule, how is it that isolated scleroblasts do not by similar means produce adventitious rays on the opposite side of the triradiates? Isolated scleroblasts must often come into the vicinity of triradiate actinoblasts, and hence, on the above assumption, might have been expected to initiate deposition under such circumstances. The answer to this question is afforded by the constancy with which the apposition of the two cells is maintained in the case of the gastral ray. This constancy of immediate apposition obviously results from the disposition of the triradiate with regard to the gastral wall in which the future gastral actinoblast is situated, and is evidently not present in the case of an isolated moving scleroblast situated on the dermal side of the triradiate. In the former case persistent maintenance of the apposition forcibly induces the co-operation of the triradiate basal cells; in the latter case the conditions do not permit of such coercion, and in this distinction doubtless lies the explanation of the difference of results in the two cases.

This forced co-operation between dermal cells, one or other (or both) of which is previously engaged in another function, is still more notably illustrated by the induced division of a pore cell, situated in the body-region of the sponge (below the oscular rim), to provide the gastral actinoblast for a

triradiate in its vicinity. Pore-cells are dermally-derived, and hence it happens that those pore-cells which happen to be situated in the neighbourhood of a triradiate are placed under conditions similar to those of the unspecialised cell of the oscular epithelium. The pore-cell itself being functionally specialised, and necessarily bearing a one-sided position with respect to the centre of the triradiate with its three basal cells (in its three angles), its division is a necessity for the end to be attained. Although it is difficult to conceive how the division of the pore-cell is forcibly induced by the proximity of the basal cells of the triradiate, yet that such is the case can hardly be doubted.

To sum up: a spherical isolated scleroblast gives rise to a spherical sclerite (especially well seen in Alcyonaria and Echinoderms); an elongated bi-polar (bi-nucleated) scleroblast, according to the same laws of spicule-formation, gives rise to a monaxon; similarly, a trio of cells ultimately produces a triradiate structure; in short, the form of the spicule is evidently related to the form assumed by the secreting calcoplasm. Further, as the foregoing statements also prove, maintained apposition of two dermally-derived cells is in Calcarea essential to, and therefore the constant percursor of, the production of a monaxon spicule of appreciable mass, and since this maintained apposition of cells occurs, apart from the instances just supplied, at only one situation in the ordinary calcareous sponge, it is only rational to attribute the gastral ray which is there produced to this cause. Granting two simple and easily verifiable propositions respecting the rationale of spicule-formation, it is thus possible to enunciate a theory consistent with the facts, or, at least, such as are at present known.

Before considering the possible causes of the secondary forms assumed by spicules and other "features" of lime secretion, I will first discuss the disposition of the spicules in Calcarea, since the former will by this arrangement be more readily comprehended.

The Modes of Disposition of the Spicules in Calcarea.

To ensure due comprehension of the explanations about to be given in connection with the several modes of disposition which the elements of the sponge skeleton assume under different conditions of life, it will be necessary to first briefly consider the sponge organism in its relation to the environment, and to this end we may select as a convenient form of sponge either of the two species of Sycon, the spicules of which have been already described.

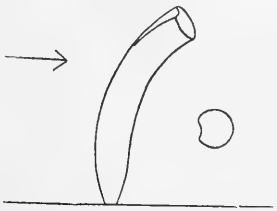
Sycons situated in shallow water which is often in motion, or planted upon rocks exposed to the action of falling waves, are in either case subjected to incident forces of considerable magnitude, and it will be readily understood that, were it not for the presence of the solid supporting structures contained within the sponge-wall, the organism could not attain to any considerable size, owing to the fragile nature of its semi-liquid gelatinous substance. Hence it follows that all the stresses to which the vertical sponge cylinder is subjected are borne by the contained spicules, and these inevitably react to the forces incident upon them.

The elongated hollow cylinder of which the Sycon[1] consists can be affected in two ways by the motion of the surrounding medium; thus, being attached by the slender base, it can either (*a*) bend vertically as a whole (just as a tree is swayed by the wind), or (*b*) the wall of the cylinder can be invaginated upon itself, so tending to obliterate the gastral cavity (text-fig. 4). This latter reaction of the sponge is obviously but another phase of the former, since invagination of the wall is merely a flexion of one half of the sponge relative to the other; nevertheless, the two reactions must be distinguished, since they constitute two separate factors in the disposition of the spicules.

[1] In Sycons the presence of the chambers interferes with the simple cylindrical form of the sponge, although the remarks sufficiently well apply to the thin-walled oscular region. Many Clathrinidæ and Leucosoleniidæ would serve as better examples of a thin-walled flexible cylinder.

To ensure that neither of these reactions of the sponge shall become excessive, i. e. detrimental, it is necessary that means of support shall be developed,[1] in order to preserve to some extent the vertical position of the sponge, and to maintain the appropriate distension of the gastral cavity. A support to protect the sponge-wall from undue vertical swaying is evidently furnished by a vertically-disposed skeleton, and likewise to maintain distension of the gastral cavity, there is needed a skeleton disposed in a horizontal manner, since flexion in either direction is resisted by skeletal material, the length of which is placed at right angles to the direction of stress in the plane in which flexion occurs. Hence the sponge skeleton must, under these conditions, be

Text-fig. 4.



constituted of both vertical and horizontal elements. Both of these elements are supplied by the numerous triradiate spicules contained within the sponge-wall, for it inevitably follows from their conformation that if one ray be vertically disposed, then the two companion rays will lie in lines only deviating from the horizontal by an inclination of 30°, and hence the three rays practically constitute two axes, respectively lying in the required vertical and horizontal directions.

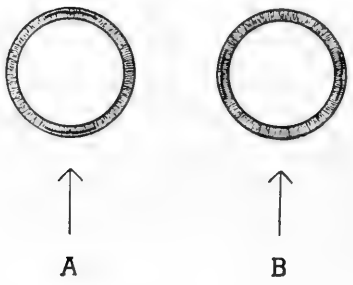
With regard to the two other forms of spicules—the monaxons and gastral rays—these probably do not in general exert a skeletal function, though the former lend considerable support to the oscular rim. The principal function of the

[1] The teleological form of the argument is merely adopted for brevity's sake.

monaxons is doubtless protective in nature,[1] preserving the sponge from the attacks of other organisms by covering its surface with a multitude of sharp spear-heads. The gastral rays, as before mentioned, are doubtless functionless.

The triradiates can be affected in two ways by the pressures incident upon the sponge wall. If (α) the triradiates be situated in those portions of the wall which are in line with the direction of the incident force (in the plane of flexion of the sponge), then each individual spicule is acted upon by the incident pressure (transmitted through the gelatinous matrix) at right angles to the plane in which the three constituent rays lie, either from the side

TEXT-FIG. 5.



adjacent to the force or from the side opposite (text-fig. 5, A). If, on the other hand (β), the triradiates be situated in those portions of the wall which are laterally placed with regard to the direction of the incident force, then each individual spicule is acted upon in the same plane as that in which it lies, i.e. laterally (B). As will shortly be shown—and as is, indeed, self-evident—pressures acting at right angles to the plane of the spicule have much more effect in determining the position of the spicule than pressures acting laterally in that plane, and, in consequence, when

[1] That is to say, the monaxons happen to possess this function. I do not wish to lend countenance to the common belief that every structural feature necessarily possesses a use or function.

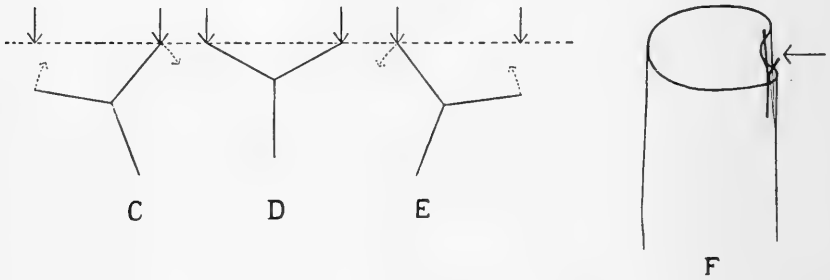
pressures are alternately incident upon the spicules in both these directions, only the effects of the former need be taken into account.

Recognising these facts, it is now possible to inquire as to the causes which have brought about the several modes of disposition of the spicules in Calcarea. And first we will consider the mode of disposition found in those sponges which are cylindrical in form and possessed of a thin wall, which are mobile about a fixed base, which possess an oscular aperture at the distal free extremity of the cylinder, and which are constantly flexed by the movements of the surrounding water (many Sycons, some Clathrinidæ and some Leucosoleniidæ). This disposition, which we may term the oscular disposition, is best observed in the Homocœla, owing to the absence of diverticula of the body-wall. In all sponges characterised as above, the triradiates are situated in the sponge-wall in such a manner that in each spicule one ray points towards the base of the sponge (in erect forms vertically downwards), whilst the two companion rays necessarily lie towards the apex (i. e. incline upwards in erect sponges at an angle of 30° to the horizontal), the whole spicule thus being symmetrically disposed with regard to the long axis of the sponge-body (p. 269, K).

An oscular aperture being present, it is only necessary to consider the forces incident upon the spicules at right angles to the plane in which they lie (text-fig. 5, A). It is obvious that, were it not for the presence of the transversely-disposed rays of the triradiates, the sponge-wall would be invaginated to a smaller or greater extent whenever the sponge was affected by motion of the surrounding water, since the vertical element of the skeleton (vertical rays of triradiates and vertical monaxons) is not adapted to resist flexion in this direction. Hence invagination of the thin wall is resisted by the two upper arms of the triradiates, i. e. the portion of the sponge-wall adjacent to the paired arms of each triradiate tends to "bulge" through the space subtended by them,

when flexion of the sponge occurs (see text-fig. 6, F, below), and the triradiates being numerous and irregularly distributed (i. e. not arranged in vertical rows), invagination of the thin sponge-wall is almost entirely prevented. This resistance offered by the paired rays of each individual triradiate is, as already implied, the means whereby the symmetrical disposition of the spicule is brought about. For if we suppose that a triradiate is not symmetrically placed with regard to the long axis of the sponge-body (as in C or E), then it will be evident that on flexion of the sponge next occurring in the appropriate direction, the spicule will at once be "righted," for the arm that is more inclined towards the vertical will be influenced by the pressure on the sponge wall sooner than

Text-fig. 6.



the lower arm, and hence the spicule will be rotated about its centre until the two arms are similarly disposed with respect to the incident force (D). This process is represented in the above diagram. If a cylinder of paper be taken, and one upper side pushed inwardly, it can easily be understood that it would tend to "right" an asymmetrically-disposed forked structure, between the arms of which the invagination occurred (F). Even if the young triradiate be so initially placed that the vertical ray points apically (towards the osculum), such a symmetrical position would not be maintained, owing to the flexion of the sponge not always taking place in an exactly vertical plane (speaking of vertical sponges); and, moreover, if the weight of the spicule be a factor in its disposition, there is still more reason

for the change from a relatively unstable to a relatively stable position, such as would obviously be the case here.[1] This supposition of a downward pressure (greater probably in its effect at one period of the growth of the spicule than another, except in the case of the apically-situated spicules of adult sponges) being brought to bear on the paired arms of the triradiates found in these sponges is confirmed when we observe that the depression towards the horizontal of these paired arms is the more marked the nearer the spicule is situated towards the apex of the (adult) sponge where flexion is greatest, the pressure on the sponge-wall having determined throughout the whole period of its activity the direction of growth of the apical actinoblast (see above, p. 236).

There is a second mode of disposition of the triradiate spicules which is typically found in the blind (without an oscular aperture) free elongated diverticula of the genus Leucosolenia which at first sight appears anomalous and antagonistic to the explanations just provided for the case of the oscular arrangement of the spicules. This second mode of disposition, which we may term the non-oscular, was first pointed out by Minchin. In this arrangement the spicules are placed in an almost exactly opposite manner to that just described, i. e. the "vertical" or longitudinally-disposed ray tends to point towards the apex of the horizontal diverticulum, and the paired rays therefore tend to lie next

[1] The process of "righting" is actually to be seen in the case of many of the young triradiates, and conspicuously in that of Sycon ciliata. In this species, as before mentioned, one ray of the young triradiate is from the first considerably larger than its two companions, and this invariably becomes the "posterior" (vertically downward) ray of the full-grown spicule. If "heredity" determined the vertically downward position of this larger ray, it might naturally be supposed that it would arise already so orientiated; but, on the contrary, the large ray is often found pointing as much as 80° from the downward vertical line. The change in position of the triradiate spicule which occurs as growth proceeds—as the spicule becomes larger—can, in my opinion only be attributed to the action of external causes, as above described; the greater weight of the larger ray is possibly also a factor.

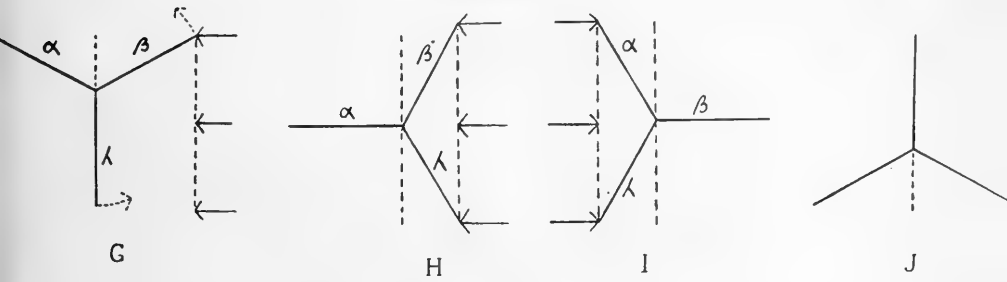
the base. As before, the triradiates are more or less symmetrically disposed (far less so than in the oscular arrangement) with regard to the long axis of the sponge body, but their position is reversed [see text-fig. 8 (L) below, p. 269].

It is clear that, owing to the horizontal position of the diverticula, considerations as to the weight of the spicules being a possible factor in their disposition must be rejected. Again, owing to the absence of an osculum, invagination of the sponge-wall is also out of the question since there is no ready exit at hand from which the contained water may be expelled, the pores being too minute to allow of ready exit. The diverticulum, in fact, here resembles a water-cushion, and pressures tending to invaginate the wall are entirely resisted by the bulk of the contained water, and not by the paired arms of the triradiates. Since pressures incident on the spicules at right angles to the plane in which they lie are here non-existent, or at least ineffective as regards the production of the oscular mode of disposition—the body of the diverticulum being wholly uninvaginable—it is evident that the only pressures which can affect the triradiates are those which are incident laterally, for although uninvaginable the diverticulum is yet freely flexible about its base. The triradiates are affected by these pressures when they are situated more or less laterally with regard to the forces incident on the sponge (see text-fig. 5, B), and the triradiates are by them caused to assume the non-oscular mode of disposition by adopting, as before, a position of equilibrium with regard to these incident forces. Thus if a young spicule be initially disposed as in G, text-fig. 7, it will be evident that the tendency of lateral pressure (exerted on flexion of the sponge) from, say, the right side is to produce rotation of the spicule about its centre (the force impinging upon β long before it can reach λ) the arms β and λ rotating to the left and right respectively (H). If pressure be exerted on the left, rotation will occur in the opposite direction (I). Also, if the triradiate be initially disposed as in J, rotation will similarly take place.

The larger the spicule grows the greater tendency will there

be for it to assume the position of equilibrium, and this position of equilibrium is evidently attained when the triradiate is in the position shown in either H or I, for when so placed, forces from neither side possess any tendency to produce rotation (the moments of the forces on β and λ about the centre of the triradiate being then equal). Whether the position of equilibrium be attained by rotation of the spicule to the left or to the right evidently depends as to whether a pressure sufficient to produce rotation of the spicule into the position of equilibrium first arrives from the right or the left, when the spicule has attained a sufficient size to be so influenced by the pressures on the sponge.

TEXT-FIG. 7.



G H I J

The spicule having attained the position of equilibrium it appears that the ray situated nearest the apex of the diverticulum (β and a in H and I) tends to lengthen somewhat, so giving the triradiate a sagittal appearance. The causes of this lengthening and the consequent tendency of the spicule to assume a more symmetrical position with regard to the long axis of the diverticulum are doubtless the same as those concerned in the lengthening of the posterior rays of the basal triradiates of Sycons (see p. 237 above), and the vertical disposition of monaxons mentioned below.

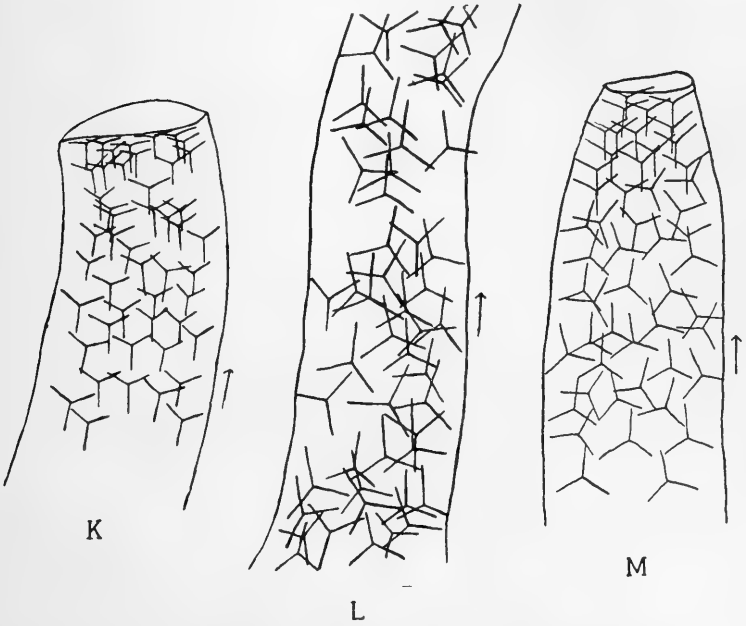
And now observe a striking confirmation of the above contention that the mode of disposition assumed by the triradiates depends, other things equal, on the presence or absence of an osculum. After the Leucosolenia diverticulum has

attained a certain length, an osculum is formed, and, as a result of this, the young triradiates in the vicinity of the osculum immediately assume the oscular arrangement (see M in text-fig. 8 below). This fact seems to me clearly to prove that the disposition of the spicules is due to the direct action of the environment and not to inheritance.

From what has been said hitherto, it would logically follow that in sponges not vertically disposed and not flexible on a slender basis, no definite arrangement of the spicules would occur. It remains to be pointed out as strong confirmation of the above general theory that such is actually found to be the case—that in those sponges which are either not subject to the pressures resulting from motion of the surrounding water or whose conformation is not such as to cause the spicules to be influenced by these pressures (as e. g. the numerous non-erect encrusting forms of the Clathrinidæ), there is no regular disposition of the spicules, and that the same is the case in the very young forms of those sponges which are erect in the adult condition, in which, before either the osculum or the sponge-wall is formed, the spicules are not only irregular in disposition, but also irregular in form, all of which facts (except the last, of course) might be anticipated on the above hypothesis.

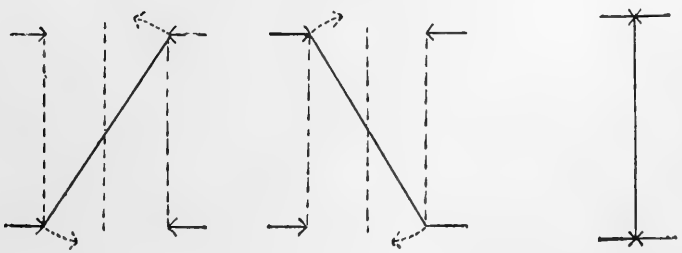
The more or less vertical position of the monaxon spicules in Sycons and other erect Calcarea can be explained in a manner similar to that adopted in the case of the triradiates of terminally-closed Leucosolenia diverticula. In brief, with the exception of those few initially disposed in an exactly transverse direction (and such are found), all young monaxons more or less inclined to the vertical will tend to be righted by the lateral pressures to which they are subjected, as the following diagram suggests (text-fig. 9). In addition to this cause, invagination of the wall will also tend to cause the monaxons to assume a vertical disposition, since (with the exception again of those few initially disposed in an exactly transverse direction) it is only when they are so disposed that they will

TEXT-FIG. 8.



The above figures K and L represent camera lucida drawings of portions of Leucosolenia diverticula, and show well the two arrangements of the triradiates—oscular (K) and non-oscular (L)—which the above hypothesis attempts to account for. M, which is not drawn from an actual preparation, though it truly represents the facts, shows the transition from the one mode of disposition to the other consequent on the form of an osculum.

TEXT-FIG. 9.



offer least resistance to the invagination of the wall, and all structures tend to place themselves in that position which enables them to offer least resistance to an incident force. Invagination of the sponge-wall, in fact, will act in the same manner as the lateral pressures above named. The protrusion of the monaxons on the sides of the sponge, or at the margin of the osculum or apex of a blind diverticulum, is an inevitable result of their elongated form, disposition and place of origin, and the thinness of the body-wall. Their protrusion at the sides of the sponge is, perhaps, also, in large part, due to the possible reflexing of the wall-substance at the margin of the osculum in elongation of the sponge cylinder during growth.

The Secondary Forms and other Features of the Spicules in Calcarea.

A few of the more conspicuous secondary features characterising the triradiate spicules found in the different groups of the Calcarea, and their possible causes must be briefly discussed.

Spicules which develop, under undisturbed conditions, in the homogeneous substance of a wall of narrow breadth, assume an approximately ideal triradiate form, i. e. equi-angular, equi-radiate, and with the rays perfectly straight and "finished." Such are to be found in the majority of the non-vertical encrusting Clathrinid sponges. Spicules, on the other hand, which develop in the body-wall of the erect Leucosoleniidæ and Sycons, i. e. under disturbed conditions (since these sponges are constantly flexed to and fro by the motion of the surrounding water), are not so regular in form as those just instanced, the rays not being perfectly straight, and in many cases (above described for Sycons) deviating more or less from the equi-radiate type. In other words, the irregular "sagittal" form of spicule "is correlated with the more erect growth of" the Leucosoleniidæ

and certain Clathrinidæ, and the finished regular "symmetrical" form of spicule in the encrusting Clathrinidæ "is doubtless correlated with the reticular form and growth of the sponges themselves" (Minchin).

Spicules, again, which develop in a mass of sponge-jelly, and which are therefore not in close proximity to two parallel surfaces, are also irregular in form; though, in these larval spicules, there necessarily exists an initial tendency to assume the triradiate form, yet, owing to the absence of the structures and hence forces which, in the adult sponge, ensure the symmetrical form of the spicule, this is not preserved in the

Text-fig. 10.[1]

ensuing growth. To speak in more detail, the thin unilaminar wall of such sponges as the Clathrinidæ must be continually subject to pressures incident perpendicularly to its surface, and, granted the presence of a young spicule of regular triradiate form, this wall must tend to be invaginated in each of the three areas situated between the rays to an equal extent, which means that a groove is formed in line with each ray of the spicule along which the apical cell must tend to travel, as

[1] I am indebted to Mr. Chubb of University College for this figure, which well illustrates my meaning.

being the path of least resistance[1] (see text-fig. 10 above). And, moreover, besides the constancy of direction of the transmitted pressures which affect the spicule of the adult sponge as contrasted with the indefiniteness of the conditions prevailing in the sponge larva, it must be remembered that the volume of the gelatinous matrix in which the spicule is embedded is considerably reduced in the adult sponge, and hence there is less space for the deviations from the typical triradiate form to occur in—the slight heterogeneities of constitution of the jelly cannot produce an appreciable effect. There is thus not only in the adult sponge a positive set of conditions tending to produce the regular triradiate structure, but also conditions which tend to negative asymmetry of form.[2]

Again, spicules vary enormously as regards size, and these differences are as evident in the same sponge as in different individuals. The factors responsible for the size of a given type of spicule seem to be (*a*) the constitutional efficiency of the scleroblast; (*b*) the initial number of scleroblasts concerned in the production of the spicule; (*c*) the amount of fission which the actinoblast undergoes during the formation of the spicule; (*d*) the character of the region of the sponge in which the spicule is situated; and (*e*) the nature of the external environment, this partly depending upon the situation of the sponge in regard to its immediate surroundings, and partly upon the distribution of the species. Illustrations of (*a*) are to be found in every individual sponge, and in the different species and genera. As an illustration of (*b*) the difference between the two kinds of monaxons in Sycon coronata and ciliata already described may be instanced. Illustrations of (*c*) are to be found in the gastral rays of

[1] A thin sponge-wall with its contained spicules may, in connection with the movements of the surrounding water, be likened to a lattice-work with a sheet spread over it on the side next the wind—the sheet, like the sponge-wall, bulging through the interspaces.

[2] See my paper on the spicules in Alcyonium digitatum (Study II). It is a rule holding for calcareous spicules generally that localisation in a matrix (always more or less heterogeneous in constitution), apart from any limiting layer, always tends to indefiniteness of form.

Clathrina cerebrum and contorta, in the former of which the gastral rays each possess two cells, and in the latter four cells, and in the huge monaxons of the majority of the Clathrinidæ, some of the more adult specimens of which possessing as many as five actinoblasts (Minchin); however, the precise development of these monaxons has yet to be determined. An illustration of (*d*) is to be found in such a genus as Heteropegma, in which the spicules situated in the cortical and medullary regions of the sponge differ largely as regards size, which difference in all probability solely results from the unlikeness of structural characters distinguishing these two regions of the sponge.[1] In any given instance, the question as to how many of these factors are concerned, and in what proportion each has contributed to the result, can at present only be answered in a very general manner.

Another subject for consideration is the relative numbers of monaxons and triradiates present in different sponges. As yet I have not sufficient data to draw any general conclusions, and for the present I will only suggest a possible solution to the problem raised by the scarcity of monaxons found in many Clathrinidæ, as e. g. Cl. contorta, in which the monaxons are very large. As pointed out before, associations of two cells are more likely to occur than associations of three, and hence, merely on grounds of probability, the scarcity of monaxons in those sponges in which they occur at all is remarkable. Again, as previously remarked, sponges differ as regards the facility with which secretion occurs. In some sponges (as e. g. the Sycons above described) secretion occurs on division of a single scleroblast; in others, it is necessary that two cells should unite (as is apparently the case with Cl. contorta); and in others, no less than three cells must unite before secretion can take place (as in the majority of Clathrinidæ). Now, if we suppose that in Cl. contorta monaxons can be produced by union of two cells, but that the stimulus to secretion is very feeble, i. e. the

[1] This is a very doubtful instance.

union of two cells only just suffices to ultimately initiate secretion, then it is easily understood how in such a case it is that such a very few monaxons are produced—the majority of the associations of two cells becoming associations of three cells in the long interval which elapses before the binary association can produce a monaxon. Such is a possible explanation of this and other like phenomena; whether it is the true one I cannot undertake to say. The fact, however, that, so far as I have been able to discover, in no calcareous sponge do there co-exist small monaxons —monaxons presumably produced by division of a single scleroblast—with triradiates, but either small and large monaxons with triradiates, or solely large monaxons with triradiates, or triradiates alone, or monaxons alone, is suggestive and confirmatory of the idea just stated. Since, if a single binucleated scleroblast can produce a spicule, then the associations of two and three scleroblasts can; if secretion, however, only occurs on the associations of two and three scleroblasts, then only large monaxons and triradiates will result; if further, secretion only occurs on union of three scleroblasts, then only triradiates will be produced; if finally, secretion occurs very easily, then only monaxons will result. Why the two mother-scleroblasts which produce Clathrinid monaxons undergo division to such an extent as to give rise to such huge structures, it is impossible as yet to say.

One more explanation may be provided. It has been seen in Part I of this paper that the inner cell (next the gastral layer) of each of the three pairs of cells constituting the sextet is chiefly concerned in the formation of the ray, and that the outer cell of each such pair is basal in position, and with its two companions constructs the central portion of the triradiate system. Why should there be this particular division of labour? The answer is doubtless largely to be found in the fact that the cells constituting the trefoil are from the first central in position, and closely adherent to each other, and therefore tend to maintain this disposition, whereas the three division-products of these are naturally

more peripherally situated, are not adherent to each other, and hence are more adapted for centripetal migration. As to the question why the more peripherally situated division-products of the trefoil should incline gastrally rather than dermally, it can only be pointed out that in all probability the sextet, like the rest of the organism, is influenced by the pressures on the sponge-wall. The sextet will, for example, always tend to be so placed in the sponge-wall as that the plane of the trefoil cells shall be parallel to that of the wall on account of the pressures transmitted through the jelly of the wall from both sides,[1] and seeing that the trefoil portion of the sextet constitutes the "body" of the cell-cluster, it is evident that all peripheral appendages (such as the future apical cells) will tend to swing to that side of it which is the less exposed to incident forces, i. e. gastrally—less exposed, since the pressures derived from the dermal surface of the sponge-wall exceed in intensity those derived from the gastral surface on account of a shield being provided by the opposite wall in the latter case. As the spicule becomes formed, the apical cell for the same reason will always incline gastrally at the extremity of the ray, the cell tending to rotate about its extremity (text-fig. 11), and this is probably the cause of the pyramidal conformation of the triradiate adapting it to the curvature of the sponge-wall.[2] Similarly the basal cells will also slide into the interspaces of the rays, in which position they are, as the figures show, normally found. In support of this conclusion may be named the fact that occasionally two cells are found in one interspace, both having slipped into the same retreat (figs. 23 and 24).

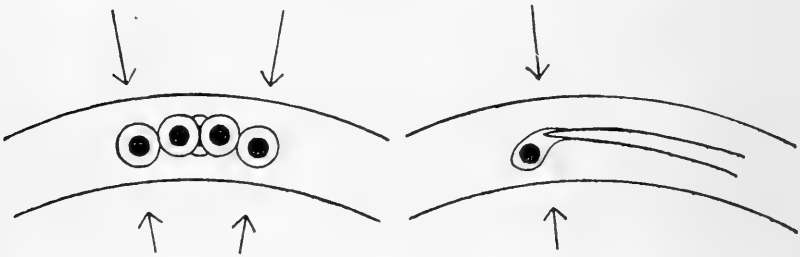
Finally, a word as to the relations between the apical and basal cells in the triradiate spicule. At first, as we have

[1] I cannot call to mind having ever seen a "sextet stage" completely edge-on, though these of course may occasionally occur.

[2] The production of the "tripod spicules" in some species of Clathrina is perhaps to be explained in a like manner, the excessive gastral inclination of the three rays being dûe to the absence of opposing pressure derived from the gastral side of the wall.

seen, each apical cell acts in conjunction with its respective basal cell in order to produce a monaxon structure, but eventually this co-operation ceases, and the basal cell commences to secrete spicular substance round the base of the ray in order to consolidate it centrally. Now the three rays once affixed centrally, the shifting of the basal cell from the base of the ray (and in young stages the basal cell is, as forming part of the trefoil, always more or less radial in position) into an adjacent interspace (which, being independent, it is at liberty to do) above noticed, is rather advantageous than otherwise, but, at the same time, it involves the loss of that cylindrical disposition of the cell-

TEXT-FIG. 11.



substance relative to the ray that commonly obtains; hence the fact cited above (p. 249), that the basal cells of triradiates alone do not possess this conformation—the cell in forming a deposit to one side of the ray loses connection with the other and does not regain its cylindrical disposition when secondarily migrating.

Many other problems remain to be solved, but the solution of these, as of those above discussed, can only be satisfactorily accomplished by means of a comprehensive survey of the vast array of facts presented by the structure and bionomics of the numerous genera and species of calcareous sponges known to exist.

The Phylogenetic Evolution of the Spicules in Calcarea.

The theory here adopted as to the origin of calcareous spicules has already been implied in the foregoing; in other words, the phylogenetic process is here held to be, in all essentials, identical with the ontogenetic process. It will tend to still further enforce the general argument if I here give a brief resumé of the above discussion from the present point of view.

Granting the presence of numerous isolated scleroblasts in the substance of the sponge-wall (generally in regions of vigorous growth), the capacity of their cytoplasm to secrete lime, and the more or less fortuitous association of these scleroblasts in twos, threes, and higher aggregates, and, as shown above, it is not only possible to show why monaxons, triradiates, and quadriradiates have been produced, but also why, in the normal course of things, spicules of other forms have not. I have further attempted to show that the modes of disposition and secondary forms of the spicules are inevitable results of environmental influences operating during the course of each individual ontogeny. Thus, e. g. as regards the mode of disposition which I have termed "oscular," we have initially in the case of each sponge an irregular arrangement of young spicules, which, during growth, becomes a regular and symmetrical arrangement, and since the rotation of solid structures implies a mechanical cause, and since this cause must be uniformly dispositioned with regard to the sponge as a whole (since the spicules are regularly distributed round the whole circumference of the sponge), there is, on this account alone, an à priori probability in favour of the conclusion that the motion of the surrounding medium so functions. In the case of localised secondary forms of the triradiates this probability is even stronger since it is inconceivable that each scleroblastic "determinant" in the sponge ovum should be guided to its appropriate situation in the

adult organism in order to there produce, or aid to produce, a spicule of a particular conformation; i. e. it seems impossible to attribute the production of these secondary forms of triradiates to inheritance. The direct action of the environment in determining the disposition of spicules is particularly well shown in the Leucosolenia diverticula mentioned above, where, owing to a change in the architecture of the sponge body, and in consequence to a different mode of action of the environmental forces, the disposition of the triradiates immediately becomes altered.

Prof. Minchin, to whom I am indebted for so many of my facts, has, in the paper before referred to, put forward an ingenuous hypothesis respecting the evolution of triradiate spicules, contending that such have arisen, through natural selection, by the apposition and fusion of primitively separate monaxons, their extremities being brought together by the agency of pore-distribution. And a somewhat similar idea has previously been expressed by Schulze, who, after asserting that "in the angle between any two rays (of the triradiates) a pore is situated" (which is only true to a certain extent for some Clathrinidæ—in all other calcareous sponges no such relationship between the pores and the triradiates being observable at any stage of growth), contends that the equiangularity of the triradiate is "to be explained in much the same manner as in the honeycomb."

I exceedingly regret having to differ from high authority on these matters, but, for the present, I must adhere to my own explanations, if only for the reason that they present to my mind more definite conceptions of the causes involved. I think it highly improbable that survival of the fittest can have had much to do either with the modes of disposition or with the primary or secondary forms which the spicules assume.

Appendix A.

A summary of the statements made by Maas in his account of the development of Sycon spicules is here given (from 'Zoological Record' for 1901, by Minchin). The misleading nature of these statements will be rendered most evident by appending to them the necessary corrigents.

(1) Each monaxon arises in a single mother-cell—which is not accurately true, since no trace of the spicule occurs until the mother-cell has constricted into two portions, each possessing a nucleus.

(2) There is never more than one cell on the smaller spicules of this class (monaxon)—whereas, in actuality, there are never less than two, however small.

(3) The large monaxons have numerous formative cells upon them, not derived from division of the mother-cell, but from the dermal layer de novo—which is demonstrably incorrect, the largest monaxons (in Sycon ciliata and S. coronata) never possessing more than two formative cells, and if, as in some Ascons, they possess four, they are, judging by analogy, probably all derived from the original mother-cell (or cells).

(4) The triradiates also arise each in a single mother-cell as a concretion, but at a later stage they bear several formative cells—a statement which misrepresents the facts about as far as it is possible to do, the triradiates being derived (as Minchin has described in the case of the Ascons, and as is shown above in the case of the Sycons) from three mother-cells which have associated together, and built up by their six division products. Additional formative cells from other sources never occur.

I may add that I treated several specimens of Sycon ciliata with absolute alcohol and ammonium carmine—the principal method adopted by Maas; needless to say, the results were the same as those obtained by me in my other preparations. Indeed, results obtained by this method are

somewhat better than those obtained with picro-carmine, the clear elongated "vacuole" or mould in the substance of the apical cell at the extremity of the spicule ray, e. g. being made more evident.

I find it very difficult on any supposition to account for the statements and figures of spicule formation supplied by Maas.

Appendix B.

I have provided two additional figures (43 and 44) which I think bear out the conclusion, based on à priori grounds, that some monaxons in these Sycons—presumably the larger kind—originate from two mother-cells. If figs. 43 and 44 be compared with figs. 3 and 5, it will, I think, be admitted that there are here presented two distinct modes of origin of the monaxon—the bi-division of a single scleroblast and the apposition of two being well distinguished by the respective cell forms. It may be objected that, as in the triradiates and monaxons already described, the original mother-cell always divides, the non-division of the two mother-cells of the large monaxons would be anomalous. But the objection has little weight, for, since "the number of formative cells produced from the original mother-cell (or mother-cells) is strictly dependent on the maximum size of the spicule attained," it is evident that, if the adult size of the spicule does not demand it, i. e. (more logically speaking) if there exists no stimulus to division, then the two mother-cells will not divide. In cases where division of the mother-cell does occur, there is always a good reason for it. Thus, in the case of monaxons produced from a single bi-nucleated scleroblast, and in the case of triradiates produced from the trefoil, the division of the one or more scleroblasts concerned is, under the conditions, a necessity for the production of the spicule. And in many Ascons, the huge monaxons possess four or more actinoblasts—there exists a stimulus (whatever

may be its nature) causing division of the scleroblasts initially concerned in the production of the monaxon.

Again, it is obviously probable à priori (as mentioned in the text) that if three scleroblasts associate, two also should associate, and in this latter case there will, under the conditions, inevitably result a monaxon structure. But if monaxons can either be produced from a single binucleated scleroblast or from an association of two scleroblasts, then, since in the latter case the initial capital is twice as great as that in the former, a difference in the size of the two classes of monaxons might be anticipated. This is the case in the Sycons, as text-fig. 1 shows. Although, of course, there exists a certain range of variation in size in each type of spicule, yet the thin and the thick monaxons are tolerably distinct from each other.

EXPLANATION OF PLATES 13—15,

Illustrating Mr. Woodland's paper, "Studies in Spicule Formation." I.

All figures magnified 1000 diameters and drawn with camera lucida.

PLATE 13.

Fig. 1.—Two ordinary scleroblasts.

Fig. 2.—The same with the nucleus enlarged.

Fig. 3.—Division of the scleroblast nucleus; in *a* the binucleated condition has probably been produced by an association of two cells, and not by a division of one; in *c* the cell-substance is more elongated than usual.

Fig. 4 shows the pale streak or "mould" in the cytoplasm.

Figs. 5—12 illustrate the further development of the monaxon spicule. Fig. 7, *b*, is slightly abnormal in its shape. The white streak is also seen in Figs. 11, *b* and *c*.

Fig. 13.—Groups of three scleroblasts in S. coronata probably about to associate to form the trefoil.

Fig. 14.—The trefoil stage.

Fig. 15.—Division of one of the trefoil cells—first stage in formation of sextet.

Fig. 16.—Division of two of the trefoil cells—second stage in formation of sextet.

Fig. 17.—The sextet stage. *a* presents the more typical appearance of this stage.

Fig. 18.—The first appearance of the young triradiate spicule—the three granules or rods being quite separate.

Fig. 19.—The junction of the three needles accomplished; thickening of the individual needles has also occurred. *c* and *d* show the hollow appearance due to corrosion by acid.

Figs. 20—24 illustrate the further development of the triradiate spicule in S. coronata. Figs. 21 and 22 are quadriradiates, the gastral ray possessing one actinoblast. In both Figs. 23 and 24 two of the basal cells have slipped into one interspace—an unusual occurrence.

PLATE 14.

Figs. 25—30 continue to illustrate the further development of the triradiate spicule in S. coronata. Fig. 25 is a quadriradiate.

Figs. 31—34.—Young stages of triradiate spicule formation in S. ciliata. The superior size of one of the three needles is manifest from the first.

Figs. 35—40 show stages of gastral ray (quadriradiate) development, as seen in longitudinal and transverse sections of the sponge-wall. In Figs. 35—38 the gastral ray possesses only one actinoblast. In Figs. 39 and 40 the gastral actinoblast has divided. In Fig. 38 the cytoplasm has been slightly stained with Kernschwarz to exhibit its full extent.

Figs. 41 and 42 show well the secondary thickening of the monaxon spicule due to the migration proximally of the distal scleroblast. In Fig. 41 is again well seen the pale "mould" in the calcoplasm in line with the extremity of the spicule.

Figs. 43 and 44 furnish evidence for the supposition that some monaxons arise from the association of two mother-cells. See also Fig. 3, *a*.

PLATE 15.

Figs. 45—49 are figures of longitudinal sections of the sponge-wall. Fig. 45 shows the division of a scleroblast before it has immigrated from the gastral epithelium. Figs. 46, 48, and 49 show monaxon spicules being produced under the same conditions. In Fig. 47 the monaxon actinoblasts still remain attached to the gastral epithelium by fine protoplasmic processes, though otherwise separated. In Figs. 48 and 49, on the other hand, are shown monaxons which show no connection with the gastral epithelium.

Fig. 50 shows scleroblasts of monaxons stained with Kernschwarz to exhibit their cylindrical conformation; only the spicule sheath is seen.

Fig. 51 shows the migrating basal cells of triradiates similarly stained to show their non-cylindrical conformation.

Studies in Spicule Formation.

II.—Spicule Formation in Alcyonium digitatum; with Remarks on the Histology.

By

W. Woodland,

University College, London.

With Plates 16 and 17.

Historical.

Considering how common a form on both English and Continental coasts is the massive colony of Alcyonium digitatum, it is the more surprising that the very conspicuous and characteristic skeleton appertaining to this species has not been more fully investigated from its developmental aspect than it hitherto has. Figures, verbal descriptions, and nomenclatures of the various forms of the adult Alcyonarian spicule are plentiful enough, but, so far as I have been able to ascertain, the manner of development of this skeletal element has, up to the present, never been completely worked out.[1]

A. von Kolliker (1), who was one of the first to study Alcyonarian spicules, supplied in his 'Icones histologicæ' (published in 1864) a full description and classification of

[1] The limited scope of the present paper—relating to the spicules of Alcyonium digitatum only—is solely due to the fact that I have as yet been unable to visit the Naples Biological Station in order to obtain and properly preserve specimens of other genera and species of Alcyonaria. I hope, however, at some future date to fully investigate spicule-formation in Alcyonaria generally when the opportunity for obtaining the material offers itself.

their various forms and a detailed account of their structure, but did not investigate their development. According to Kolliker, an Alcyonarian spicule consists of an organic and an inorganic part, the former, constituting the bulk of the spicule, being crystalline in nature and with a complicated structure, the latter consisting merely of a cuticular spicule sheath. Kolliker thus denied the existence of one of the most conspicuous features of this type of spicule, viz. the large central organic axis; this, however, is not surprising since he also failed to observe the still more conspicuous fact of the enclosure of these spicules within a well-defined protoplasmic layer containing granules and one or more nuclei, and therefore of their intra-cellular origin. Nevertheless, many of Kolliker's observations on the more intimate structure of the Alcyonarian spicule are correct, as instance, e. g. his statements as to the concentric lamellar structure of the spicule body when seen in transverse section, the breaking up of the spicule into smaller crystalline elements on treatment with weak acids, and the "axial" continuity of the processes of the spicule with the main trunk, resembling, as Bourne remarks, the origin of secondary roots in a plant.

G. von Koch (2) was the next investigator who contributed to our knowledge of Alcyonarian spicules. He ascertained to some extent, and for the first time, the development of the spicules in Clavularia prolifera, giving in his paper on the histology of this species highly-coloured figures of the earliest stages of spicule formation and of the subsequent growth. He rightly insisted on the endoplastic development of the spicules, showing that these not only originate in scleroblasts derived from the ectoderm, but remain conspicuously enveloped by the cell-substance throughout their existence. Koch's figures show well the presence of nuclei—never exceeding two in number it is important to note—in the granular protoplasmic layer investing the spicule, and also incidentally prove that the change in shape of the spicule from the spherical to the elongated is strictly correlated with the division into two of the nucleus of the original mother-

cell. It is also shown that spicules situated in different portions of the colony (and so subject to different environmental influences) are different in form—spicules situated near the surface being monaxon and unbranched, spicules more deeply situated being more or less branched and irregular.

Kowalevsky and Marion (**3**) in 1883 confirmed Koch s statements as to the intra-cellular growth of Alcyonarian spicules, although they gave no figures in support of their statements.

In 1892 K. C. M. Schneider (**4**), in a very short paper, and also without giving any figures to justify his statements, asserted that in development the scleroblast gradually assumed the form of the adult spicule, which latter was then produced by the calcification of the cell-substance. As Hickson (**5**) remarks, the appearance of developing spicules does not by any means justify this assertion, and it is, moreover, grossly improbable that the actual protoplasm should become converted into the crystalline substance of the skeleton.

In 1899 G. C. Bourne (**6**) made some observations on the formation of the spicules in Alcyonium digitatum and Gorgonia cavolinii preparatory to investigating the minute structure of the Madreporarian corallum, and, although only incidentally made, these observations of Bourne up to the present afford the most complete account of the subject extant.

In the following lines I shall occasionally have to refer to certain of the statements made by Bourne and less often by Hickson.

Preliminary Remarks: Methods of Preparation, Histology, etc.

Bourne's method of preparing microscopic specimens of Alcyonium digitatum for examination of the developing

spicules was as follows:—"The specimens were killed in an expanded condition by rapid immersion in a ·5 per cent. solution of osmic acid in sea water, were thoroughly washed with distilled water and stained for twenty minutes with Ranvier's picro-carmine. The expanded polyps were cut off close to their bases, placed in dilute glycerine, and laid open. The ectoderm and endoderm having been removed with a camel's-hair brush, very satisfactory flat preparations were obtained illustrating the formation of the spicules in the lower moities of the exserted portions of the polyps." My experience of this method of making flat preparations of Alcyonium digitatum for the study of spicule formation is that it is by no means a satisfactory one—certainly not so satisfactory as that which I have adopted, and which I will shortly describe. For, in the first place, spicules do not occur to any great extent in the region of the retractile polyps—as, indeed, Bourne admits when he states that "one of the smallest sclerites which I was able to discover is shown . . . ," whereas in my own preparations I have been able to count such by the dozen—and hence there exists a difficulty in observing all the stages of spicule formation; secondly, what spicules do occur here are not very characteristic in shape of the greater number of the spicules found in the mass of the colony—there existing a tendency for spicules situated in the region of the polyps to be smooth and spindle-shaped—and hence the development of these others cannot from these data be assured.

Hickson remarks that "Alcyonium digitatum is not a favourable form to take for the study of the development of the spicules, as it is a matter of very great difficulty to make a thin section of the surface of the colony before decalcification," but, owing to the transparency of the mesoglœa, it is not necessary to cut thin sections for this purpose, as I think is proved by the fact that all the figures supplied in the plates accompanying the present paper were carefully drawn by means of a camera lucida from free-hand sections which measured anything from 10 μ to 50 μ in thickness. The

method of preparing microscopic slides of Alcyonium digitatum[1] which I adopted was practically identical with Bourne's as regards the fixation and staining of the specimens, but different as regards the region of the colony selected for examination and the manner of obtaining thin portions of it. Young colonies only were selected (an important point), and these were simply fixed by sudden immersion in 1 per cent. osmic; they were then thoroughly washed in distilled water, and deposited in either Ranvier's or Weigert's picro-carmine for three hours. When thus effectually stained the colonies were again washed and carefully graded up to absolute alcohol. The spicules offering too great an obstruction to microtome section-cutting, the colonies were transferred straight from the absolute alcohol to coco-butter, in which substance they were held whilst free-hand sections of them were made. These sections were then placed in xylol, and finally mounted in Canada balsam. By this method excellent preparations were obtained, the nuclei, cell-plasma, and organic axes appertaining to the numerous spicules being rendered very evident. I occasionally substituted paraffin-wax for the coco-butter, but the latter is preferable in many respects, and, moreover, is more economical as regards time.

Before proceeding to the description of the development of the spicules in Alcyonium digitatum, it will be as well to give a brief account of the general histology, if only merely in order to distinguish the spicule-forming cells or scleroblasts from neighbouring endoderm-cells, etc.—a distinction that has not always been made. The ectoderm, which forms the limiting layer of the mesoglœa "consists," according to Hickson, "of a number of columnar, spindle-shaped, and flask-shaped cells . . . connected at their outer borders, but

[1] These specimens were obtained from Plymouth, and the method above described was employed in consequence of the failure to obtain the polyps in an expanded condition (as recommended by Bourne) by narcotization. I subsequently fixed the expanded polyps, like Bourne, by rapidly immersing them in 1 per cent. osmic; but these, as above explained, did not give good results, as compared with those obtained from the free-hand sections of the mass of the colony.

free from one another for the greater part of their course. At the base of the epithelium there are a few spherical interstitial cells of different sizes." In the course of my observations of the spicules I came across a number of exceedingly small cells—some even smaller than the nuclei of other cells—which were situated in the peripheral layers of the mesoglœa. These cells, which I have represented in Pl. 17, fig. 20, were slightly granulated, and many contained a distinct though faintly-stained nucleus; in others, however, it was impossible to distinguish one. It is probable that these are the interstitial cells of Hickson, more especially since, owing to variations in size, it would be possible to trace cells intermediate in size and appearance between these and the sclero-blasts they in all probability give rise to. Hickson says that "it is difficult to determine with certainty the origin of the cells that give rise to the spicules, but, for many reasons, I am inclined to agree with von Koch's results on Gorgonia and Clavularia, and attribute them entirely to the ectoderm. Among the interstitial cells of this tissue one frequently finds large spherical cells which lie beneath their neighbours, and cells very similar to these may be seen isolated in the subjacent mesoglœa." These scleroblastic mother-cells are represented in Pl. 17, fig. 19, and Pl. 16, fig. 1.

In addition to the epidermal cells, the interstitial cells, and the scleroblasts which they probably give rise to, and two or three other kinds of cells, which I shall mention, there exist, according to Hickson (and I can confirm his statements), in the peripheral portion of the colony, the endodermal canals, the "solid cords of endoderm," and "isolated cells connected with one another and with the endoderm by fine anastomosing fibrils." "The cords [see fig. 18] are, in some, fairly compact, resembling a canal in all respects except the presence of a lumen, but in others the cells are only loosely connected with one another, become elongated or star-shaped, giving off fine fibrils at their angles. There may be only a single row of oval or cubical cells, or in some cases the row may be drawn out into a chain of elongated

spindle-shaped cells." Bourne mistook these strands of endoderm cells for scleroblasts—"the scleroblasts have the form of irregularly polygonal, ovate, or amœbiform cells, varying very much in size and shape; they run in strands and patches through the mesoglœa at the bases of the expanded polyps, and may be found, though they are not easily studied, in the thickened mesoglœa of the cœnenchyme" (**6**)—but the endoderm cells as a whole, besides often being multi-nucleated (which the scleroblasts never are until spicule-formation is fairly advanced, and then only two nuclei are present) are also considerably larger and much more irregular in shape than the true scleroblasts, which are approximately spherical. It is true that in many cases these endoderm cells possess large vacuoles, which, but for the absence of refringency, might be mistaken, at first glance, for young spicules, but, personally, I cannot call to mind having ever seen a real spicule contained in one of these irregular cells.

Bourne describes, in the paper before referred to, two other kinds of cells—one possessing refringent granules and the other containing the ovoid bodies—only the latter of which Hickson refers to. Bourne remarks that the cells of the first kind are "rather smaller than, but of similar shape to, the scleroblasts," which, since Bourne reckons the endoderm cells as scleroblasts, must therefore be somewhat irregular in form, as his figures indicate. These cells are said to be "filled with minute highly-refringent granules," and their nucleus is "rarely to be seen, being hidden by the granules." Bourne believes that "their function is to secrete the gelatinoid substance of the mesoglœa." To some extent I can confirm Bourne's statements, since I also have found peculiar cells containing very distinct minute granules and a faintly-staining nucleus, and which are "of similar shape to the scleroblasts." The cells I recognise as scleroblasts are, however, spherical, and so are these cells containing the distinct granules; whether they are the same as the jelly-secreting cells of Bourne I cannot decide. I have shown three of these cells, which are somewhat bladder-like in appearance, in fig. 21.

Hickson states that the "nematocysts of Alcyonium are extremely small (0·0075 mm.) and all of one kind," and describes those situated in the ectoderm of the tentacles. In addition to these nematocysts Hickson describes certain "oval bodies lying in and among the cells of the endodermic cords." He adds that "they may be readily distinguished from the endoderm cells by their dark but homogeneous appearance," and that "in one or two instances he succeeded in making out a somewhat irregular body in the centre, which may be a nucleus." Hickson suggested that "they may be some form of parasitic sporozoon." Bourne also noticed these "ovoid bodies,"[1] as he calls them, and stated quite correctly that "each . . . is surrounded by a protoplasmic sheath, and has a relatively large nucleus on one side of it," and that they are not calcareous in nature. "They stain deeply with hæmatoxylin," and (contrary to Bourne's statement) also with picro-carmine, methylene blue, and some other stains. Bourne hazards a guess that these "ovoid bodies" are degenerate nematocysts. I also have observed these very conspicuous bodies, illustrations of which I have supplied in figs. 22 and 23. In one respect only can I improve upon the description of Bourne, and that is the observation of a structure distinguishing the cells containing "ovoid bodies," situated at the

[1] As Bourne says, these "ovoid bodies" occur in other Anthozoa, and Mr. E. T. Browne has shown me globular bodies of the same nature in Solmundella bitentaculata (one of the Narcomedusæ)—bodies which exactly resemble those shown in fig. 22, save that they are spherical instead of oval. No cnidocil could be detected in connection with the cells containing them. With respect to the cells containing "ovoid bodies" found in Alcyonium digitatum, I may add that those possessing cnidocils have, as shown in the figures, the greater part of the cytoplasm associated with the cnidocil, which is wholly composed of it; also, in these cells generally the nucleus is situated at the cell periphery, and somewhat flattened out in its contact with the cell-wall. In one case I have observed the stained "ovoid body" to be contained within a fairly large spherical cell situated in the ectoderm—doubtless a cnidoblast not yet reduced to the normal dimensions. Quite recently I have observed "ovoid bodies," similar in every respect to those of A. digitatum, in teased preparations (stained with methylene blue), of the common Hydra!

edge from those situated more or less deeply in the mesoglœal substance. In the former I have observed in nearly every instance the presence of a distinct cnidocil, as shown in fig. 23, but I cannot detect this structure in those more internally situated, and it doubtless does not exist in their case. The presence of this cnidocil I think distinctly proves the nematocyst nature of the "ovoid bodies" of Alcyonium, which thus, according to Hickson's account, possesses two kinds of nematocysts—one restricted more to the region of the tentacles, the other to the mass of the colony.

Thus, in Alcyonium digitatum, in addition to the scleroblasts, there exist in the mesoglœal substance, endoderm cells, the spherical "jelly-secreting" (Bourne) cells, the small interstitial cells and the nematocysts or "ovoid bodies," all of which latter are more or less distinguishable from the former.

The Origin and Development of the Spicules.

The scleroblasts, as already stated, are granular, more or less spherical cells situated at the periphery under the ectoderm, and probably derived, as Hickson suggests, from the interstitial cells of that layer. Further, "the spicules are far more numerous at the periphery than in the deeper parts of the colony. This suggests very forcibly that the spicules are only formed at the periphery, and that with the growth of the mesoglœa they become more and more separated from one another"—a suggestion I can amply confirm. The spicule first appears in the cytoplasm as a small spherical concretion (figs. 1 and 2), and remains approximately spherical in its further enlargement until the division into two of the nucleus (fig. 3).

This last statement, I am aware, is contrary to the account of early spicule formation given by Bourne, who supplies figures representing the young spicule as of very irregular shape long before nuclear division occurs. One possible

explanation of this difference of form in the two cases is the difference of situation of the young spicules drawn by Bourne and of those drawn by myself—Bourne's spicules being localised in a mobile portion of the colony (the bases of the polyps, where the mother-cell is obviously subject to disturbing influences which might very possibly lead to irregularity of form of the contained spicule) and mine in a quiescent. But, apart from this, some of Bourne's figures (e. g. his fig. 4) certainly suggest the idea of his spicules having been subjected to rough treatment of an artificial kind, such as the stretching open of the polyp, and the brushing of its internal wall might by chance involve, but whether the fractured appearance of the spicules suggested by Bourne's figures is due to this, or to the above-mentioned cause or to the mode of drawing, I am unable to say. Certainly the young spicules observed by me did not exhibit this broken irregular appearance.

As just stated, up to the division of the scleroblast nucleus, the form of the young spicule remains smooth and approximately spherical, but when this change in the nucleus occurs, the spicule becomes elongated and somewhat dumb-bell in shape (not dumb-bell in shape in the sense in which Hickson refers to adult spicules), i. e. thickened and rounded at the two extremities, and the two nuclei, which form centres for the aggregation of the cytoplasm, in general travel to its opposite ends. This simple dumb-bell then enlargens and its two extremities become amphicœlous by the development of a broad rim round the terminal surface of each "head" of the dumb-bell (figs. 3 and 4). Following on this again, the rim on the terminal surface becomes developed into two, three, four, or more processes, some, or occasionally all, of which afterwards become the main branches of the spicule; other smaller processes may also appear, and the spicule now assumes the form depicted in figs. 5 and 7. This stage thus reached forms in the vast majority of cases the basis of all future development, since it is a ground plan common to, and recognisable in, all spicules whatever shape they may ultimately assume (with the possible exception of the spindles

presently to be described), and is, in fact, the starting point of the morphological differentiation of the spicules. In form it somewhat resembles, as Bourne suggests, a caudal vertebra, and like all preceding and succeeding stages is enclosed in a granular protoplasmic sheath containing two nuclei, which, in the vast majority of cases, are situated at the two extremities of the dumb-bell basis.

It is an interesting fact that in all succeeding stages of spicule-formation—in all the varied and complicated forms which adult spicules assume—only two nuclei are present. It is important to insist upon this point since Bourne states that "in older and more complicated spicules I have [he has] counted three or four nuclei." Personally, in all the hundreds of spicules which I have observed I have only once, possibly twice, chanced upon one possessing more than two nuclei; in one case the spicule was at the "caudal vertebra" stage of development, and of the ordinary form, although perhaps with some extra processes developed, and possessed as many as six nuclei, all apparently contained within the protoplasmic investment of the spicule; in another case the spicule possibly possessed four nuclei, although of this I am not so certain. In both of these cases I may of course have been mistaken—may have misjudged adjacent free scleroblasts as cells appertaining to the spicule,—but in the former example, which I most carefully observed and drew, I do not think I was; in any case the rarity of such multinucleated spicules quite disproves Bourne's statement that such are of common occurrence. The fact that Bourne mistook endoderm cells for scleroblasts[1]—"the scleroblasts are often cœnocytes containing two, three, or more nuclei"—was probably the source of his erroneous assumption on this head, despite the admission that "the nucleus apparently divides when the sclerite has attained a certain size," and the erroneous statement that "this division is repeated as growth [of the spicule] continues." I may here point out in this connection that von Koch also

[1] It must not be inferred from these little criticisms that I fail to appreciate the high value of Bourne's excellent paper.

figures two nuclei as the maximum number attained in the formation of the comparatively simple spicules of Clavularia prolifera. Why only two nuclei are concerned in the formation of these Alcyonarian spicules I cannot at present say, and I have therefore no à priori objection to the existence of multinucleated spicules, and am quite ready to accept provisionally, e. g. Bourne's other statement that, so far as he has been able to ascertain, the "scale-like spicules in Primnoa and Plumarella are formed by several cells, or at least by a comparatively large cœnocytial investment containing many nuclei;" all I at present contend is that, strange as the fact may appear, the huge spicules of Alcyonium digitatum never, in the ordinary course of things, possess more than two nuclei embedded in the wall of the protoplasmic sac which envelops them.

Starting from the "caudal vertebra" stage, different spicules develop in different directions assuming unlike forms. Nearly, if not all, the different forms are derived from the "caudal vertebra" condition by the special development either (1) of the large processes (see text-fig. C below) derived, as just described, from the rims of the amphicœlous extremities (some of the minor processes occasionally replace these, however, as the figures show), i. e. of some or all of the four angles or corners of the spicule basis when this is viewed from a lateral aspect, or (2) of one or both of its two ends, or (3) of the two sets of processes combined. In fig. 8, e. g. two of the diagonally opposite angles have become specially developed, and similarly in fig. 9, though here, owing to the spicule lying edge-on, the two processes resemble the elongated ends of the spicule and not its corners; in fig. 10 two of the angles on the same side of the spicule, but not in the same plane; in fig. 11 two of the angles at the same end of the spicule and the opposite extremity, and similarly in fig. 12; in fig. 13 three of the four angles; in fig. 14 all of the four angles, two more so than the opposite pair. It will be seen from the figures that in the growth of these angles or ends, the actual prolongation may not, as before pointed out,

be derived from the original "corner" or end, but from a process developed to one or the other side of it. In some cases also the rounded extremity of the simple dumb-bell divides into (i. e. the rim develops into) many more than three or four regions, and the resulting spicule is then more complicated at its extremities, but this is rare in A. digitatum.

In every case I failed to observe any relationship between the position of the nuclei and the development of certain processes, and it seems certain that after the first elongation of the spicule the nuclei play no further part in determining its form.

Remarks on some other Features of the Spicules.

On observing a section of Alcyonium digitatum containing spicules, preferably under a low power of the microscope (say × 500 diameters), one cannot fail to notice that each of the larger spicules is contained in a distinct cavity apparently formed in the substance of the mesoglœa by, and during the growth of, the spicule itself, since the thickened edges of the mesoglœal substance constituting the walls of this cavity are distinctly supported by the spicular processes —like tent canvas on a pole (see figs. 16 and 17),—and hence must have been displaced by their formation. This simple fact[1] serves to throw some light upon the physical consistency of the mesoglœal substance, and possibly has a bearing on the problem as to the causes of the various forms which spicules assume (see further).

The figures of the spicules provided in the plates illustrate well another fact of importance, and that is the large amount of organic matter contained in the substance of the spicules. Fig. 6 shows in optical section the concentric structure (probably indicating periodic growth) of the spicule axis, which stains pink with picro-carmine, and it is owing to the presence of this axis that all the spicules appear pink in colour. The

[1] Possibly an artefact.

width of the axis, as compared with the width of the spicule, is variable in different spicules, it being noticeably small, e. g. in the lancet-shaped or monaxon spicules (fig. 15).

I have never observed any horny sheath enclosing the spicule, such as that, e. g. figured by von Koch in the case of the spicules of Clavularia prolifera, and since, up to the time when the spicule attains its maximum size, the whole surface-area of the spicule is having in a varying degree fresh calcareous matter deposited upon it by the investing protoplasmic layer, I am inclined to think it does not possess one—at least not one of any considerable thickness.

With regard to the position of the nuclei on the more adult spicules, there is only one remark to make, and that is that in general one nucleus is to be found at one extremity of the "caudal vertebra" body of the spicule, and one on a process at the opposite end, but this position of the nuclei is not an absolutely constant one, and is probably of little or no significance.

The intimate structure of the Alcyonarian spicule has been investigated by Bourne and others, and since I have nothing to add to their accounts, there is no need for me to here describe this.

Remarks on the Forms of Alcyonarian Spicules.

Like most, if not all, calcareous spicules which originate in connection with an isolated more or less spherical mass of protoplasm containing one nucleus—a single cell—the Alcyonarian spicules at their first appearance are approximately spherical in form. Further, as is also universally the case, bi-division of the nucleus of this cell[1] containing the spherical sclerite is followed by an elongation of the spicule more or less in the direction of nuclear division. And, in Alcyonium at least, it is not difficult to see why this should be so, for the scleroblast resembling all other isolated cells

[1] Bi-division of the calcoplasm must also occur.

in its mode of fission—the mass of the cytoplasm becoming concentrated at two centres (each with its nucleus), and therefore attenuated in the region situated between these,—and lime salts being deposited most freely where the bulk of the cytoplasm is greatest, the sclerite must obviously, under these conditions, not only, like the cytoplasm, become elongated, but also, like the cytoplasm, become dumb-bell shaped, as we know it does (text-fig. A). So far, then, the Alcyonarian spicule follows the normal course of development. But, as before stated, beyond the dumb-bell stage of growth, the position of the two nuclei does not seem to exert any influence on the form gradually assumed by the spicule—processes being developed on all sides quite irrespective of the two

TEXT-FIG. A.

thickenings of the general protoplasmic investment containing these bodies,—and the further morphogenesis appears to be solely related to external factors. In what manner, however, environmental conditions produce the various forms characteristic of adult Alcyonarian spicules is a question somewhat difficult to answer, and at present I can only supply a suggestion or two towards the solution of this problem.

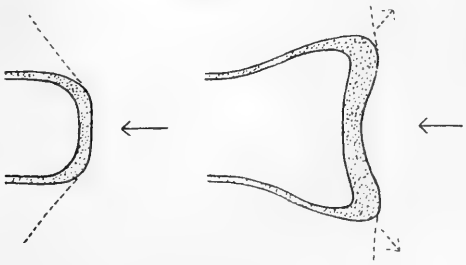
Two general principles at once present themselves for consideration, the first of which is that growing spicules situated in a mass of mesoglœal substance far removed from any limiting surface must, owing to the proximity of other spicules, endodermal canals, and other heterogeneities of constitution of the surrounding medium, necessarily be subject to an aggregate of influences which tend to produce irregularity of form; and the second is that the extension of a growing body into a surrounding resistant medium is most easily effected by the protrusion of more or less acute processes

which, in virtue of their acuteness, are best able to cleave a passage. In connection with this last principle, it may be pointed out that the form of the Alcyonarian spicule certainly suggests the idea that the minor processes (doubtless due to that localised activity of the protoplasm which remains unabsorbed in the prolongation of the main branches) may be attributable to the same cause as say the lobose pseudopodia of a rhizopod—the protoplasmic investment of the spicule being comparable to the ectosarc in which the protrusive movement originates, and the deposited calcareous matter to the stream of endosarcal granules which follows in its train,—and in all probability there does exist an affinity between the two kinds of emergencies.

As has already been described, the extremities of the young dumb-bell spicule quickly become amphicœlous in character, or, in other words, a broad rim usually develops around the hemispherical "head" of each dumb-bell rendering the terminal surface more or less concave (fig. 3), and a few minor processes developing at the "corners" of the spicule, the entire structure comes to resemble, as also before remarked, a caudal vertebra. A possible explanation of this change from the simple dumb-bell to the amphicœlous condition is to be found in the fact stated above, viz. that the consistency of the mesoglœal substance is largely solid, and not liquid in nature (figs. 16 and 17), from which it follows that in the elongation of the binucleated scleroblast and its contained sclerite, the resistant pressure offered by the mesoglœa is greatest at the extreme ends of the spicule, and therefore less towards the sides (i. e. the pressure is not the same at all points as would be the case were the medium liquid), and seeing that, at the same time, the mass of the protoplasm is situated towards the extremities (being pushed somewhat to the sides however by the terminal pressure), further deposition of the calcareous substance must, under these two conditions, take place at the sides and towards the ends of the spicule, i. e. the terminal surfaces will become amphicœlous (see text-fig. B).

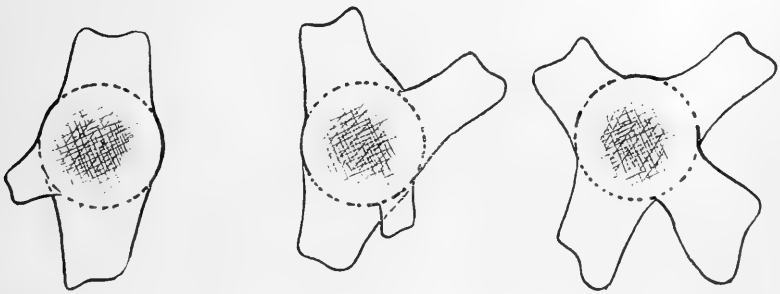
Now, according to the second of the two above stated principles, further growth of the spicule substance, i. e. further protrusion of the protoplasmic investment into the surrounding medium, will take place at that portion of the spicule surface possessing the greatest angularity or rather

Text-fig. B.



convexity, i. e. at the rim of the amphicœlous extremity, or in other words, at any or all of the four "corners" always visible when the spicule is viewed from a side aspect. If the external medium were absolutely homogeneous in constitution,

Text-fig. C.



extension of the spicule substance would, under such conditions, take place uniformly at the rim of the spicule extremity, and a hollow cone-shaped structure would be produced, but in Alcyonium digitatum and the vast majority of other Alcyonaria, the surrounding mesoglœa is not by any means homogeneous in nature, and hence the

extension of the amphicœlous extremities of the spicule is not uniform; on the contrary, the "head" of the spicule, or rather rim, becomes divided into two, three, four, or more regions producing the large processes in every case observable (figs. 4, 5, and 7, and text-fig. C above). Any spicule at this stage seen sideways from any aspect will thus present the appearance of the ordinary "caudal vertebra," with its concave-headed dumb-bell basis, and more or less well-developed processes projecting from two, three, or all of its four angles according to the number of these developed, and to the side of the spicule presented to observation.

From the "caudal vertebra" stage onwards, it apparently depends entirely on the influence of the heterogeneous constitution of the surrounding medium which of the angles or corners shall develop into the elongated branches proceeding from the main body or basis of the spicule, and which shall remain inconspicuous. In some cases indeed, the extremities of the dumb-bell elongate, but only very occasionally in deeply-situated spicules. Adjacent spicules and other structures must exercise an influence on a developing spicule, and therefore determine to some extent at least its mode of growth, and seeing that the extreme irregularity of form found among the numerous spicules ("no two spicules in the field of the microscope are alike," Hickson) is alone congruous with the view that such is related to the irregularity of the surrounding conditions—different conditions necessarily obtaining in the case of each spicule—this view is doubtless the correct one.

The alternative view that the furcations of the Alcyonium spicule are to be accounted for in the same manner as, e. g., the dichotomy of the lower Cryptogams, seems to me unlikely since, in this latter case, the branching is mostly related to food-supply—a large surface area being essential to the efficient nourishment of a large bulk—but in the case of the spicule the internal mass does not require nourishment and hence the increase of surface area of the cell-substance cannot be related to that end. I regard the cell-substance investing

the spicule as unavoidably secreting lime—the cell "cannot help it"—and the faster it secretes the better is it nourished, and therefore able to secrete, since additional secretion of lime implies further increase of surface area, and this (although the scleroblast is not a plant) is favourable to the nourishment of the cytoplasm. However, as there is a limit to the divisibility of a Paramæcium, however well nourished,[1] so there is a limit to the growth, and therefore secretory power of the enormously-distended scleroblast with its two nuclei, which, judging from appearances in some of the full-sized spicules I have seen, like the Paramæcium, eventually degenerates.

The massiveness and irregularity of the majority of the spicules in Alcyonium digitatum is due to the fact already suggested that all older spicules are situated in mesoglœal substance far removed from any limiting layer, the colony of A. digitatum being, as is well known, exceedingly massive in form and not racemose. When, however, spicules have existed for the greater part of their development in close proximity to the external ectoderm or to the endodermal layer of a gastral canal, irregularity of form largely disappears and the spicules assume the form of a simple manaxon either with very minute processes or without any (fig. 15). As Hickson says, "there may be seen a certain number of long unbranched lancet-shaped spicules covered with irregular tuberosities. They vary very much in shape, some being like a thick pin (without its head) others lancet- or spindle-shaped, and others again slightly curved like a boomerang. These long unbranched spicules occur chiefly in the tentacles and disc of the polyps [where the mesoglœa is evidently of narrow width]. They do occur in other parts of the colony [in proximity to the endoderm canals], but I do not remember to have seen any dumb-bell-shaped spicules in the extensible portion of the polyps." Again, in the other English species, Alcyonium glomeratum, which is much more racemose in form than A. digitatum, and in which consequently the

[1] In view of Calkin's experiments, I suppose I ought to add "on one diet."

mesoglœa does not form such a solid mass enabling the spicules to be distantly situated from any limiting layer, "the majority of the spicules are elongated needles and spindles, and there is an entire absence of the small dumb-bell-shaped forms, very few Ks. and crosses [i. e. both resembling figs. 13 and 14], and there are several club-shaped forms [essentially monaxon spicules with fairly conspicuous processes and thickened towards one end, which occur in the most deeply situated portions of the mesoglœa], which I have never seen in any preparation of A. digitatum." "It is true that many of the elongated and spindle-shaped spicules of A. glomeratum are almost exactly the same shape as the spicules of the tentacles and disc of the polyps of A. digitatum, but the clubs are peculiar to it, the dumb-bell [not my developmental dumb-bell] absent, and the Ks. and crosses very rare." Further evidence in support of this general conclusion that the nearer a spicule is situated to a limiting layer the more regular will be its form, is supplied by von Koch in his account of the spicules in Clavularia prolifera already referred to, where he gives figures showing that spicules situated near the surface are quite regular in form (stick-like), but that spicules situated in deeper layers of the mesoglœa become irregularly branched, but never massive and tree-like, as in A. digitatum, since the colony of Cl. prolifera is very racemose in form. Other Alcyonaria supply like evidence, as may be seen, e. g., by glancing through the plates in the 'Challenger Report' on the Alcyonaria. It is also worthy of note that when as in Calcareous Sponges and certain Holothuria the body-wall is very thin, and the contained spicules therefore necessarily in close apposition to both external and internal epithelia, these are in general remarkable for their definiteness of form.

List of the Works referred to in the Text.

1. A. von Kolliker.—'Icones Histologicæ,' Leipsig, 1864.
2. G. von Koch.—"Anatomie der Clavularia prolifera," 'Morph. Jahrb.,' Band vii, 1881. (There are other papers by von Koch on Alcyonaria in the same journal from 1878 to 1881.)
3. A. Kowalevsky and Marion.—"Documents pour l'histoire embryogénique des Alcyonaires," in 'Ann. du Musée d'Hist. Nat. de Marseille,' i, 1883.
4. K. C. M. Schneider.—"Einige histologische Befunde an Cœlenteraten," in 'Jena. Zeitschr.,' xxvii, 1892.
5. S. J. Hickson.—"The Anatomy of Alcyonium digitatum," in 'Quart. Jour. Micr. Sci.,' vol. 37, 1895.
6. G. C. Bourne.—"Studies on the Structure and Formation of the Calcareous Skeleton of the Anthozoa," in 'Quart. Jour. Micr. Sci.,' vol. 41, 1899.

EXPLANATION OF PLATES 16 & 17,

Illustrating Mr. Woodland's paper, "Studies in Spicule Formation." II.

Figs. 1—7 and 18—22 magnified about 950 diameters.
Figs. 8—15 magnified about 475 diameters.
Figs. 16 and 17 magnified about 450 diameters.

PLATE 16.

Fig. 1 shows scleroblasts containing spicules at their first appearance.

Fig. 2 shows young spicules, in which the pink-staining central organic core has become distinguishable from the peripheral layer.

Fig. 3.—The scleroblast nucleus has here divided, and the young spicule has in consequence assumed an elongated form. The nuclei, it will be noticed, tend to be situated at the opposite extremities of the sclerite.

Fig. 4.—The extremities of the spicule are becoming divided up into processes.

Fig. 5 shows the typical "caudal vertebra" form of spicule.

Fig. 6.—A young spicule placed end-on. The concentric lamellar structure of the organic axis shows well.

Fig. 7.—An older stage of growth.

Figs. 8—12.—Various forms of adult spicules.

PLATE 17.

Figs. 13 and 14.—Various forms of adult spicules.

Fig. 15.—Monaxon unbranched spicules found in the vicinity of limiting layers.

Figs. 16 and 17 show the mesoglœal cavities in which the spicules are situated.

Fig. 18.—An "endodermal cord" with several multinucleated cells.

Fig. 19.—Scleroblasts.

Fig. 20.—Interstitial cells.

Fig. 21.—Spherical "jelly-secreting" (Bourne) cells.

Fig. 22.—Nematocysts ("ovoid bodies") from the mesoglœa situated some distance under the ectoderm.

Fig. 23.—Nematocysts from the surface of the colony showing cnidocils.

Studies in Spicule Formation.

III.—On the Mode of Formation of the Spicular Skeleton in the Pluteus of Echinus esculentus.

By

W. Woodland,
University College, London.

With Plates 18 and 19.

Introductory.

Though the mode of development of the skeleton of the Pluteus larva has already been ascertained, as regards the main features, in the case of several genera, as, e.g., in Echinus (miliaris) by Selenka (**17**) in 1879, and Théel (**23**) in 1902, in Arbacia (Echinoid) by Garman and Colton (**6**) in 1882, in Ophiopholis (Ophiuroid) and Echinarachnius (Echinoid) by Fewkes (**5**) in 1886, and especially in Echinocyamus (Echinoid) by Théel (**21**) in 1892, yet since this subject has not hitherto been investigated in detail in any one instance (excepting the case of Echinocyamus), it is well that the following description of the process of spicule formation as it occurs in the larva of Echinus esculentus should be published. This is further desirable in that I have both several new facts of importance to add to our existing knowledge of this process and a few minor suggestions to make relevant to the factors concerned.

The material on which I worked was procured during my

stay at the Plymouth Marine Biological Laboratory in April, 1904, and was produced by carefully rearing larvæ (derived from artificially-fertilised ova) in large jars, each provided with a Browne plunger, and daily supplied with fresh sea water obtained from outside the limits of Plymouth Sound. There is no necessity for me to describe in detail the methods adopted in rearing the larvæ of E. esculentus since the whole process has already been admirably described by MacBride in his important paper relating to the development of this species (**15**, pp. 288—292), and to this account I refer the reader. I wish to add, however, one or two remarks supplementary to MacBride's description. Prof. MacBride carried out his work at Plymouth five years previously to my own visit there, and I was, on this account, able to benefit by his experiences as communicated to me by Mr. Smith, assistant at the Laboratory, who kindly initiated me into the practical methods of larva rearing. In my own case I was fortunate enough to secure at the first haul three or four ripe males and females out of about a dozen specimens of E. esculentus, and seeing that this was in April—a month earlier than the time selected by MacBride, when, as he says "one was lucky if one obtained a single ripe pair out of a haul containing over a hundred specimens"—I think the fact is noteworthy. Again, I may mention that during the early stages of development it was found necessary, by means of Bolton silk, to strain off from the daily supplies of fresh sea water the flagellate alga Phaeocystis globosa, since this was liable to become entangled with the larvæ. Later on, however, when the plutei were about half grown, the same alga, broken up so as to release the spores, served as food, and, indeed, the plutei principally subsisted on this diet. As regards MacBride's tabular scheme of the succession of events given on page 293, I found it in general correct, so far as my observations of the earlier stages went, but Mr. Smith writes me that "some of the plutei (the eggs were fertilized on April 20th, 1904) hung on till the beginning of September, never looking robust but still healthy"—a period after ferti-

lisation of nineteen weeks, i. e. 133 days—whereas in MacBride's experience, metamorphosis occurred about the fiftieth day. This abnormal prolongation of the larval stage was, without doubt, due to the fact that pressure of work at the laboratory prevented very much attention being bestowed upon the larvæ during the summer months—the somewhat stale water and scarcity of food resulting from this inattention retarding the normal rate of development; however, by September 12th all the plutei had undergone metamorphosis, and one jar alone contained "quite a hundred" small Echinus. As might have been expected, most of these died later through sheer lack of food, and by December 12th only four Echinus were left. Had it been possible to properly superintend the later development of these specimens, I have no doubt but that they would have attained a very considerable size before succumbing to laboratory conditions of life.[1]

The only method of efficiently preserving the plutei at different stages of development—the desideratum, of course, being the preservation of the skeleton—which I found practicable was as follows:—A dozen or more larvæ were removed from one of the large bell-jars employed by means of a small pipette (the Browne plunger having, for the purpose of collecting, previously been removed for about half an hour or less to enable the larvæ to aggregate in the upper layers of water), and placed in a test-tube. The problem now was to get rid of the great bulk of the water in which the dozen or more larvæ were suspended, and this was easily solved by means of an ordinary centrifuge—the larvæ settling at the bottom of the tube, and so allowing all but one or two drops of the water to be poured off. The test-tube was then filled with absolute alcohol, and this was usually a sufficient quantity to render negligible the small volume of sea water originally present; when, as occasionally happened, the mixture thus formed contained a conspicuous precipitate of calcium sulphate, the centrifuge was again used. The larvæ being

[1] I understand that two of these Echinus were still living in August of the present year (1905), and were about as large as walnuts.

thus fixed and preserved in absolute alcohol, were then simply packed in small tubes duly labelled until it was convenient to examine them. I found that it was of no use adopting the method of preparation usually employed in the study of spicule formation, viz. fixing the tissues with osmic acid and staining with picro-carmine, since these reagents destroyed the skeleton within a very short time. Indeed, like the spicules of most calcareous sponges, the pluteus skeleton is very susceptible to traces of acid, and the utmost care must be taken to exactly neutralize all reagents employed in its preparation. The larvæ preserved in neutral absolute alcohol were doubly stained with safranin (the larvæ remaining for at least a week in a saturated solution in absolute alcohol) and nigrosin (the larvæ remaining half an hour or more in a similarly saturated solution), though, judging from my later experience in preparing holothurian spicules, I have no doubt but that licht grün (about fifteen minutes' immersion in the same saturated solution) would form a satisfactory substitute for the latter stain. The result was that the nuclei assumed a pink colour and contrasted well with the slaty hue of the cytoplasm.

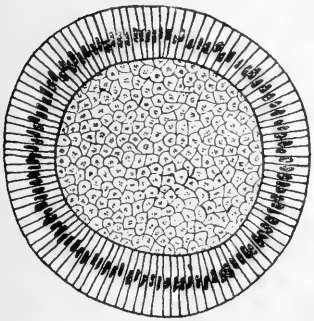
Before proceeding to the subject proper of the present paper I wish to express my warmest thanks to Dr. E. J. Allen, who very kindly afforded me all facilities for carrying out my work at Plymouth, to Prof. Minchin for general advice, and to the Council of the British Association who, at the kind suggestion of Mr. Garstang, granted me a free table.

The Origin of the Spicules in the Pluteus of E. esculentus.

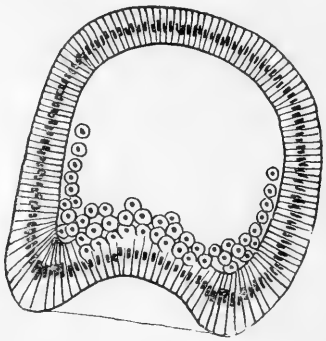
As is well known, the mesenchyme cells, the great majority of which are skeletogenous in function, arise in the blastula (text-fig. 1) of E. esculentus, as in Echinoderm blastulæ generally, by the migration into the blastocœle of cells at first situated in the flattened posterior wall of the larva (text-fig. 2), and later at the convexity of the hypoblastic invagination (text-

figs. 3 and 4). This internal budding commences as soon as the flattening of the posterior wall has become apparent and

Text-fig. 1.

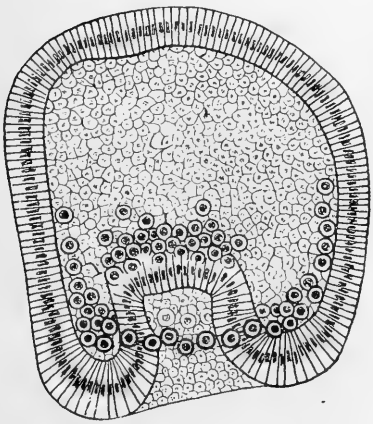


Text-fig. 2.

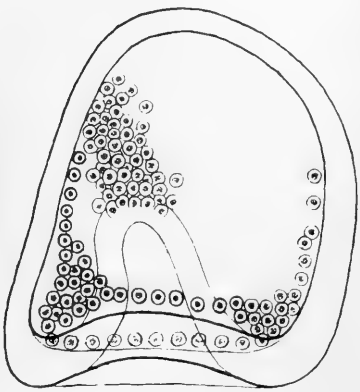


continues up to the formation of the enterocœle or true mesoderm. It has been stated by Boveri (1) from observations made on the pigmented and unpigmented blastomeres of

Text-fig. 3.



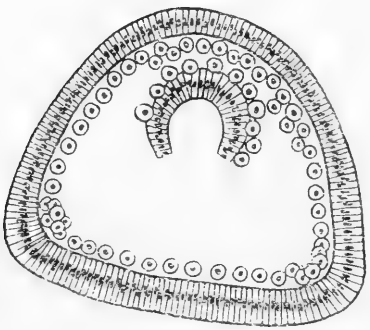
Text-fig. 4.



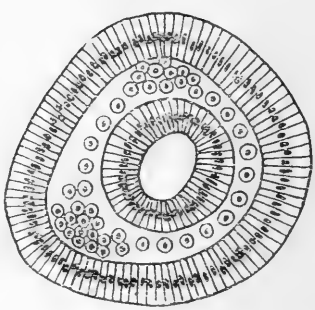
Strongylocentrotus lividus that these free mesenchyme cells are all derived from that portion of the blastula wall which extends from the posterior pole as its centre upwards

for about a quarter of the distance to the equatorial line, and the researches of numerous other observers on this and other genera—Echinus, Echinocyamus, Toxopneustes, Ophiopholis, Echinarachnius, Arbacia, etc.—tend to confirm this conclusion. These budded-off mesenchyme cells, though free in the sense that they are not attached to adjacent structures, are yet not free in the sense that they are at liberty to wander into all regions of the blastocœle; on the contrary all observations show that they take up a very definite position in this cavity. In E. esculentus, e. g. as the accompanying semi-diagrammatic figures (text-figs. 3—6)

Text-fig. 5.



Text-fig. 6.



indicate, they first aggregate in two positions, situated one on each side of the larva, where they form two elongated strands lying close to the body-wall and running parallel to the long axis of the gastrula. Later these two longitudinal cellular strands, which start from the posterior end of the larva, become connected by the formation of two chains of cells (in single series), which, extending round the archenteron on both sides and in each case joining on to the bases, i. e. posterior extremities, of the strand, thus form a complete circle (blastoporic ring) of cells lying immediately underneath the gastrula wall where it bends in to form the archenteron (text-figs. 3, 4).

From the fact that these mesenchyme cells thus take up their position at all those regions of the body-wall where

sharp curvatures are formed—the two longitudinal strands, e. g. being situated in the two corners, one at each end of the flat side, of the gastrula when this is viewed in a transverse plane (text-figs. 5 and 6), and the ring of cells being placed, as above mentioned, just where the posterior wall bends in to form the archenteron, i. e. round the blastopore,—one who had not previously observed how strictly localised the area of immigration is, would with reason suppose that the cells had been budded off in these regions—the cells being squeezed in, so to speak, by the foldings of the wall. But this is certainly not the case, as all observers agree. At the same time, it is an undisputed fact that in E. esculentus, and most, if not all, other genera, all these free mesenchyme cells migrate into all the corners of the gastrula, thus giving rise to the conformation described above. As Théel (**21**), who has observed the process in living larvæ, says: "It is a sight of the greatest interest to follow these cells, to see how they move towards these two places [the positions of the two longitudinal strands] as by word of command."

The cause of this directed migration is not easy to ascertain. Herbst attributes it to the desire for oxygen on the part of the cells, and certainly, if we imagine oxygen as uniformly diffusing through the gastrula walls, those cells situated in corners will doubtless obtain the greatest share. Driesch (**3**) also advocates a chemotactic solution to the problem, and he performed an interesting experiment in connection with this subject. Stated briefly, this experiment consisted of shaking up Echinoid gastrulæ in a test-tube in such a manner as to displace the mesenchyme cells forming the blastoporic ring and two longitudinal strands from this their normal disposition and to cause them to become irregularly dispersed throughout the blastocœle: the result was that, after the lapse of a certain time, Driesch found that the cells had returned to their original position in the blastocœle, and, as if nothing had happened, proceeded in their further development quite normally.

One interesting fact concerning the cells of the blastoporic

ring which I do not think has been definitely described before is the protoplasmic continuity existing between them. This protoplasmic continuity is shown in Pl. 18, figs. 8, 11, 12, 15, etc., in which the cells form a single series, the members of which are conspicuously joined together by long protoplasmic threads into a continuous circle. These connections between the cells of the blastoporic ring are primarily and not secondarily formed, since the ring originates by the outgrowth from each longitudinal strand of two chains of cells (i. e. the cells are already connected), one on each side, which extend round the archenteric invagination and meet in the median ventral plane of the larva the corresponding two of the opposite strand. Until one of these chains of cells has met its fellow of the opposite side its foremost cell often has the appearance of protruding in front of it a long pseudopodial process which perhaps acts as a feeler. Connections (though not elongated) between some of the adjacent cells situated in the longitudinal strands doubtless also exist, though it is not always easy to see them.

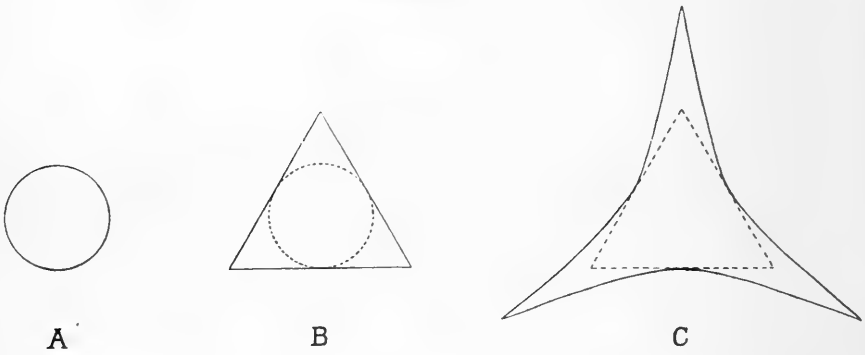
Starting, then, from this disposition of the mesenchyme cells in the blastocœle, viz. a blastoporic ring attached at two points to two longitudinal strands (text-figs. 3 and 4), the young spicules first make their appearance at the centres of the two longitudinal strands of cells, and in the form of one or more spherical granules contained in one or more cells (Pl. 18, figs. 1—5, 8, 15). In E. esculentus, as elsewhere, "the simplest form of spicule is a minute granule, generally more or less spherical" (Sollas, **18**), and thorough agreement on my part with this general statement leads me to here protest against that prevalent dogma which, in my opinion, most unwarrantably asserts that in all cases the calcareous spicule first, or, at least, early in the development, assumes the shape of a tetrahedron. In E. esculentus there is not at any stage a trace of such a crystalline form of the young spicule, nor have I detected such either in the young spicules of Cucumariidæ, of A. digitatum (**25**), or of Sycon sponges (**24**). Semon (**20**) himself, who is the chief authority

for the assertion that the tetrahedron is the primordial form of calcareous deposits in Echinoderms, does not figure any such stage in the development of the wheel-and-anchor spicules of Synapta inhaerens, and, indeed, admits that such does not here occur. Fewkes (**5**) does not describe such in Ophiopholis aculeata, nor does Gerould (**7**) in Caudina arenata, nor Garman and Colton (**6**) in Arbacia punctulata, and many other cases might be cited. Minchin (**16**) is equally positive on this point for Ascon sponges, Chun (**2**) for Auricularia larvæ, and von Koch (**12**) for the Alcyonarian genus Clavularia. On the other hand, Semon (**19**) describes most distinctly the young tetrahedron spicule for the holothurian Chirodota venusta, also Herouard (**11**) for certain other holothurians, and Théel (**21**) in his excellent paper on the development of Echinocyamus pusillus. Now all of these last three authors admit that calcareous spicules are deposits of mesenchymatous cells, and, indeed, Herouard and Théel endeavour to trace the form of the later deposits to the disposition of the secreting cells, and yet, this being the case, it is deemed necessary to have initially a visible crystalline structure as the basis upon which the future spicule is to be developed! Probably having in mind the "integrant molecule" or crystal particle of crystallography, the young stage of the triradiate spicule, which, from a crystallographer's point of view, might at first sight be mistaken for a tetrahedron (see text-fig. 7 below), is deemed to be such. Now this assumption is a very doubtful one for two reasons: (1) because Semon (with Fewkes, Selenka, Ludwig [**14**] and others) admits that the calcareous deposit originates in the interior of a cell as an approximately spherical grain, and that this grain forms the nucleus of the future spicule: hence the tetrahedron can at most only be the first "definite" form to be assumed by this grain,[1] from which it is to be

[1] Théel (**21**) disputes this in the following passage:—"According to Selenka and Semon the tetrahedron not only has originated in a single cell, but also arises directly from the calcareous granule of uncertain shape which is present in it, and which consequently should form the centre in the future

inferred that a geometrical, i. e. crystalline, structure is interposed between two stages of spicule growth which are non-crystalline in form—the spherical granule and the adult triradiate. This is evidently grossly improbable à priori. There is nothing very objectionable in commencing with a crystal as a nucleus for the future spicule, but to interpose one in an otherwise uniform series of non-crystalline metamorphoses is a proceeding to be regretted; and (2) because of the rarity of the occasions on which this tetrahedron is stated to have been observed. For these two reasons, then, I think it more feasible to suppose that the observers named

TEXT-FIG. 7.



mistook the small three-cornered mass which represents the transition from the spherical granule to the young triradiate

calcareous spicule. For certain reasons I do not think this to be the case. Firstly, it may be remembered that before the formation of the tetrahedron takes place there are several cells heaped together in Echinocyamus, each with one or more calcareous granules of uncertain shape. Now it seems rather singular that only one of these granules should be transformed into a tetrahedron, while the remaining ones are probably dissolved, and by successive depositions give rise to the further increase of the calcareous body. Besides, it is common to all calciferous cells to possess such granules." (The italics are mine.) The further description and figures given below of what occurs in Echinus esculentus offer a sufficient answer to this argument of Théel, who evidently had not observed the phenomena I have described.

(B in text-fig. 7; also in figs. 9, 11, 22) for the crystalline form in question, and that this last does not occur in the development of any calcareous spicule.

The Triradiate Spicules.

To continue the description of spicule formation in E. esculentus. As already stated, the skeleton of one side of the larva not infrequently (in say 20 per cent. of larvæ) arises in several centres, i. e. as several spherical granules situated in more than one cell, and seeing that the longitudinal strand usually consists of from half a dozen more or less closely-apposed cells, this is only what we should expect, but normally a single spherical granule is produced in the interior of one of the centrally-situated cells.[1] This spherical granule thus situated becomes after a short time three-cornered, and these three corners later assuming the form of three rays, there is thus produced a young triradiate spicule[2] (figs. 6, 7, 13, 16, etc.).

When we consider the conditions under which calcareous spicules are in general developed—when we study their development in distinct groups of animals (**12**, **16**, **24**, **25**, etc.)—it will seem probable that the triradiate form is correlated in the pluteus larva, as in other instances, with the disposition of the secreting cells. That this correlation roughly exists in the present instance is obvious from the most superficial inspection of the figures provided. In the region of a lateral longitudinal strand it is evident that, given

[1] As to why lime should be first deposited in this particular region, it may be remarked that this deposition always occurs where the scleroblasts are most thickly clustered (see my paper on the Sycon spicules—**24**), these scleroblasts "during their lively activity supplying themselves with calcareous salts [i. e. with water containing these salts] in such a degree that it becomes impossible to keep them in a state of solution" (Théel), the mutual apposition of the cells doubtless facilitating the deposition.

[2] In Arbacia punctulata "the first spicules to appear are four-rayed" (Garman and Colton, **6**).

a position situated just above the junction of the strand with the blastoporic ring as a centre, the cells are arranged in groups diverging from this centre in three directions—one group (the strand) extending longitudinally upwards and away from the blastopore, and two series of cells stretching round the hypoblastis invagination on both sides. Hence it follows merely from this general disposition of the cells that further growth of the initial deposit must occur more or less in these three directions, i. e. in the directions which the three rays of the triradiate actually take. In other words, the form of the triradiate, viewed from a general standpoint, undoubtedly, though roughly, does correspond to the disposition of the scleroblasts.[1] However, there is another possible factor which must be considered. It is easily observable that in the growth of the four spicule rays (two belonging to each triradiate) which extend some distance round the region of the blastopore, the actual deposition occurs in the course of the long protoplasmic processes connecting the adjacent cells (figs. 12, 20, 25, 26), or, in other words, the deposition takes place in a mould already formed for it. Since this is the case for this distal portion of the skeleton, it is therefore possible that the moulding takes its origin from the cell which contains the initial granule, i. e. centrally, and that therefore the initial triradiate form is immediately due to this. It is certainly possible on occasion to trace a faint streak directly continuous through the mass of cells from the tip of the ray of the young triradiate to the protoplasmic "cord" on which the cells of the blastoporic ring are "strung," and therefore it would seem that the protoplasm in the course of the growing spicule is modified in connection with its further extension. At the same time, it happens on occasion that several

[1] In the figures supplied by Selenka (**17**) of the young triradiate spicules of E. miliaris only three or four cells are shown on each side of the gastrula. If a longitudinal strand is in this case composed of such a small number of cells it is extremely remarkable; but, merely judging from Selenka's other figures, and from the appearance presented by the adult skeleton in plutei which I possess of this species, I doubt if this be the case.

triradiate spicules (only one of which persists as a part of the mature larval skeleton, the others becoming gradually absorbed) are developed from the several granules initially deposited in the longitudinal strand of cells (figs. 17, 20, 22), and if the single spicule possesses a triradiate mould in which to deposit its substance, so also must the several. From these facts, then, it would seem that, instead of the mould determining the form of the spicule, the contrary is rather the case, since the number of moulds is determined by the number of spicules. The elimination of this factor of a mould leaves us the disposition of the secreting cells as the only known cause to which we can attribute the triradiate conformation. We must imagine that the cells extending in any one direction from the centrally-situated granule or granules co-operate to exert a species of tractive influence on the freshly-deposited lime, tending to cause this to be deposited in a direction which is that of the resultant of their individual "pulls," and which the cells eventually place themselves in line with: such a conception, indefinite though it may be, seems at present alone capable of accounting for the facts. Compare the development of a triradiate spicule in a calcareous sponge (**16, 24**). Here the triradiate, unlike that of the pluteus, originates in three centres—in three pairs of cells, three cells of the three pairs being closely apposed centrally (the "trefoil"), and three being more distally situated—and each of the three lime deposits is, owing to the fact that it originates in one cell with another in close apposition, elongated from the commencement—the influence of an adjoining cell is obvious. Again, in the sponge triradiate, owing to each deposit, i. e. each ray, having only one cell in its vicinity in addition to that in which it originates, this is fairly straight; on the other hand, in the pluteus the initial deposit has, in any one of the three directions, not one, but many cells in its vicinity, and the resulting ray is correspondingly curvilinear. It will be seen from what has just been stated how impossible it would be to assume that in the pluteus triradiate the direction of a given

ray is at any point determined by the cells in its immediate neighbourhood,[1] for, if such were the case, the curves of the ray would be numerous and sharp, whereas, in actuality, they are comparatively few and gradual. It may be added in connection with the hypothetical tractive influence of the cells on the growth of the initial calcareous granule that the undoubted influence which the basal actinoblasts of the triradiate spicule of Calcarea exert on an adjacent pore-cell or cell of the oscular rim (**16, 24**) supplies a very fair analogy for my supposition.

The above seems to me to be the only possible explanation, in our present state of knowledge, as to the cause of the triradiate form of the pluteus spicule. Consider the argument: triradiate spicules very alike in form, but not exactly alike, arise in all plutei in a certain position; the disposition of the secreting scleroblasts is in all larvæ substantially the same en masse, but is very different in different larvæ as regards the arrangement of individual cells—from which premises the only logical conclusion to draw is that which I have stated above.

Théel suggests an explanation of the triradiate form which, though it seems to me wholly untenable, I think is worth while quoting, since it contains several very true observations. "I could never observe," he says, "that the tetrahedron [the three-cornered granule] becomes visible before at least three calciferous cells have arranged themselves in a heap close to the blastoderm. But after this is done the formation of the tetrahedron takes place in a clear pseudopodial plasm situated between these cells and the ectoderm [see my fig. 15, e. g.] and evidently derived not from one cell but from all three cells, the pseudopodia of which have united into a small clump . . . Thus, according to my opinion, the calcareous tetrahedron is a result of the activity of several cells, which

[1] Judging, however, from the different directions which the three rays of the young triradiates in figs. 7 and 16 e. g. have assumed, it would seem that adjacent cells have a lot to do with the origin of young triradiates, if not so much with their future growth.

deposit calcareous salts in a liquid state in the common pseudopodial clump, where the formation of the tetrahedron afterwards takes place . . . The minute tetrahedron grows rapidly to a small star with three very short arms, acquiring a shape almost completely corresponding to the interspace between these [three] close-lying calciferous cells. Thus one is almost tempted to think that there exists a certain relation between the form of the deposit and the interspace in question. The calciferous cells having placed themselves close to the ectoderm, the deposit becomes pressed between them and the latter. If it be so, that this interspace decides the outline of the star, one would expect always to find it in its early developmental stage placed just between the calciferous cells. This seems, however, rarely to be the case. Mostly I have noticed the star situated by the interspace with the arms upon the three cells and not between them, and sometimes I have seen the star itself somewhat displaced. Notwithstanding this, I cannot free myself from the thought that the cells mechanically exercise influence on the outline of the tetrahedron, and the star in the earliest stages of the development."

There yet remains, of course, a third possible supposition, viz. that the form of the triradiate spicule is due to some agency at present unknown, as e. g. that complex of physical causes termed heredity, but since this supposition certainly does not constitute an explanation—does not affiliate the phenomenon under discussion to other phenomena whose mode of production is known—I am not prepared to discuss it.

Further Growth of the Triradiate Spicules, etc.

To proceed once more with the description. Though, in E. esculentus, the skeleton of the larva primarily originates in two centres—the two triradiates of the longitudinal strands—yet it often happens that isolated granules, rods, and (occasionally) triradiates arise independently in other regions

of the skeletal area marked out by the presence of scleroblasts, i. e. in the course in which further extension of the arms of the original triradiates will occur. These isolated deposits, which mostly originate in the pseudopodial processes connecting the cells of the blastoporic ring, are shown in figs. 8, 12, 25. The rod in fig. 25 has doubtless originated between two cells once closely apposed but which have since separated—the apposition of two cells being, at least in calcareous sponges, an essential condition to the production of an elongated structure. In fig. 25 also the triradiate, in all probability, arose in a clump of cells—at least three—which have since dispersed to join the adjacent longitudinal strand. It is probable that these independent deposits largely contribute to the small processes and other irregularities characterising the adult larval skeleton.

The three rays of each of the two triradiate spicules once produced gradually elongate in their three respective paths. The curved transversely-disposed rays (the "recurrent apophyses") ultimately meet ventrally in the median plane, but are not in the majority of cases, contiguous in the same line (figs. 27. 28); the rays pointing anteriorly—away from the blastopore—extend upwards to form the supports of the antero-dorsal arms; the rays pointing downwards, or towards the blastopore, extend posteriorly for a short distance and then curve suddenly dorsally (tending to thus complete dorsally the calcification of the blastoporic ring already accomplished ventrally by the recurrent apophyses) to form later the "baguettes de corps" or main terminally-thickened posterior supports of the larva; thus the blastopore becomes (relatively) shifted to the ventral side. At the point where these postero-dorsal arms of the original triradiate bend dorsally a pair of new arms originate to form the supports of a pair of postero-ventral arms.

The projection of a new pair of arms from any point of the previously formed skeleton is always preceded at that point by a small cluster of scleroblasts. As Théel says, "As a rule, calciferous cells are present at such parts of the increasing

spicule where branches protrude, apparently caused by them, but this seems not always to be the case in the formation of unimportant spines." I do not know whether the fact that the rods of the postero-ventral arms appear at the bends of the previously deposited skeleton, cells having previously collected there, is of any significance, but it may at least be pointed out that such is the case; moreover, in the other pluteus which I have carefully examined (that of E. miliaris), the same thing happens, the skeletal rods of the dorsal arms arising from sudden bends which here occur in the recurrent apophyses. The same phenomenon occurs in other plutei, though whether secondary branches always arise in such positions[1] I am unable to say.

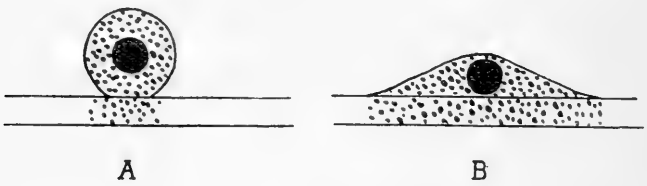
That the formation of spicular processes is essential to the production of the arms characteristic of plutei is now well established. Herbst (**8**), e. g. showed that arms are not developed in plutei reared in sea water deprived of calcium salts—a skeleton necessarily being absent under such conditions. He also showed (**9**) that if, by immersion in lithium water, the calcareous needles are displaced from their normal position in the pluteus, arms are produced from portions of the ectodermal surface which do not normally share in the formation of these outgrowths—a result confirmatory of the same conclusion.

As regards the relation between the scleroblast and the lime deposited, I can amply confirm the statements of Théel as to the deposition occurring in the "clear homogeneous hyaloplasm or ectoplasm" of the cells, and not in the vacuolated endoplasm, and the young triradiate originating in a "clear pseudopodial clump" formed by the fusion of the ectoplasms of three or more cells. Also, as Théel remarks, it is "on account of the transparency of the pseudopodial plasm and the opacity of the granular [very much vacuolated in E. esculentus] main portion of the cells [that sometimes] one gets the impression that the tetrahedron is extra-cellular

[1] In many cases, of course, the secondary arms arise from the angles of the already formed skeleton.

in position." This fact explains why it is that the outline of the pluteus scleroblast bears such a very different relation to the spicule ray compared with that which the actinoblasts of calcareous sponges assumes. In Calcarea the actinoblast is elongated in the direction of the ray-length, and is in most cases cylindrical in form, i. e. envelopes a certain length of the ray on all sides; also on one side the cytoplasm forms a mound of gradual slope containing the nucleus (text-fig. 8, A). In the pluteus larva, on the other hand, the actinoblasts are spherical in contour, and are attached to the skeletal rod by only a very small portion of the entire circumference, i. e. the base of attachment is very limited (text-fig. 8, B). In other words, the deposition of lime in Calcarea occurs in the mass of the cell-substance, whereas in the pluteus larva it solely occurs in the ectoplasm or thin peripheral layer; hence the difference of cell-outline in the two cases. As the skeleton of the larva

TEXT-FIG. 8.



(both in E. esculentus and miliaris, and doubtless all other plutei) assumes its adult form, however, the scleroblasts become more closely applied to the spicule ray (fig. 29), and then resemble the actinoblasts of the Calcarea in form: this change in contour being probably correlated with the exhaustion of the cell-substance, the vacuolated cytoplasm becoming gradually absorbed and changed in constitution as the active deposition of lime proceeds.

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EXPLANATION OF PLATES 18 & 19,

Illustrating Mr. Woodland's paper, "Studies in Spicule Formation." III.

All figures magnified 1000 diameters except Fig. 5 (× 2000) and Figs. 25 and 26 (× 500).

PLATE 18.

Figs. 1—3 show the initial granules deposited in the cells of the longitudinal strand which is viewed from the surface.

Fig. 4.—The granule in the longitudinal strand when this latter is viewed edge on, as also in Fig. 15. In Fig. 8 the strand is viewed end-on.

Figs. 4, 8, 10, 11, 12, 15 (and 19 and 20 of Plate 19) show well the pseudopodial processes connecting adjacent cells in the blastoporic ring.

Fig. 5.—The scleroblast containing the granule, magnified 2000 diameters.

Figs. 6, 7, 10, 11, 13, 14, 16.—The young triradiates derived from the initial granules.

Fig. 9 shows well the young "tetrahedron"—the three-cornered granule; also seen in Figs. 11 and 22 (Plate II).

Fig. 15 shows well the position of the spicule relative to the wall of the larva.

Fig. 16 shows two young triradiates dissimilarly aurientated (p. 316); also Fig. 7.

PLATE 19.

Figs. 17, 20, and 22 illustrate the production of two large triradiates in the longitudinal strand. In Fig. 25 as many as three triradiates have been formed.

Fig. 19 shows half a dozen granules produced at the centre of the longitudinal strand.

Figs. 21 and 25 illnstrate the rare occurrence of small triradiate spicules in the course of the blastoporic ring. In Fig. 25 the cells have deserted the spicule after forming it. In Fig. 22 they are still present.

Figs. 22, 24, and 26 show the typical fully-formed triradiate spicules with their actinoblasts. As is evident, the precise position of the scleroblasts is in no way constant, at least at this stage, for the given form of spicule—the relation is only a general one.

Fig. 25 is remarkable for the number of independent deposits which have arisen in the blastoporic ring. The ring is viewed from the posterior end of the larva.

Fig. 26.—Spicule formation well advanced. The recurrent apophyses have not yet met in the mid-ventral line.

Figs. 27, 28 show the junction of the recurrent apophyses. Despite the completeness of the blastoporic ring, the apophyses are not contiguous in the same line.

Fig. 29 shows the "mound" form which the actinoblast of the adult larval skeleton assumes (p. 320).

The Digestive Organs of the Alcyonaria and their Relation to the Mesoglœal Cell Plexus.

By

Edith M. Pratt, D.Sc.(Vict.).

(With Plates 20—22.)

Contents.

Introduction.

The research in connection with this paper is based upon a study of a very comprehensive collection of specimens of Alcyonaria from many localities, now in the possession of the Victoria University of Manchester, and kindly placed at my disposal by Professor Hickson. This includes Mr. J. Stanley-Gardiner's excellently preserved collections from the Maldive Islands and Funa Futi respectively; Dr. Willey's collection from New Guinea, New Britain, and Lifu; Professor Haddon's collection from Torres Straits; Mr. Gilchrist's collection from the Cape of Good Hope; and Professor Herdman's collection from Ceylon. I have compared the results of my investigations on these forms with a study of the British representative of the family Alcyonium digitatum in the living as well as the preserved condition.

In two former papers (1903, 1905) I have described the general anatomy and relationships of Alcyonium, Sarcophytum, Lobophytum, and the new genus Sclerophytum. The present paper is devoted to a more detailed account of the minute anatomy of the digestive organs, and records an attempted investigation of the physiology of digestion in these genera.

Little is known of the food supply and mode of digestion in the Alcyonaria, and, as the accounts in other groups are very conflicting, experimental evidence has been sought in the hope of obtaining enlightenment as to the nature of the food supply, the physiology of digestion, and the distribution of nutriment in this family. During the spring of 1902 and

1903 numerous feeding experiments were carried out at the biological station of Port Erin on the British Alcyonium digitatum. These yielded very interesting results which are briefly described.[1]

I have examined the plexus of mesoglœal cells of Alcyonium, hitherto regarded as a nerve plexus, in the living as well as in the preserved condition, and after a careful comparison with other Alcyonaria, have come to the conclusion that it can be no longer regarded as a differentiated nerve-plexus. We have at the present time no experimental evidence of the existence of a specialised nervous system in the Alcyonaria.

The Zooids.

In form, and to a very considerable extent also in structure, a zooid of the monomorphic Alcyonium is very similar to a fully-developed autozooid of the dimorphic Sarcophytum and Lobophytum.

The zooids vary considerably in size. As they are extremely contractile it is frequently impossible to form any true conception of their actual size from preserved specimens. Forms inhabiting tropical waters, however, frequently have smaller and fewer zooids than their relations in temperate seas. The fully expanded living zooids of Alcyonium digitatum are usually very different in form, size, and apparent structure from those in the preserved condition.

A fully-expanded living zooid of Alcyonium digitatum frequently measures from 6—9 mm. across the crown of tentacles, and the anthocodia often attains a length of from 10—12 mm. The tentacles are from 3—6 mm., and the pinnules about ·5 mm. in length.

[1] These experiments have been repeated on several Alcyonaria, including Corallium rubrum, and in Actinians, including Anemonia sulcata, at the Zoological Station at Naples during the month of April, 1905. The results of these experiments confirm the observations recorded in the presen paper.

In all expanded zooids grooves occur between the bases of the tentacles (fig. 3); they probably serve for the escape of waste fluid and solid matters.

In all the genera the tentacles and pinnules are hollow when expanded. The surface of the latter is dotted with innumerable excrescences, which are due to the presence of batteries of cnidoblasts, each battery in Alcyonium digitatum contains some hundreds of these cells, but they are not usually so numerous in tropical members of the family.

In Alcyonium, Sarcophytum, and Lobophytum the tentacles are fringed laterally with a single row of pinnules. In the genus Sclerophytum the tentacles of some species have one row, and others (Scl. capitale and palmatum) have two, while those of the genus Xenia, according to Ashworth, have three rows of pinnules.

The tentacles and pinnules are apparently shortest in Sclerophytum and longest in Alcyonium, although it is extremely difficult to form an opinion as to their exact size from a study of preserved material alone.

The Food of the Alcyonaria.

In consequence of the many difficulties which attend the physiological investigation of the food supply and mode of nutrition in this family, little systematic work on this subject has hitherto been attempted. The accounts of the digestive processes in other groups of the cœlentrates are also very conflicting.

Milne-Edwards and Wilson maintain that digestion in the Alcyonaria is intra-cellular, while Hickson states that the food is acted upon by a digestive secretion before it is ingested by the cells lining the digestive tract, i. e. an intercellular digestion occurs in Alcyonaria as well as an intracellular digestion.

In an investigation of the physiological processes of digestion it is necessary that the study of the minute anatomy of

digestive organs should be very largely supplemented by observations of the actual digestive processes in living zooids. "Alcyonium digitatum"[1] provides an excellent subject for the study, for not only is it easily obtainable, but the transparent nature of the body walls of the arthocodiæ enables one to observe many of the stages in the digestion of food within the living zooid, especially if brightly-coloured food material be employed. To obtain a suitable food which could be stained by an innocuous colouring matter was found, however, to be no easy task. It is well known that food material has very seldom been observed in the zooids in collections of preserved material, while in tropical forms it has rarely, if ever, been seen, even in living specimens. In the hope of obtaining some information as to the natural food of the Alcyonaria, some hundreds of autozooids of genera from many localities (including the tropics) were examined, but food was observed only in very few instances in the cœlentera. It then consisted of small partially-digested masses of organic matter, containing fragments of minute crustacea, zoochlorellæ, and portions of algal filaments, the last-named, however, were apparently unaffected by a digestive ferment.

Several freshly-captured specimens of Alcyonium digitatum were examined, but with the exception of the occurrence of a few fragmentary copepods the cœlentera of the zooids were invariably empty. Certain feeding operations on this form were then attempted, which were at first abortive, but finally successful. These experiments were carried out in the laboratory of the Biological Station at Port Erin where I was successful in keeping colonies of this species for a considerable time under healthy conditions.

[1] Corallium rubrum was found to be equally suitable for this purpose, and yielded identical results.

Feeding Experiments on Alcyonium.

I.

Several freshly taken and apparently healthy specimens of Alcyonium were placed in wooden tanks, through which a gentle but constant stream of filtered sea water was running. Conditions of light and temperature were carefully observed and maintained as nearly normal as possible; the temperature of the water in the tanks never fell more than two degrees below, and never exceeded that of the sea.

Several colonies were also kept in tanks of unfiltered sea water and submitted to the same treatment.

At the end of forty-eight hours most of the zooids of all the colonies were seen with their tentacles completely extended, and were found to be very sensitive to contact.

A fairly well-grown colony was transferred from filtered sea water to a glass vessel containing a concentrated surface tow netting consisting chiefly of Nauplii, small Copepods, Daphnids, and Diatoms. The colony quickly recovered from the transference, and, at the end of half an hour, the tentacles, with their delicate fringes of pinnules, were again extended.

A nauplius, actively swimming so near a tentacle as to lightly brush against the pinnules, was instantly captured by them and paralysed by the innumerable poisoned threads of the nematocysts, and in a very short time the surface of the tentacles was dotted with hundreds of paralysed Nauplii and Copepods. Occasionally a tentacle would curl inwards and deposit its captured prey within the mouth. Usually, however, the zooids, with tentacles outspread, remained expanded for quite an hour, then the colony slowly contracted, and, at the end of a second hour, all the zooids were withdrawn below the surface.

Fifteen hours later the zooids began to slowly expand, and, when expansion was almost complete, the colony was fixed

and preserved by a fairly hot 7 per cent. aqueous solution of formalin.[1]

After fixing, the colony was submitted to microscopic examination, when minute fragments of Nauplii, of chitinous cases of Copepods and Daphnids were seen to be partially, and in some cases completely extruded from several zooids. Comparatively large specimens of Daphnids and Copepods (Cyclops, etc.) were observed in the cœlentera of several zooids, enfolded and supported by the mesenterial filaments.

The crustacea which had not been swallowed were found to be excellently preserved by formalin, but the specimens observed in the cœlentera of the zooids were generally found to exhibit unmistakable signs of disintegration. In many cases the empty chitinous shell of a Daphnid still supported by the mesenterial filaments was apparently complete.

I have observed no instance of the ingestion by the filaments of any Copepod, or other fairly large form of prey in a complete state. In many cases the filaments were distended with food material, which, however, was always observed to be in a finely divided condition.

II.

In a second experiment colonies of Alcyonium were confined in running filtered sea water for twenty-four hours. Ripe ova of the flounder were then gently placed upon the extended tentacles, and were immediately enfolded by them. In a few cases only, however, were they swallowed. Usually they were grasped tightly by the tentacles for about a minute and then released. Ova of the plaice, whiting, and cod were substituted with the same result. Believing the ova to be too large to pass through the mouth, extremely small embryos of the crab "Galathea" were offered. These were eagerly taken, enfolded by the tentacles, and afterwards

[1] This method of fixing, recommended to me by Mr. J. T. Wadsworth, of the Victoria University of Manchester, yielded excellent results for histological purposes.

rejected in exactly the same way. The zooids, therefore, exercise considerable choice in the selection of food.

Colonies confined in unfiltered sea water for the same period also refused to feed on fish ova.

An ovum of the plaice which had been swallowed by a zooid was kept under observation for several hours. The comparatively hard shell of the egg remained rounded and apparently intact, but the yolk rapidly became reduced in quantity, and, after five hours, had almost completely disappeared, the egg case being still grasped by the ventral mesenterial filaments. From these experiments it is evident that by some means large food bodies are either broken up into small particles, or at least partially dissolved, in the cœlentera before ingestion by the mesenterial filaments.

III.

In a third feeding experiment a fairly solid homogenous jelly was obtained by pounding the flesh of the whiting. A very small portion of this gently brought in contact with a pinnule was immediately seized, transferred to the mouth by the tentacles, and slowly swallowed. Other colonies which had been kept without food for thirty hours in filtered sea water partook of the food with equal avidity. The pounded flesh of cod, flounder, and plaice were also substituted for whiting, and proved equally acceptable to the zooids.

In order to observe the course of the food within the zooids, the flesh of the fish before pounding was brightly stained by a dilute solution of borax carmine, and afterwards carefully washed in running sea water. The colonies were found to feed with equal avidity on the coloured fish food.

When the colonies were expanded the course of the food could be easily observed through the transparent body wall of the zooids (figs. 2 and 3 illustrate the seizing and swallowing of food, which, on entering the cœlenteron, is grasped and squeezed by the ventral mesenterial filaments, and rapidly disappears). On microscopical examination the food was found to be ingested in such quantity by the filaments as

to materially increase their size and to impart to them a red colour (figs. 2—4). The dorsal filaments were observed to take no part in the digestive function.[1]

This particular colony was fed five times between 11 a.m. and 7.30 p.m., and was then fixed and preserved in a fairly expanded condition (fig. 1) by the hot formalin method. All the colonies which had been fed with the coloured fish food were found to be in a healthy condition at the end of the experiments, which lasted fourteen days.

Several colonies, kept in tanks of running but unfiltered sea water, were submitted to the same treatment. After three days the anthocodiæ of these colonies were observed to be slightly swollen, and were apparently less sensitive to contact than their neighbours in filtered sea water, and, although expanded, refused to feed. The swollen condition increased; on the sixth day the colonies were fully twice their former size, and were found to be almost insensible to contact. Preserved sections of these colonies were found to be quite useless for histological purposes.

In his account of the Oban Pennatulida, Marshall (1882) remarks upon the swollen condition and loss of sensitive power of specimens in captivity, and states that, as the Pennatulids inhabit deep water, the swollen condition is due to a difference in pressure. All the specimens of Alcyonium which exhibited this condition, however, were taken from shallow water, many of the colonies being exposed at low tide, while three specimens from forty fathoms, and kept in filtered sea water, remained in a normal condition for several days. The swollen and insensible condition in Alcyonium is doubtless pathological, and is quite independent of either increase or decrease in pressure.[2]

[1] By feeding greenish-brown specimens of Anemonia sulcata on carmined fish food they gradually acquired a pinkish hue, which at the end of fourteen days was intense. The specimens apparently suffered no inconvenience from this mode of diet, and seemed to be quite healthy and vigorous at the conclusion of the experiment.

[2] W. May (1899, p. 44) describes a new species of Clavularia, which he names C. inflata because of its swollen and bloated appearance. As it

A few colonies confined in filtered sea water for seven days without food were fixed and preserved for comparison with well-fed colonies.

The Mouth Disc.

The histology of the mouth disc closely resembles that of the stomodæum (figs. 5 and 6), from which it differs in that the ectoderm is slightly thinner. It differs from that of the tentacles and general ectoderm in the scarcity of nematocysts, and in the presence of numerous granular gland cells similar to those of the stomodæum and mesenterial filaments.

The Stomodæum.

In Alcyonium, and in all other Alcyonaria, with the exception of Xenia, the function of the stomodæum has been believed to be limited to the conveyance of food material to the cœlenteron; it has therefore not been considered a portion of the digestive tract.

The stomodæum of zooids, which had been fed just before fixing and preserving, apparently contained no gland cells of any description, but in starved zooids the stomodæum showed a considerable number of gland cells with granular contents identical with cells occurring in the mouth disc and ventral mesenterial filaments (figs. 5 and 6). These cells are very clearly indicated in sections from 2—4 μ in thickness, stained with iron brazilin, when the granules become intensely black.

From the presence of gland cells in the stomodæum of starved zooids, and their apparent absence in recently fed zooids, it may be assumed that the food has received a secretion from the cells, in its passage through the stomodæum. (Reference is again made to the subject of secretion in connection with the description of the gland cells of the mesenterial filaments, pp. 342—345.)

appears to agree with C. viridis in every other respect, however, it cannot be regarded as a distinct species.

In the presence of gland cells in the stomodæum Alcyonium resembles Xenia. The cells, however, differ from those described by Ashworth, 1898, in that genus, in form and in their granular character. In Xenia they are swollen, flask-shaped, and, according to Ashworth, give rise to a mucous secretion. As I have observed both mucous and granular gland cells in the stomodæum of every species of Sarcophytum (fig. 5), Lobophytum, and Sclerophytum (fig. 6) which I have examined they doubtless occur throughout the family.

Gland cells occur in the stomodæum of several Zoantharia, and have been described in Flabellum by Stanley Gardiner (1902), and in Mæandrina by Duerden (1903). The cells differ slightly in shape and size in the two groups of Cœlentrates, but doubtless have the same function.

In several members of the Zoantharia the stomodæal ectoderm is raised into ridges at the insertion of the mesenteries, where in Mæandrina it differs in character from the thinner ectoderm between the ridges. In all the members of the Alcyonaria which I have had the opportunity of examining, the ectoderm of the stomodæum, though usually convoluted in the preserved condition is similar in character and of apparent uniform thickness throughout, no thickenings being apparent at the insertion of the mesenteries.

The stomodæal ectoderm of Alcyonium, Sarcophytum (fig. 5), Lobophytum, and Sclerophytum (fig. 6) is made up histologically of the following elemental cells, which occur in varying proportions, the relative abundance of granular gland cells being in many cases dependent on the condition of the zooids with regard to the supply of food material:

(*a*) Granular gland cells, usually irregular in shape, and containing a varying number of rounded granules, which become intensely black when subjected to stains containing iron. These cells are histologically identical with the granular gland cells of the mouth disc and mesenterial filaments. Their function is discussed on pp. 343—345.

(*b*) Mucous gland cells with deeply staining nuclei.

Each cell contains also a delicate reticulum of protoplasm and a deeply staining mucous secretion, which occupies the middle and upper portion of the cell, often in the form of a dense deeply staining reticulum. The secretion in some cases may be seen exuding through the outer wall into the stomodæum. In some instances the cells are almost empty. The mucous cells appear to be more numerous in tropical (figs. 5 and 6) forms than in the British species.

(*c*) Nematocysts, similar to those occurring in the tentacles, are frequently imbedded in the outer ectoderm.

(*d*) Scleroblasts with minute spicules are occasionally observed, but are never numerous.

(*e*) Columnar cells, usually with a single flagellum, more or less fill up the spaces between the superficial cells.

(*f*) Interstitial cells, more deeply seated than any of the above-mentioned cells, are of varying shape, and may give rise to any of the superficial forms of cell.

(*g*) Stellate cells, with long processes, are often attached to interstitial and other cells. They often extend into the mesoglœa, and have been described as nerve cells, and the processes as nerve fibrils. They have not, however, been experimentally shown to be nervous in function, but are identical in form, and probably also in function, with certain stellate cells which occur in the mesoglœa (pp. 351—356), and are probably not ectodermic in origin.

The Siphonoglyph.

The siphonoglyph extends through the entire length of the stomodæum in Alcyonium, Sarcophytum, Lobophytum, and Sclerophytum, and is apparently lined throughout with flagella of uniform size.

The stomodæum of Xenia, Ashworth (1898, p. 443), is described as having a well-marked siphonoglyph, in which only the cells of the lower third bear long flagella. In Lemnalia (Bourne, 1900, p. 532) the siphonoglyph is present as a shallow ciliated gutter, but in some cases entirely

disappears, the epithelium of the stomodæum being then ciliated and of the same character throughout. Bourne regards this as a primitive condition from which the siphonoglyph has been derived. In his account of the siphonoglyph in Alcyonaria (1883, p. 699) Hickson states that the tendency of dimorphic forms is to throw the siphonic function upon the siphonizooids, and to eliminate it from the autozooids.

The siphonoglyphs of the autozooids are not so pronounced in the dimorphic Sarcophytum and Lobophytum as in Sclerophytum and the zooids of Alcyonium. In Scl. hirtum the flagella of the siphonoglyph are fully ·03 mm. in length. In the siphonozooids of well-marked dimorphic genera the siphonoglyphs are very large, but are absent in the degenerate siphonozooids of Sclerophytum.

Mesenteries.

The mesenteries have the same fundamental structure throughout the Alcyonaria. Hickson's description (1895 and 1900) of the mesenteries of Alcyonium will apply with certain modifications to the zooids of monomorphic, and to the autozooids of dimorphic forms. The ventral mesenteries of the siphonozooids of the latter are extremely small, and even in well-marked cases of dimorphism seldom project beyond the lower end of the stomodæum. In some species of Sclerophytum they are so minute in the siphonozooids as to be almost unrecognisable as such; while in other species of this genus they may be entirely absent (Pratt, 1903, p. 531).

In the autozooids of Sarcophytum the mesenteries are relatively larger than in Lobophytum and Alcyonium. In Sclerophytum they are usually smaller and more feebly developed. The extreme prominence of the mesenteries in Sarcophytum is due to the presence of mesoglœal thickenings near the free edge, which, when examined with low powers of the microscope, have the appearance of enormous mesenterial filaments. The thickenings, however, are

due to a localisation of mesoglœal tissue alone (fig. 12), and in cross section have a rounded appearance. The thickenings vary in different species, in "S. ehrenbergi" they are ·05 mm. in diameter, in "S. glaucum" ·03 mm. They occur also in Xenia (Ashworth, 1898), but are much more feebly developed. They probably serve as additional supports to the autozooids when in an expanded condition.

The musculature is typically Alcyonarian, but is much more strongly developed in the dimorphic Sarcophytum and Lobophytum than in Sclerophytum. In Alcyonium it is usually well marked, but is more strongly developed in the British species "A. digitatum" than in the tropical "A. pachyclados." As in Alcyonium the retractor muscles are much larger than the protractors. The pleating of the mesoglœa varies according to the development of the muscles. In Sarcophytum, Lobophytum, and Alcyonium the folds are numerous and very prominent, in Sclerophytum they are smaller and vary in size in different species. In the species "Scl. palmatum" and "Scl. capitale" they are not numerous, but are fairly large, but in the species "Scl. polydactylum" and "Scl. gardineri" the folds are very few and extremely small. The musculature of the mesenteries is more strongly developed in the upper than in the lower portions of the zooids. In the genus Sclerophytum it seldom extends below the terminal portion of the stomodæum. The musculature of the mesenteries of the siphonozooids is always feebly developed, as these individuals are only very slightly contractile. In the siphonozooids of Sclerophytum it is entirely absent.

In the Alcyonaria the stomodæum is continuous with the mesenteries. Of the three layers which compose the stomodæum, only the endoderm and mesoglœa are continuous with the ventral mesenteries. There can be no doubt of the termination of the ectodermic epithelium of the stomodæum at its aboral opening (fig. 8). Further reference is made to this fact (pp. 341 and 342).

Mesenterial Filaments.

E. B. Wilson (1884, p. 12) has shown that the dorsal mesenterial filaments have almost precisely the same structure throughout the Alcyonaria. As they are fully described, and their ectodermic origin established by him, it is necessary to add but little to his excellent account of these structures.

Throughout the family these filaments are very long. In the siphonozooids of well-marked dimorphic genera they are proportionately very much longer and more strongly marked than the ventral filaments, and are proportionately less pronounced in the autozooids.

In his account of the mesenterial filaments of the Alcyonaria E. B. Wilson (1884, p. 22) states:

"There can be no doubt that the compound Alcyonaria are derived from solitary forms, which probably possessed eight similar filaments, each consisting of an ectodermic circulatory part and an entodermic digestive part. As the colony-forming habit became established, bringing with it the need for specialised organs of circulation, a physiological division of labour took place among the filaments. In the dorsal pair the ectodermic part gradually supplanted the entodermic, while the reverse process took place in the other six." He further states that we have no embryological evidence of this, but suggests that the portion of the ectoderm (*ect.*) of the stomodæum which is in immediate continuity with the ventral mesenteries probably represents the original ectodermic part of the ventral filament.

A study of vertical sections of the stomodæum shows (fig. 8) this ectodermal tissue (*ect.*) to be the ectoderm of the lower end of the stomodæum which has become fused with the mesenteries. Between the mesenteries the ectoderm becomes thinner towards the free edge, and is identical in every sense with that portion which has fused with the mesenteries.

We have, therefore, no evidence in favour of Wilson's hypothesis of an ancestral identity of form, origin, and multiple function of the dorsal and ventral mesenterial filaments.

The ventral mesenterial filaments of the Alcyonaria exhibit considerably more variety in form, size, and, to a certain extent, in structure than do the dorsal filaments.

In his account of the anatomy of Cœnopsammia Stanley Gardiner (1900) maintains that the mesenterial filaments, together with the stomodæum, are ectodermic in origin in that form, and says:

"The stomodæum of the Zoantharia, and necessarily also of Alcyonaria, is not comparable to the stomodæum of the Triploblastica, but rather is, with the mesenterial filaments, the homologue of the whole gut. The so-called endoderm, giving rise to the muscular bands and generative organs, and performing also the excretory functions, is then homologous with the mesoderm of Triploblastica. In the terms of the layer theory, of whatever value it may be, the Actinozoon polyp must then be regarded as also a Triploblastic form having ectoderm, endoderm, and mesoderm."

E. B. Wilson (1884, p. 7) states in the development of Funiculina that the ventral mesenterial filaments arise quite independently of the stomodæum, and are endodermic in origin. What Wilson has shown for Funiculina may be true of other Alcyonaria. I have already shown (fig. 8) that the ectoderm terminates with the aboral opening of the stomodæum in the adult condition, and only the mesoglœal and endodermal tissues are continued downwards into the mesenteries. Yet a histological study of the mouth disc, stomodæum, and ventral mesenterial filaments in several members of the family reveals many points of similarity, if not identity, in their elemental constitution. Both granular and mucous gland cells, as well as nematocysts, occur in all these structures.

Milne-Edwards in 1835 was the first to attribute a digestive function to the mesenterial filaments of the Alcyonaria. The

presence of gland cells in the ventral mesenterial filaments of Paralcyonium was first observed by Wilson, 1884, who states that they are similar to those observed by the Hertwigs in the Actinians. He also describes the occurrence of ingested foreign bodies in the filaments.

Hickson (1895, p. 367) described two kinds of gland cells in the ventral filaments of Alcyonium digitatum, one kind being large, unciliated, and deeply staining with hæmatoxylin, the other consisting of elongated columnar cells filled with numerous minute granules. He further states (1901, p. 12) that the function of the ventral filaments is to secrete a digestive juice upon particles of food which have passed through the stomodæum.

J. Stanley Gardiner (1900 and 1902) describes the occurrence of granular and mucous cells in the mesenterial filaments of the Madreporaria, and in his description of Flabellum says:—"Every stage of ingestion and protrusion of foreign matter could be seen in the swollen-out endodermal bases of the mesenterial filaments, but elsewhere was not observed. The storing up of round, fat globules, not only in the endoderm at the bases of the mesenterial filaments, but anywhere in the endoderm, indicates that there must be a true digestion—due to the secretion of the gland cells of the mesenterial filaments—and absorption over the whole endoderm, as well as ingestion at the bases of the filaments. No absorption would, however, seem to occur in the mesenterial filaments, the concentration of fat, etc., in the endoderm at their bases being correlated with this."

The Zoantharia are well known to be widely separated genetically from the Alcyonaria. Nevertheless, the record of the secretion of a digestive juice is of great importance, for in a recent publication Mesnil (1902) states that digestion in the Actinians is entirely intra-cellular, and denies the occurrence of an inter-cellular digestion in the group. This statement is based on the result of a number of experiments of a chemico-biological character.

On comparing sections of the ventral filaments of recently-

fed zooids with similar sections of starved zooids, a considerable amount of histological difference was observed (figs. 9—11).

The filaments of starved zooids were densely crowded with gland cells containing numerous rounded granules, which became so intensely black on staining with iron brazilin and iron hæmatoxylin, that their histological structure could only be observed in very thin sections (3—5 μ). Each gland cell was then seen to contain a deeply-seated nucleus and a delicate reticulum of protoplasm, in which the granules are imbedded. The gland cells near the surface of the filament usually contain more granules than the younger more deeply-seated cells (fig. 10). It is worthy of note that the granules and ingested food matter have not been observed together in the same cell.

Gland cells identical in structure, and, doubtless, also in function, have also been observed in the stomodæum and mouth disc.

A few mucous cells are interspersed between the granular gland cells and amœboid endoderm cells.

Sections from 15—20 μ in thickness were cut through the filaments, distended with carmined fish food (fig. 4), and microscopically examined without staining. The food was observed to be ingested in an amœboid manner by the endoderm cells covering the filaments (fig. 9). Particles of food were observed in the act of ingestion (*f. f.*), and particles of waste matter were also seen to be extruded from the cells (*f. u.*).

Within the cells the food material quickly became enveloped in food vacuoles, and speedily disintegrated; the red colour disappeared, and the process of digestion was apparently completed.

Similar sections were also stained with iron brazilin and examined in the same way. These were found to contain numerous gland cells, which were either empty or contained only a few granules (fig. 11).

From the feeding experiments the following facts are

gleaned, which have an important bearing on the question of the occurrence of an inter-cellular digestion in Alcyonium:

1. Large food bodies are rapidly broken up into small particles, and in some cases apparently acted upon by some digestive ferment in the cœlentera of the zooids before they are ingested by the ventral mesenterial filaments.

2. The mesenterial filaments of hungry zooids are crowded with gland cells containing numerous granules.

3. These gland cells also occur in the stomodæum and mouth disc of hungry zooids.

4. The mesenterial filaments of zooids immediately after feeding contain very few granular gland cells in which the granules are numerous, many cells contain very few granules, and several gland cells are empty.

5. The stomodæum and mouth disc of zooids immediately after feeding are usually devoid of granular gland cells.

The only inference to be drawn from a consideration of these facts is that the gland cells of recently-fed zooids have poured on to the food, during its passage through the stomodæum and envelopment by the filaments, a digestive secretion, which has brought about its disintegration and partial solution before its ingestion by the mesenterial filaments. Therefore, we have evidence in the Alcyonaria, as in the Madreporaria, of an inter-cellular digestion by the secretion of a digestive fluid into the cœlentera of the zooids, as well as an intra-cellular digestion which occurs throughout the Cœlenterates.

I have already drawn attention to the fact that food material is seldom observed in the cœlentera of the zooids in Alcyonaria (p. 331). This is especially the case with regard to tropical forms, and has been commented upon by several authors (p. 347).

Histologically, the stomodæum and ventral mesenterial filaments differ to a greater or less degree from those of the British Alcyonium digitatum. Several tropical species, Sarcophytum glaucum, Sclerophytum capitale, palmatum, densum, etc., give rise to a copious mucous secre-

tion. In these forms mucous gland cells are extremely numerous in the stomodæum (figs. 5 and 6).

In tropical forms the mesenterial filaments are frequently small compared with those of colonies inhabiting temperate waters. This is particularly noteworthy in the tropical specimens of Alcyonium pachyclados. In specimens from the Cape which have been attributed to this species the mesenterial filaments are fairly well developed, but in colonies from the Maldive Islands they are either extremely small or entirely absent. The enormous size of the filaments in Sarcophytum I have shown to be entirely due to the thickening of the mesoglœa of the mesentery near the free edge (fig. 12), and cannot in any sense be regarded as an increase of digestive surface. In many cases of this genus the mesenterial filaments are small compared with those of the British genus (figs. 10 and 12), and contain few granular gland cells.[1]

The filaments are apparently larger in Sarcophytum glaucum than in any other species, but it is to be regretted that several specimens are not sufficiently well preserved for the study of the histology of the filaments; when gland cells are present they are similar to those of Alcyonium (fig. 10), and doubtless fulfil the same function.

The mesenterial filaments of Sarcophytum latum closely resemble those of Lobophytum, but as this species resembles Lobophytum in several other respects (Pratt, 1903) it should henceforth be included in this genus.

These filaments in Lobophytum are more like those of the British form than any other tropical genus in the collection. They are, however, smaller than in our species of Alcyonium, and it is interesting to note that zoochlorellæ are by no means numerous.

The ventral filaments of Sclerophytum vary considerably in different species (cf. Table, Pratt, 1903, p. 531). They are

[1] The scarcity of granular gland cells in the mesenterial filament (fig. 13) is not to be confused with the empty condition of these cells in Alcyonium after feeding (fig. 11).

smaller than in Lobophytum, and in some cases their presence is extremely doubtful.[1] They differ from those of Sarcophytum and Lobophytum in that they are frequently crowded with zoochlorellæ, and from the latter genus also in that granular gland cells are very scantily distributed (fig. 13, Scl. capitale). Fragments of zoochlorellæ sometimes occur in the filaments, and there is little doubt that these cells are digested by the zooids. I have examined several specimens of the species of this genus, and have been unable to find other food material in the cœlentera, or in an ingested condition in the mesenterial filaments. The scarcity or complete absence of food material in the cœlentera of tropical corals is well known (p. 331), and has been commented upon by Hickson for Hydrocorallines, by Hickson, Bourne, Fowler, and Duerden for Madreporaria. Brandt and Hickson suggest that zoochlorellæ contribute nutriment in a state of solution to the corals in a mature condition.

After experimenting on Radiolaria and Anemones Famintzin (1891) maintains that these cells can only afford nutriment to the animals by the actual digestion of their tissues.

Gamble and Keeble (1903) have experimented on Convoluta roscoffensis, a Turbellarian which contains green cells in great abundance. They find that Convoluta feeds voraciously from the time of hatching to the period of maturity when it adopts a new mode of nutrition, and "derives all its food directly from the green cells by digesting them, and possibly also indirectly by extricating plastic nutriment from them." Dr. Gamble informs me that since the publication of this paper he has obtained evidence in favour of the last supposition.

The foregoing comparative description of the histology of the genera Alcyonium, Lobophytum, and Sclerophytum indicates a reduction of the digestive surface of the autozooids in tropical forms associated

[1] In a specimen of Scl. Gardineri ventral mesenterial filaments were absent in many mature autozooids, but extremely small ones were observed in the case of young zooids.

with a corresponding increase in number of zoochlorellæ,[1] and may be summarised as follows:

1. The ventral mesenterial filaments of Lobophytum more closely resemble those of the British Alcyonium, and are only slightly reduced. Food material has been observed in the cœlentera of this genus. Zoochlorellæ are never numerous.

2. In Sarcophytum the mesenteries are modified by mesoglœal thickening near the free edge, but the filaments are smaller than those of Lobophytum, and are provided with few gland cells. Zoochlorellæ are fairly numerous.

3. The filaments of the tropical species of Alcyonium are extremely small, and contain few gland cells. Food material was not observed. Zoochlorellæ are very numerous.

4. The ventral mesenterial filaments in Sclerophytum are either very small or entirely absent.[2] When present, gland cells are so few in number that their physiological function must be extremely limited (fig. 13). No foreign food material was observed. Zoochlorellæ are extremely numerous.

From the comparatively small number of zooids in Sclerophytum and the minute size of the tentacles it is obvious that the amount of food captured by the latter must be extremely small and totally inadequate to supply the growing needs of a colony. Furthermore the minute mesenterial filaments—(the degenerate representatives of the principal organs of digestion in the British Alcyonium)—and the stomodæum are together incapable of digesting a sufficient amount of food to serve for the nutrition of an entire colony.

I have already experimentally shown (p. 332) that the natural food of the British Alcyonium appears to consist chiefly of small living crustacea, which, captured by the long and extremely contractile tentacles, are paralysed by the poisoned threads of innumerable nematocysts before being swallowed. The absence of food in many tropical Alcyonaria may be

[1] A short account of the zoochlorellæ in Alcyonaria, their nutritive function and geographical distribution is appended, p. 349.

[2] Ashworth (1898) states that Xenia Hicksoni from North Celebes has no mesenterial filaments.

attributed to the degeneration of the zooids, which appear to have not only lost the power of capturing living prey but also of killing and digesting it.

I have already shown that zoochlorellæ are most numerous in the genus Sclerophytum, in which the reduction of the digestive surface reaches its extreme limit. It therefore seems very possible that these algal cells indirectly contribute nutriment in a soluble condition to the corals they inhabit, as well as directly by their actual digestion. This matter is further discussed in the following portion of the paper devoted to the description of the structure and function of the zoochlorellæ.

Zoochlorellæ.

I have already described the occurrence and relative abundance of these algal cells in Sarcophytum, Lobophytum, and Sclerophytum (1903), and have drawn attention to the fact that while they usually occur in colonies inhabiting shallow water they are found to be fairly abundant in specimens from 24—34 fathoms, so that their numbers do not appear to be affected by bathymetric variations within certain limits. I have suggested that their presence in enormous numbers in the superficial tissues in certain species of Sclerophytum is correlated with a reduction in size of the tentacles and mesenterial filaments.

The geographical distribution of zoochlorellæ is interesting. These algal cells commonly occur in tropical Alcyonaria, but are usually absent in corals inhabiting the temperate and cold waters of British seas and the South Atlantic (Cape of Good Hope). Hickson (1894), however, describes them as being extremely abundant in species of Clavularia from the Victorian Coast of Australia.

In a preceding part of the present paper I have discussed the more or less gradual reduction of the digestive surface in tropical members of the Alcyonaria, and the accompanied increased abundance of zoochlorellæ in connection with the scarcity or absence of food in these forms.

As these cells are frequently observed in a partially-digested condition, they no doubt serve as a direct source of nutriment to the corals they inhabit.

The zoochlorellæ of the Alcyonaria are very similar to those described by Duerden (1903) in the Madreporaria, and do not apparently differ in any essential respect from those inhabiting other tropical corals. A cell usually has one, but many have two chromatophores. The presence of starch in these algal cells may be easily demonstrated by treating with a dilute solution of caustic potash followed by iodine solution. A starch ring, sometimes incomplete, is then seen to surround the pyrenoid, and in many cases starch grains are scattered about the middle of the cell. The presence of reserve food material in the form of starch in these algal cells indicates a superabundance of nutriment. This insoluble food material can only be converted into a soluble form, such as sugar, by the action of diastase secreted by the protoplasm of the alga or possibly also by the animal cell which it inhabits. [1] It is well known to botanists that the vegetative cells of plants may convey nutriment in soluble form from one cell to another. In lichens nutriment of a carbohydrate, and possibly also of a nitrogenous nature is prepared by the algal cells and is conveyed to the symbiotic fungus through the walls of the algal cells and fungal hyphæ.

Zoochlorellæ occur only in those portions of the corals which are exposed to light, and are most abundant in the endodermal cells and spaces, continually bathed with sea water, which circulates more or less freely within the colony through the zooids and canals. It is obvious that the circulating sea water rapidly becomes charged to a generous extent with carbon dioxide and other products of animal metabolism. The presence of carbon dioxide in considerable proportion would enable the rapid formation of carbohydrate food-material in the algal cell under the influence of sunlight. These cells have also the power to build up organic nitrogenous

[1] For information on the nutrition of vegetable cells I am indebted to Professor Weiss.

compounds from the inorganic nitrogen salts contained in the sea water, but they may also make use of the waste nitrogenous animal matter, in which case the alga too would derive some benefit from the symbiosis.

In the reduction in size and function, or complete loss of the organs of digestion in corals greatly infested with zoochlorellæ, we have evidence that these algal cells nourish the coral to a considerable extent by contributing carbohydrate, and possibly also nitrogenous food material in a soluble condition.

The Mesoglœal Cell Plexus.[1]

The cells and fibrils which compose the so-called "mesoglœal nerve plexus" of the Alcyonaria were observed to be extremely numerous in some members of the family and comparatively rare in others, while in some instances they appeared to be of an unusually large size.

In his account of the anatomy of the Alcyonaria, Hickson (1895, p. 371) calls attention to the fact that, while this system of cells and fibrils has not been experimentally shown to be nervous in function, yet it is undoubtedly homologous with the "nervenschicht" described by the Hertwigs in the Actineæ (1879). Ashworth (1898, p. 209) describes and gives admirable figures of a nervous plexus in Xenia which he states to be homologous with that of Alcyonium.

Kassianow, 1903, gives a preliminary account of the nervous plexus of "Alcyonium."

In preserved specimens of Sarcophytum, Lobophytum, Sclerophytum, and Alcyonium the cells and fibrils vary considerably in size, shape, and relative abundance. In certain specimens the cells have a stellate form, and are provided with numerous long and short fibril-like processes (fig. 15, Sclerophytum durum). In some forms they are polygonal, with fewer processes (fig. 16, Lobophytum

[1] Preliminary account of nerve plexus. Pratt, 1902, p. 545, and 1903, Sect. D.

pauciflorum), while in others they are less numerous, are somewhat spindle shaped, and have only two or three processes (fig. 17, Sclerophytum densum). The fibril-like processes usually have a hyaline structure, are sometimes very long, and frequently fuse with each other, so as to form a more or less complete network (figs. 18 and 21), which is known as the mesoglœal nerve plexus. Where fusion has taken place the processes have a granular protoplasmic appearance similar to the cell contents.

Many of the cells are intimately connected with the endoderm cells of the zooids and canals[1] (figs. 17 and 18), but the connection between the cells and the ectoderm is less intimate (fig. 16). Occasionally a cell may be connected by means of its processes, with an ectoderm cell on one side, and an endoderm cell on the other.

These cells, with their long fibril-like connections with ectoderm and endoderm, present, in the preserved condition, a remarkable likeness to nerve cells and fibrils occurring in other groups, but, beyond this resemblance, we have no evidence of their nervous character; moreover, the very intimate connection existing between the plexus and the endoderm (fig. 18), and its less intimate connection with the peripheral ectodermal tissues (fig. 16), throws considerable doubt upon the theory of its having a special nervous function.

In order to ascertain, therefore, its true nature and function, I examined the plexus in living specimens. A comparison of preserved preparations with the living plexus of cells in Alcyonium digitatum yielded results which are as interesting as they were unexpected.

Thin free-hand sections of Alcyonium were examined in the living condition with moderately high powers,[2] when the

[1] The connection between the nerve fibres and the endoderm and ectoderm cells has been noted for the Alcyonaria by Hickson in Alcyonium, and Ashworth in Xenia and for the Madreporaria by Stanley Gardiner in Flabellum.

[2] Zeiss, No. 6 eyepiece, $\frac{1}{12}$ oil imm.

cells of the "mesoglœal plexus" could be observed without difficulty. The stellate cells were then seen to withdraw and thrust out the processes which have been called nerve fibres. Several cells were sketched with the aid of a camera lucida at intervals of from twenty minutes to half an hour, and in every case they were observed to be in an amœboid condition, the pseudopodial processes being more or less numerous, long and slender in form, and frequently branched (figs. 19 and 20).

The rapidity with which the cells change their outline varies considerably. The cell shown in fig. 19 was moving much more quickly than that of fig. 20. The so-called nerve fibres are simply the pseudopodia of the amœboid cells, which vary in size according to the cell's mode and rate of progression. The curious stellate appearance of the cells in the preserved condition (fig. 15) is doubtless due to their contraction on fixing.

Acting on the advice of Professor Hickson, I carried out the following experiments:

Minute particles of carmine were suspended in the sea-water in which living colonies of Alcyonium were kept. For three days clouds of carmine were squirted, by means of a pipette, about the expanded zooids. Thin free-hand sections were then cut, and, on examination, minute particles of carmine were observed in an ingested condition in the endoderm cells of the ventral mesenterial filaments and in the endoderm of the body walls of the zooids.

It is interesting to note that the ingestion of foreign particles of carmine is amœboid, and is identical in every respect with the ingestion of food material (fig. 9).

The experiment was continued for seven days. After the fourth day carmine particles were observed, first in the cells of the endoderm canals, and then in the cells of the solid cords of endoderm in the mesoglœa. In both instances some of the cells containing carmine particles were seen to be in an amœboid condition (fig. 22*a* and 22*b*) and to thrust out processes into the mesoglœa.

Finally, particles of carmine were also seen in the stellate and spindle-shaped amœboid cells forming the so-called "mesoglœal nerve plexus" (fig. 24). Some of these cells were kept under observation for a considerable time. They frequently remained in an active amœboid condition for quite an hour, then the pseudopodia would be withdrawn, and the cells would become rounded and inert. Such a condition is comparable with the rounded cells frequently observed in the mesoglœa of stained preparations.

These experiments substantiate the following facts:

1. Solid particles of carmine are ingested by the endoderm cells of the mesenterial filaments, body wall, and endodermal canals (figs. 21, 22, and 24) in a manner precisely similar to the ingestion of food particles by the mesenterial filaments (fig. 9).

2. The endoderm cells of the ventral mesenterial filaments, the body wall, the canals, and cords in the mesoglœa are frequently amœboid.

3. The presence of ingested carmine particles in the cells of the mesoglœal plexus indicates that they have been conveyed from the cœlenteric cavities of the zooids to portions of the colony apart, or even remote, from the zooid.

4. The cells composing the so-called "mesoglœal nerve plexus" are amœboid, and the so-called "nerve fibres" are really the pseudopodia of the amœboid cells.

5. The mesoglœal plexus is more intimately connected with the endodermal than with the peripheral ectodermal tissues, while the cells, apparently of the same nature as the endoderm, are frequently larger in the neighbourhood of the endoderm than near the ectoderm.

These facts afford evidence that the so-called "nerve cells and fibres" are really amœboid endoderm cells which wandered into the mesoglœa, forming with their pseudopodial processes the more or less dense protoplastic meshwork known as the "mesoglœal nerve plexus."

Although it is well known that, in the embryonic stages of higher forms of life, ganglion cells have a certain power of

movement through the tissues, yet we have no reason for believing that nerve cells retain this power when maturity is reached. Amœboid nerve cells and pseudopodial nerve fibres are unknown. We have no evidence that the Alcyonaria are more nervously sensitive than other lowly organised groups, so that it is impossible to regard this extremely well-developed system of amœboid cells with coalescing pseudopodia as a specially differentiated "nervous plexus."

1. Functions of the Mesoglœal Plexus.—The ingestion of the inorganic particles of carmine by the endoderm cells is identical with the ingestion of organic food material. The distribution of ingested foreign matter by means of the wandering amœboid cells, usually most abundant about the digestive centres, is no doubt similar to the distribution of food material in a digested condition. I would, therefore, suggest that the distribution of nutriment is effected in the following manner:—Certain amœboid endoderm cells loaded with nutriment wander, or have wandered, into the mesoglœa, where they form an amœboid plexus of cords and strands of cells which extends throughout the colony. The intimate connection between the digestive endoderm cells of the zooids and the plexus is maintained. If we suppose that throughout the plexus the nutritive protoplasm may be transferred from cell to cell—and the presence of carmine particles in the mesoglœal plexus affords substantial evidence for believing this to be the case—then this system of amœboid cells must be regarded as a nutritive as well as a sensitive plexus, and by its means nutriment may be conveyed from the digestive endoderm cells of the zooids to every portion of the colony.

2. Excretory Function.—As the amœboid endoderm cells were observed to eject foreign bodies in the form of carmine particles (fig. 9, *f. u.*), it is very possible that the plexus has also an excretory function, the amœboid cells, which are to be regarded as the carriers of nutriment may also convey waste products to the cœlentera or lumen of the canals.

3. Nervous Function.—As a stimulus affecting one polyp may be transmitted with gradually diminishing effect to its neighbours, it is probable that stimuli or impulses travel through this unspecialised amœboid protoplasmic plexus. As the speed of transmission might possibly be retarded by the presence of nutrient matter in the protoplasm, the colonies would therefore become less sensitive to stimuli during the distribution of the digested food material. This may explain the fact that the colonies under experiment (p. 332) withdrew their anthocodiæ shortly after feeding, and remained in a contracted condition for several hours.

In their amœboid character and multiple function these cells are homologous to the phagocytes occurring in other groups.

The apparent lack of differentiation in their structure and function must be considered a secondary feature. Certain cells, at one time forming a constitutional part of the endoderm, have reverted to a more primitive amœboid condition, in which they are capable of fulfilling any function which the demands of the colony may require them to perform.

The research in connection with this paper has been carried out in the Zoological Laboratories of the Victoria University of Manchester, in the Biological Laboratory of Port Erin, and in the Zoological Laboratory at Naples.

I am greatly indebted to Professor Hickson for much valuable advice and kind supervision of my work.

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EXPLANATION OF PLATES 20—22,

Illustrating Edith M. Pratt's paper, "The Digestive Organs of the Alcyonaria and their Relation to the Mesoglœal Cell Plexus."

List of Reference Letters.

Amb. c. Amœboid cells. *b. cn.* Batteries of cnidoblasts. *car. p.* Carmine particles. *c. g. c.* Clear gland cells. *ch.* Chromatophore. *cn.* Cnidoblast. *cœl.* Cœlenteron. *col. end.* Columnar endoderm. *c. v.* Ciliated vessel. *c. z.* Contracted zooids. *d. mg.* Dense layer of mesoglœa surrounding zooids. *d. mes.* Dorsal mesentery. *d. m. f.* Dorsal mesenterial filament. *ect.* Ectoderm. *em. g. c.* Empty gland cell. *end.* Endoderm. *end. b. w.* Endoderm body wall. *end. can.* Endodermal canals in mesoglœa. *end. co.* Endodermal cords in mesoglœa. *f. c.* Flagellate cell. *f. f.* Carmined fish food. *f. u.* Par-

ticles of extruded undigested food. *f. v.* Food vacuoles. *g. c.* Golden cells. *gr. c.* Granular gland cells. *in. mes. sp.* Intermesenterial space. *int. c.* Interstitial cell. *int. c. s.* Internal canal system. *l. c.* Longitudinal canals, *l. m. f.* Lateral mesenterial filament. *m.* Mesentery. *m. ap.* Mouth aperture. *mg.* Mesoglœa. *mg. b. w.* Mesoglœa body wall. *muc. c.* Mucous gland cell. *nem.* Nematocyst. *nu.* Nucleus. *nuc.* Nucleolus. *ov.* Ovum. *v. mes.* Mesenteries coloured red by ingested fish food. *s.* Stomodæum. *sc.* Scleroblast. *si.* Siphonozooid. *sp.* Spicule. *sph.* Hole left by spicule after decalcification. *sta.* Starch. *st. c.* Stellate cell = amœboid cell. *tent.* Tentacle. *ts. s. c.* Transverse superficial canal. *v. m.* Ventral mesentery. *v. m. f.* Ventral mesenterial filament. *zo.* Zoo-chlorellæ.

PLATE 20.

Fig. 1.—Alcyonium digitatum. A fairly young colony which has not yet assumed the digitate form. The zooids have been fed on carmined fish food, which can be seen through their transparent body walls. The colony was fixed and preserved in a fairly expanded condition by the hot formalin method. × 4½.

Fig. 2.—Alcyonium digitatum. Diagrammatic representation of the capture and course of food within the zooid.

a. Capturing food.

b. Tentacles contract slightly and enclose food.

c. Swallowing food.

(The next stage—the grasping and squeezing of food by the ventral mesenterial filaments—is shown in Fig. 3.)

d. The ventral mesenterial filaments are coloured red by ingested particles of carmined fish food.

The dorsal mesenterial filaments take no part in the digestive process. × 10 (cam. luc.).

PLATE 21.

Fig. 3.—Alcyonium digitatum. Anthocodia of a zooid which has been fed on carmined fish food. The excrescences on the pinnules of the tentacles are batteries of nematocysts. Grooves are shown between the bullate bases of the tentacles. The ventral filaments are shown embracing the food as it emerges from the stomodæum. × 10 (cam. luc.).

Fig. 4.—Alcyonium digitatum. A mesenterial filament of a zooid which has been fed for two days on carmined fish food. The red patches are ingested particles of coloured food. × 67 (cam. luc.).

Fig. 5.—Sarcophytum glaucum. Transverse section through stomodæal ectoderm to show granular and mucous cells. × 620 (cam. luc.).

Fig. 6.—Sclerophytum densum. Transverse section through stomo-

dæal ectoderm to show mucous gland cells. The mucous secretion is seen to be oozing from many of the cells. × 608 (cam. luc.).

Fig. 7.—Sclerophytum hirtum. Transverse section through the siphonoglyph, showing the extremely long flagella, which are about ·03 mm. in length. × 608 (cam. luc.).

Fig. 8.—Lobophytum pauciflorum. Longitudinal section through the lower portion of the stomodæum of an autozooid, showing the termination of ectoderm and the continuation of endoderm into the ventral mesenterial filaments. × 312 (cam. luc.).

Fig 9.—Alcyonium digitatum. Transverse section through an unstained ventral mesenterial filament, showing amœboid ingestion of carmined fish food by the endoderm cells. Some of the amœboid cells are shown with pseudopodia projecting into the cœlenteric cavity. Particles of undigested food (*f. u.*) are being extruded from some of the cells. Gland-cells are not indicated, as they cannot be seen without the aid of staining reagents. × 930 (cam. luc.).

Fig. 10.—Alcyonium digitatum. Transverse section through a ventral mesenterial filament of a starved zooid, stained with iron brazilin. Granular gland cells (*gr. c.*) are extremely numerous. A few mucous cells are interspersed between the granular gland cells and the amœboid endoderm cells. There are no spaces between the gland cells at the periphery of the filament, but spaces are numerous in the middle of the filament. × 930 (cam. luc.).

Fig. 11.—Alcyonium digitatum. Slightly oblique section through a ventral mesentery of a recently fed zooid, similar to the one shown in Fig. 10, after staining with iron brazilin. This filament differs from that of a starved zooid (Fig. 11) in that the granular gland cells are remarkably few in number and contain very few granules. The spaces occurring between the cells at the edge of the filament are probably empty gland cells which have discharged their secretion on to the food when it was embraced by the filaments. A few nematocysts, similar to those of the tentacles, are present. × 930 (cam. luc.).

Fig. 12.—Sarcophytum ehrenbergi. Transverse section through a ventral mesentery to show the mesoglœal thickening near the free end, and the scarcity of gland cells in the feebly marked filament. × 416 (cam. luc.).

Fig. 13.—Sclerophytum capitale. Slightly oblique transverse section through a ventral mesenterial filament in which zoochlorellæ are extremely numerous and granular gland cells very few in number. This drawing illustrates the reduction of the digestive surface in tropical forms, and the corresponding increased abundance of zoochlorellæ. × 930 (cam. luc.).

FIG. 14.—Lobophytum pauciflorum. Slightly oblique transverse section through a ventral filament. This is a tropical form which contains comparatively few zoochlorellæ. The filament is fairly large, and has fairly numerous granular gland cells. (Compare fig. 10.) This is the only tropical species in which I have observed the presence of food material. × 416 (cam. luc.).

PLATE 22.

FIG. 15.—Sclerophytum durum. Stellate cells with fibril-like processes, which compose the mesoglœal "cell plexus," from a stained preparation. × 826 (cam. luc.).

FIG. 16.—Lobophytum pauciflorum. Transverse section through the wall of an autozooid at the base of a tentacle to show the processes from the inner ends of the ectodermal cells, and their connection, in some cases, with the stellate cells of the mesoglœal plexus, which have fewer processes than in fig. 18. × 826 (cam. luc.).

FIG. 17.—Sclerophytum densum. Section through the terminal portion of an endodermal canal in the mesoglœa showing its intimate connection with the cells of the mesoglœal plexus. Many of the cells are more or less spindle-shaped, and have very few processes. (Compare with figs. 15 and 16.) × 826 (cam. luc.).

FIG. 18.—Alcyonium digitatum. Drawing showing the intimate connection between the stellate cells of the plexus and the endodermal canals in a living colony. × 706 (cam. luc.).

FIG. 19.—Alcyonium digitatum. Two drawings of a living amœboid cell of the mesoglœal plexus. Half an hour elapsed between the drawings. The cell is moving in an upward direction, and changes its outline more rapidly than in fig. 20. *a* × 930, *c* × 960 (cam. luc.).

FIG. 20.—Alcyonium digitatum. Three drawings of a single living amœboid cell with long pseudopodia of the mesoglœal plexus. (Twenty minutes elapsed between *a* and *b*, and thirty minutes between *b* and *c*.) The pseudopodia (so-called "nerve fibres") of the lower part of the cell are being withdrawn while long new pseudopodia are being thrust out from the upper part. The cell is obviously moving in an upward direction. × 723 (cam. luc.).

FIG. 21.—Alcyonium digitatum. Diagrammatic representation of the course of carmine particles suspended in the sea-water in which living colonies were confined. After two days these particles were observed to be ingested by the endoderm cells of the ventral mesenterial filaments and the endoderm lining the body-walls, and after from four to seven days were observed in the cells of the endoderm of the canals and cords in the mesoglœa, and finally in the amœboid cells which have hitherto been regarded as nerve cells.

Fig. 22.—Alcyonium digitatum.

a. A portion of an endodermal cord in the mesoglœa in a living specimen. The cells contain ingested carmine particles.

b. The same cord after an interval of half an hour. Two of the cells containing carmine are in an amœboid condition, and are beginning to wander into the mesoglœa. × 547 (cam. luc.).

Fig. 23.—Sclerophytum densum.

a. Group of endodermal canal cells, one of which is in an amœboid condition, and has an extremely long, branched pseudopodium thrust into the mesoglœa. (Compare with fig. 25.) × 800 (cam. luc.).

b and *c* are amœboid endoderm cells which have wandered into mesoglœa. × 800 (cam. luc.).

All from stained preparations.

Fig. 24.—Alcyonium digitatum. Living amœboid cells of mesoglœal plexus containing particles of carmine. These have hitherto been known as nerve cells. × 717 (cam. luc.).

A Contribution to the Morphology and Development of the Pectoral Skeleton of Teleosteans.

By

H. H. Swinnerton, D.Sc., F.Z.S.,

University College, Nottingham.

With Plate 23, and three Figures in the Text.

Introductory.

The following pages contain the results of an investigation conducted chiefly on the salmon and the stickleback. These types were chosen as representative of lowly and of advanced teleosts respectively. The adult sturgeon and developmental stages of other teleosts were also examined in order to correct, confirm, and extend results.

The method pursued was that of reconstruction in wax from serial sections as described in my previous papers.

Swirski, Wiedersheim, and Ducret are the only workers who have dealt with the same subject during recent years. Of these the first alone attempted to reconstruct the pectoral skeleton at different stages; the others confined themselves to descriptions and to figures of sections.

Before proceeding to the main body of the paper I should like to record my indebtedness to the Research Committee of the Royal Society for a grant towards the expenses incurred, to Prof. T. W. Bridge and Prof. W. C. M'Intosh for much useful material, and finally to Mr. G. A. Boulenger for his kindly criticism.

Descriptive.

The Development of the Pectoral Skeleton of Salmo salar.

Younger stages than any at my disposal were described by Ducret. From his observations it is seen that the cartilaginous skeleton first begins to appear as procartilage in embryos 9·6 mm. long. When they are 13·4 mm. long the skeleton has become cartilaginous, and consists of a lamina lying in the fin, and of a basal plate in the body wall.

Stage I (figs. 1 and 2).—At this stage the fin is a half-moon-shaped fold which, on account of the relative smallness of the embryo as compared with the yolk-sac, points dorsally. Consequently the morphologically ventral side of the fin faces laterally. It is largely supported internally by a thin concavo-convex plate of hyaline cartilage (*f. p.*) with its concave face towards the inner side. Its shape calls to mind that of the larval Polypterus (Budgett), and it differs chiefly in the breadth of its base of attachment. The fin-plate is continuous with the girdle (*sc.* and *co.*), which lies in the body wall, and is also hyaline. These two regions are far from being sharply marked off from one another either by histological structure or difference of orientation. The scapulo-coracoid region is but a continuation of the fin-plate, and with that forms the same concavo-convexity. Such figures as that of Ducret's (pl. II, fig. 12) give the impression that the fin-plate is attached at right angles to the plane of the girdle or "plaque vasale," and therefore that the portion of the girdle dorsal to the line of attachment is the scapula, and that which is ventral is the coracoid. Such an impression is erroneous and is due to the study of sections without the aid of reconstructions.

The boundary between these two regions can therefore be ascertained only by following the line of attachment of the fin-fold to the body. This is represented by a dotted line (*g. b.*) in the figures, and may be spoken of as the glenoid line. It is along this that the glenoid articulation is formed

at a later stage, though there is no sign of its formation at present. At this early stage the glenoid line is practically parallel to the long axis of the animal's body.

The girdle is triradiate with a large fenestra (*fn.* 4) at the junction of the radii. Through this there passes a branch of the fourth spinal nerve to supply the ventral muscles of the fin, and also a small artery and vein.

The dorsal radius is the scapulo-coracoid region (*sc. co.*). It is continuous with the fin-plate along its upper, and is folded inwards on its lower, border (fig. 2). The inner edge of the fold bears a slight outgrowth—a kind of scapular process (*sc.*) near its anterior end. Posteriorly it is much thickened, and bears a much stronger outgrowth (*m.*), which is the first trace of the mesocoracoid bar. At a point (*fn.* 1—3) lying a short distance internal to this folded edge, and between the two outgrowths, a stout nerve forks, sending one branch to the true dorsal, and the other to the ventral, surfaces of the fins. This nerve is formed by the union of the first three spinal nerves.

The anterior radius (*pr. p.*) is long and rod-like, and looks almost like an antero-ventral continuation of the mesocoracoid thickening. At a later stage it trends medianly and meets its fellow of the opposite side in the middle line. At this stage, however, owing to the size of the yolk-sac, it bends somewhat laterally. Gegenbaur, and Swirski following him, calls this the procoracoid. The latter describes it as arising independently from the rest of the pectoral skeleton in the pike. But though Wiedersheim has investigated the same fish he finds no evidence for this independence. Neither from Ducret's observations nor my own on the salmon is there anything in support of this. It will be sufficient, therefore, to regard it as a process of the coracoid, and to call it the præcoracoid process.

The posterior radius (*po. p.*) stands nearly at right angles to the general plane of the girdle and the fin-plate. It runs for a short distance from the post-axial border of the latter into the body wall. It corresponds to the "coracoid" of

Swirski, the "Sockelstück," or "processus posticus" of Wiedersheim, and the "processus ensiformis" of Ducret. For the sake of uniformity it may be called the post-coracoid process.

Stage II (figs. 3 and 3*a*).—The skeleton, as a whole, is no longer concave towards the inner side, but both fin-plate and girdle are flat. The boundary between them is marked by an angle (fig. 3*a*, *g. b.*) due to the fact that the fin is no longer vertical, but stands out laterally.

The glenoid line is still practically parallel to the long axis of the body.

The fin-plate has undergone a considerable change in shape (cf. figs. 1 and 3), consisting mainly in a rounding off of the posterior angle due to a relative shortening of the posterior border (*P.*) and a growing out of the distal border. Near the front border (*A*) a large foramen has appeared, which marks off the region of the future first radial (*r.*).

The girdle is still triradiate, but now all its radii lie in the same plane, which is nearly vertical (3*a*, *co.*, *pr. p.*), tending to slope inwards towards the mid-ventral line. The foramen for the branch of the fourth spinal nerve (*fn.* 4), which loomed so large at the previous stage, is now extremely small, and has taken up its final position near the base of the post-coracoid process, and may, therefore, be conveniently referred to as the post-coracoid foramen.

The cartilage of the scapular region has grown forwards so that the ventral branch from the common trunk of the first three spinal nerves has become half surrounded. The deep notch thus formed (*fn.* 1—3) eventually becomes a foramen—the scapular foramen.

The coracoid region has grown more rapidly than the scapular, as may be seen by comparing the region anterior to the post-coracoid foramen in figs. 1 and 3. This growth has brought the anterior border (*f. b.*) close against a blood-vessel which supplies the ventral muscles of the fin.

The mesocoracoid swelling, which at the previous stage was situated near the anterior margin of the coracoid, is no

larger, and is situated just ventral to the middle of the glenoid line (fig. 3 *a. m.*), and halfway between the scapula and postcoracoid process.

The præcoracoid process has undergone no change beyond a relative increase in size and a slight median rotation.

The postcoracoid process also is slightly larger, and is now in a straight line with the præcoracoid process.

Stage III (figs. 4 and 5).—Hitherto the fin-plate has tended to be the dominant feature of the pectoral skeleton, but now it has yielded to the girdle. Both regions are still continuous, and the line between them horizontal. They are both orientated in the same way as in Stage II, though the hinder half of the fin-plate has begun to turn in such a way as to face somewhat posteriorly.

In the fin-plate the first radial element (*r*) has become a separate piece of cartilage. Another fenestra has appeared in the centre of the plate, and depressions on either side of it indicate the position of future fenestræ. Thus the four radials are blocked out.

Along the distal border of the second and third radials three nodules of cartilage have been differentiated. These are the distal radials. The first two belong to the first radial, the third distal to the third radial. There is a close similarity in everything but size between these distal elements and the first radial.

The scapulo-coracoid has now grown round the nerve and blood-vessel which lay in front of it, and thus the scapular (*fn.* 1—3) and præcoracoid (*f. b.*) foramina have been formed. In consequence of this growth the large space which formerly existed between the coracoid and cleithrum (*cl.*) has almost disappeared.

The mesocoracoid process (fig. 5, *m.*), though still small, is larger than before. Owing to the growth of the coracoid it is no longer situated close under the glenoid line. The apparent reduction of the præcoracoid process is due to the fact that its proximal portion has been taken up into the extending coracoid. The distal portion is remarkably slender,

but does not yet meet its fellow of the opposite side. For the greater part of its length it is in contact with the cleithrum.

The most striking feature at this stage is the great length of the postcoracoid process, which rivals even the præ-coracoid process. It is interesting to note that this process forms a posterior continuation of the cartilage bordering the glenoid line.

Stage IV (figs. 6 and 7).—The pectoral skeleton now bears a close resemblance to that of the adult, and differs from the previous stages chiefly in the orientation of parts. Whilst the scapular region still retains an upright position, the coracoid region, and with it the fin, has rotated inwards into an almost horizontal position (fig. 6). Consequently the glenoid line, which has hitherto been parallel to the long axis of the body, now slopes obliquely backwards and inwards towards this axis. This change was foreshadowed in Stage III, when the hinder part of the fin-plate faced slightly posteriorly.

The fin-plate has now broken up completely into the four radials indicated in Stage III. These are now rod-like, and increase in length from the pre-axial to the post-axial element. At its distal end each bears a pair of distal radials —mere nodules of cartilage. These increase in size in a reverse direction, viz. from post- to pre-axial border, a fact which suggests that the first formed radial element also belongs to the distal series. In support of this it may be pointed out that like the other distal radials it appears before the radials themselves; that it is not in the same line with the latter, but with the former; and finally that, like all the distal elements, it is enclosed by the base of the dermal fin-ray.

The scapular region has now, for the first time, risen above the pre-axial corner of the fin skeleton, which is thus removed some distance from the cleithrum (cf., figs. 4 and 7).

The coracoid region has extended still further forward, so

that the præcoracoid foramen is a considerable distance from its anterior border.

The mesocoracoid (fig. 6, *m.*) is now fully developed, having extended up to and united with the top of the scapula. It is a significant fact that the mesocoracoid remains dormant so long and then develops so rapidly. The rapidity of its development may be judged from the fact that the young in my Stages I—III vary in length from 15·5 mm. to 22 mm., whilst those of Stage IV are only 25 mm. long. This is no doubt associated with the equally rapid rotation of the coracoid into the horizontal position; and just as the scapula rises to form a buttress for the outer end of the glenoid border, so the mesocoracoid does likewise for the inner end. In this connection it is interesting, as further evidence, to compare the position of the base of the mesocoracoid as seen in figs. 5, 6, and 8, and to note its increasing proximity to this border. Ducret observed the late appearance of the mesocoracoid (p. 21).

The growth of the præcoracoid process has been almost stationary, and so in consequence of the forward growth of the scapulo-coracoid region it has been pulled away from that intimate relation to the cleithrum which it has had up to this stage. Nevertheless it now meets its fellow in the middle line.

The postcoracoid process is still present, but is greatly reduced.

The Adult (fig. 8).—Already in the salmon 52 mm. long, the girdle is as fully ossified as in the adult, and unfortunately I have no stages between Stage IV and this. Concerning the adult little need be said here, for it has been well described by Parker, though he overlooked the præcoracoid foramen.

The Development of the Pectoral Skeleton in Gasterosteus aculeatus.

Stage I (fig. 9).—Comparing this with the corresponding stages of the salmon one is struck at one time by the great

resemblances, at another by the equally great differences. In both the girdle and fin together form a continuous whole; concavo-convex in shape with the concavity facing inwards. In both the fin predominates over the girdle, though here this predominance is carried much further. This no doubt is associated with the fact that the stickleback begins to lead an active free-swimming life at a much earlier stage than does the salmon.

On the other hand in the stickleback there is an entire absence of both præ- and meso-coracoid processes. The glenoid line tends to be more vertical. The postcoracoid process is enormously developed, being about the same size as the whole of the remainder of the girdle. The postcoracoid foramen is unrepresented, and the fourth nerve passes down to a muscle which runs along by the side of the process. The level at which it reaches this muscle is indicated by an asterisk (*n.* 4).

The common trunk for the first three spinal nerves forks at the position indicated (*fn.* 1—3).

Stage II (fig. 10).—Owing to the growth of the girdle the disproportion between it and the fin-plate has diminished considerably. There is still no trace of a division between them, and together they are concavo-convex as before.

The fin-plate shows no sign of differentiation into radials.

The scapulo-coracoid region has grown much further anteriorly and dorsally, thus bringing about a rotation of the glenoid line into an almost vertical position. The præcoracoid process has no actual existence, but is represented by the antero-ventral angle of the coracoid.

The postcoracoid process is relatively larger than before and has become bent downwards.

Stage III (fig. 11).—The fin and girdle still form a continuous piece of cartilage without any sign of either foramina or radials. It is now much flatter.

The glenoid line makes about the same angle with the vertical as it did in Stage II, but its dorsal end comes much nearer to the upper corner of the fin-plate.

The scapulo-coracoid is very little altered, and the whole of its anterior margin is applied to the slender spicular cleithrum. Its antero-ventral corner is much more prominent (*pr. p.*), and may with reserve be spoken of as a process.

The postcoracoid process has attained astonishing proportions. It has rotated upwards. Its exact position is between the anterior portion of the trunk muscles and the skin (figs. 13—14). It is evidently a structure of considerable importance, but in what way is difficult to tell.

Stage IV (fig. 12).—The skeleton now bears a close resemblance to that of the adult, and, as in that, the scapulo-coracoid forms by far the greater portion. It is flat and plate-like, and stands in a vertical position comparable with that of the scapula in Stage IV of the salmon. The glenoid articulation has been formed by the breaking down of the cartilage.

The tendency for the glenoid line to rotate from a nearly horizontal into a vertical position which we have followed through the earlier stages has now reached its consummation.

The fin-plate has been perforated in three places preparatory to the formation of the four radials.

The cartilage of the scapular region has extended dorsally and anteriorly with the result that its upper end is on a level with the top corner of the fin skeleton. Its dorso-anterior border has been folded inwards and continued into a process (*sc.'*). A similar infolding was noted by Swirski in the pike. He cautiously suggested that it might be a relic of the meso-coracoid (p. 31). I have also seen it in the adult pike, and it seems to me more likely that it serves to supply a larger surface of contact with, and secures a firmer hold to, the cleithrum.

The forward growth of the scapula has brought about the complete enclosure of the nerve, which up till now has run round its anterior border.

The præcoracoid process is now more worthy of the name, though still very small as compared with that of the salmon. It has no relation to its fellow. The whole front border of

the girdle from the tip of the scapula to the end of the process is in the closest contact with the cleithrum, thus furnishing a striking contrast to the state of affairs in the salmon.

Perhaps the most interesting feature at this stage is the ossification of the still very long postcoracoid process. This extends later to the coracoid region, thus giving rise to the bone of that name.

Close to, but by no means continuous with, the postcoracoid process, is the "interclavicle" (*i. cl.*), which is a true dermal bone as opposed to the coracoid which, as development shows, is a cartilage bone.

The Adult.—The pectoral skeleton of the adult has been accurately described by Parker. It differs from that of Stage IV in the much greater extension of the scapulo-coracoid in an antero-posterior direction. Also the scapula has risen high above the fin skeleton, and the foramen has become extraordinarily large.

The "interclavicle" is now completely co-ossified with the coracoid.

General Considerations.

The Postcoracoid Process.

This process was first seen and described by Swirski in the development of the pike. In that fish it is the most striking feature of the earlier stages, and remains prominent until a fairly late stage. By comparison with Silurus and through that with sturgeon he came to regard it as homologous with Gegenbaur's "third process," and Parker's "coracoid." Wiedersheim found it also in developmental stages of grayling, catfish, and pike, and called it "processus posticus." He absolutely disagreed with Swirski in his interpretation, because the process was directed caudally, and ran parallel to the long axis of the body. He also examined larvæ of the sturgeon, and not finding it in these he regarded it as a new structure peculiar to the Teleosts, developed for purposes of fixing and support (p. 170). Ducret found it also in the salmon, and called it "processus ensiformis."

It is present during development in the cod and herring, but I have failed to find it in either Siphonostoma or Anguilla. Both the latter fish, however, are of a very special type, and are characterised by the great reduction of cartilage in all parts of the skeleton. In a larva of Amia 19 mm. long there is no sign of it.

To Wiedersheim's objection to Swirski's interpretation it may be added that whereas in those forms which, according to Gegenbaur, have a well-developed "under process" it is closely associated with the cleithrum, the postcoracoid process is never, at any stage, related to that bone.

Owing to the researches of Humphrey, Thacher, and Regan, no doubt remains concerning the similar nature of the median and paired fins. This is perhaps best shown by the pelvic fin of Psephurus (Regan). In this fish there is very little to choose in the anatomical details between the pelvic and the anal fins. In both each ray of the fin consists of three segments, which—using Bridge's terminology—may be called proximal (axonost), mesial (baseost), and distal (marginal) radials. In both there is a tendency towards the fusion of the anterior proximal radials. This is carried further in the pelvic than in the anal fin. The plate formed by the fusion of these elements is the very primitive pelvic girdle. The essential community of structure between the pectoral fins and the median is not so obvious; but, bearing in mind the researches of palæontologists, e. g. on Cladoselache and Cladodus, we have no difficulty in seeing it between the pectoral and pelvic fins of Psephurus. The distal and mesial elements are still present in the fin, and, as pointed out by Regan, the metapterygium and girdle represent the proximal elements, though in a much more specialised condition than in the pelvic fin. The metapterygium, therefore, has the same origin as the girdle, and is fundamentally the posterior continuation of it. The postcoracoid process has precisely the same relationships (e. g. fig. 9), and may be regarded as the homologue of the metapterygium.

Further facts may be given in support of this conclusion,

In the adult sturgeon the metapterygium lies almost in the region where the base of the fin passes into the body-wall, and has not yet rotated much outwards to form part of the free fin. It thus occupies the same position as the process.

It is significant that in the Ganoids which possess a metapterygium, development, so far as known, has revealed no postcoracoid process. In the Teleosts, which have no metapterygium, the process is very generally present, and present in widely diverse types.

The fact that the process is continuous with the girdle is not a serious objection to the view, for in early development the radials are continuous with one another and with the girdle.

This view does not agree with Gegenbaur's conclusion (p. 161) that the metapterygium of Ganoids has become one of the radials of the Teleosts. Based, as it is, upon the study only of adult living forms, his conclusion is so far sound. But all diversities of structure are not wrapped up in Lepidosteus and Amia. Other types have existed, and many facts of Teleostean morphology will probably find their explanation only among them.

The Evolution of the Pectoral Skeleton of Teleostomes.

At its first appearance the pectoral skeleton of the sturgeon consists of five short rays and a plate, the "primäres basale." From Mollier's figures it is evident that the line of attachment of the rays to the plate is parallel to the long axis of the body. The skeleton at this stage, then, bears a close resemblance to the pelvic skeleton, and presents such a condition as the fin-fold theory of the origin of paired fins requires. Mollier tells us that the second to the sixth spinal nerves converge to the "primäres basale," and bifurcate medially to it, sending one branch to the dorsal and the other to the ventral side. In the adult (Pl. 23, fig. 15) the second and third nerves, united with a branch from the hypoglossal or first spinal, form a common trunk (*n.* 1—3), which bifurcates

in the muscle canal (*m. c.*), sending one branch (*n.* 1—3*d*) to the dorsal muscles, and the other (*n.* 1—3*v*) through the scapular foramen to the ventral muscles.

The fourth nerve (*n.* 4) likewise bifurcates, sending one branch to a plexus which supplies the dorsal muscles, and the other through a passage (*fn.* 4) to the ventral muscles. The entrance to this passage was seen by Parker and called the "coracoid foramen," but was overlooked by Gegenbaur. It evidently corresponds to the postcoracoid foramen. The fifth spinal nerve (*n.* 5) repeats the conditions of the fourth with this difference, that its ventral branch has no foramen, but passes to the ventral fin muscles by way of the inner side of the metapterygium. Thus the anterior part of the "primäres basale" is represented by that portion of the girdle which lies between the glenoid facettes and the scapular and postcoracoid foramina, whilst the posterior part becomes the metapterygium. This is proved to be the case by Mollier's figures.

Whilst the metapterygium has retained the primitive position parallel to the axis of the body, the girdle has rotated so that the glenoid border, whilst still horizontal, is nearly at right angles to this axis. The radial elements upon it no longer project at right angles, as they must have done in early fishes, but are almost parallel to the metapterygium. Herein, then, lies a cause for the reduction in number and importance of the radials borne by the metapterygium. Thus the latter, instead of rotating out to become the main bearer of the fin skeleton, as in other fishes than the Teleostomes, remains in the body-wall, and becomes a minor factor.

All these changes are probably associated with the increasing importance of the dermal fin rays, and with the acquirement of the power to lay that portion of the fin flat against the side of the body, or to extend it so that it may act as a brake instead of a keel.[1]

[1] I hope to publish soon an account of some experiments which illustrate this point more fully.

It is much to be regretted that Mollier did not make careful reconstructions of the developing pectoral skeleton in the sturgeon. The study of sections alone cannot give a clear idea of the changes in place relations.

In both salmon and stickleback at the earliest stage the glenoid approximates to the most primitive position, viz. parallel to the long axis of the body. In the former, as development advances, the fin-plate and coracoid region rotate, so that the glenoid border slopes obliquely inwards. In the latter no such movement takes place, and the glenoid line rotates into a vertical position.

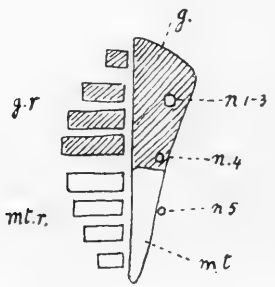

Fig. 1.—Diagram to illustrate the hypothetical pectoral skeleton of a very primitive teleostome.

Unfortunately, Swirski, in making reconstructions for the pike, did not insert the fin plate; nevertheless, examination of his figure shows that in it the movements of the glenoid line are the same as those in the stickleback.

We thus have two absolutely divergent tendencies exhibited by the developing pectoral skeleton of the salmon and sturgeon on the one hand, and by the stickleback and pike on the other. These find their concisest expression in the statement that, whilst both start with the glenoid line in practically the same position, in the one it rotates horizontally round the pre-axial end, in the other it rotates vertically round the post-axial end. The following diagram will serve to make the movements clear.

Attention has already been drawn (p. 369) to the close relationship which exists between the presence of a mesocoracoid and the horizontal rotation of the glenoid border. If the facts of development are any guide at all, it is very

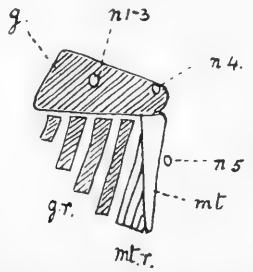

Fig. 2.—Diagram of pectoral skeleton of Acipenser. *g.* Girdle. *g. r.* Radial elements belonging to the girdle. *mt.* Metapterygium. *mt. r.* Radial elements belonging to metapterygium. *n* 1—5. First five spinal nerves.

difficult to see how the type of girdle without a mesocoracoid has been derived from that with a mesocoracoid, and vice versa. In the development of the former there is no sign of

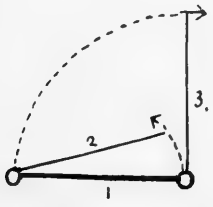

Fig. 3.—Diagram to illustrate the movements of the glenoid line during development. (1). Primitive position parallel to long axis of the body. (2). Final position in the salmon. (3). Final position in the stickleback.

a vanishing mesocoracoid or of an inwardly moving glenoid border.

In the most recent classification of Teleosts (Boulenger) all the non-ostariophysous fishes with a mesocoracoid are placed in the sub-order Malacopterygii. The lowliest forms without

it are placed in the Haplomi. In the definition of this sub-order this sentence occurs:—"The absence of the meso-coracoid arch distinguishes the Haplomi from the Malaco-pterygii, with which they are united by various authors." This statement alone suffices to show that, apart from the absence of this arch, the Haplomi, at least as represented by the pike and Galaxias, are as primitive as the general run of Malacopterygii. If a list be made of the features which indicate lowly affinity among the latter, it will be seen that most, if not all, are found among the Haplomi. Again, in my paper on Cromeria (p. 270) these words about Galaxias occur:—"In some respects, e. g. forward extension of the cranial cavity, and the condition of the articular head of the hyomandibular, it is as lowly as, or even more lowly than, the salmon."

Whatever may be said about the classification of other Teleosts, it is generally acknowledged that the Ostariophysi form a natural group. Though every conceivable modification of form and external character occurs amongst them, yet the mesocoracoid and the features associated with its presence remain constant. Again, among Malacopterygii there are forms which are extraordinarily specialised, but they nevertheless retain the mesocoracoid. These facts speak strongly for the stability of this structure.

The absence of a mesocoracoid in Polypterus was pointed out by Gegenbaur, and indicates the possibility that forms may have existed even among Holostei exhibiting the same peculiarity. Unfortunately fossils have as yet yielded no information on the primary pectoral girdle of these fishes.

If there be any truth in the view just propounded, then we are able to recognise among Teleosts three distinct offshoots from the Holostean stock, viz. one including the Ostariophysi, another the Malacopterygii, and a third the Haplomi and all those teleosts with the same type of pectoral girdle.

The "Interclavicle."

This bone was first described in the stickleback by Parker, and because of its resemblance to the true clavicle (interclavicle) of the sturgeon he called it "interclavicle." Recently Starks has disputed this decision, and after a careful study of the pectoral skeleton of Hemibranchii has come to the conclusion that this bone has no separate existence, but is only an extension of the coracoid (hypocoracoid). A glance at Pl. 23, fig. 12 shows him to be wrong in this conclusion for there it will be seen that the "interclavicle" is quite separate from the coracoid.

This reopens the question of its homologies once more.

This bone is related to the post-coracoid process, which, as we have seen, very probably represents the metapterygium. Now the "interclavicle" of the sturgeon is related only to the lower tip of the coracoid (Pl. 23, fig. 15, *co.*), and does not come near the metapterygium. The two bones, therefore, are not homologous, and the "interclavicle" of Gasterosteus and its allies should be called by a different name, viz. "infracleithrum."

Mr. Regan has called my attention to the presence of a similarly placed bone in Eurypholis (Woodward, p. 207). There can be little doubt that it is an infracleithrum also.

Summary.

1. The earlier stages in the development of the pectoral skeleton of the salmon are fundamentally the same as those of the stickleback (p. 370).

2. The mesocoracoid appears late in the development of the salmon, and is associated with the rotation of the glenoid border into a transversely horizontal position (p. 368).

3. There is no sign of a mesocoracoid during development in the stickleback, and this is associated with the rotation of the glenoid border into a vertical position (pp. 370 and 371).

4. Those Teleosts without a mesocoracoid probably consti-

tute a group separate and not originating from those Teleosts with a mesocoracoid (pp. 377 and 378).

5. The postcoracoid process, which is so prominent a feature during development, represents the metapterygium (pp. 373 and 374).

6. The so-called "interclavicle" of the stickleback is not a part of the coracoid, but arises as a separate dermal ossification. It is not homologous with the bone of the same name in the sturgeon, and should therefore receive a different name, e.g. infracleithrum (p. 379).

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EXPLANATION OF PLATE 23,

Illustrating Dr. H. H. Swinnerton's paper, "A Contribution to the Morphology and Development of the Pectoral Skeleton of Teleosteans."

List of Reference Letters.

A. Preaxial border of the fin. *cl.* Cleithrum. *co.* Coracoid or coracoid region. *f. b.* Foramen for blood-vessel, or præcoracoid foramen. *f. n.* Foramen for spinal nerve. *fn.* 1–3. Scapular foramen. *fn.* 4. Postcoracoid foramen. *f. p.* Fin plate. *g. b.* Glenoid line or border. *i. cl.* Infracleithrum. *m.* Mesocoracoid arch or bone. *m. c.* Canal for dorsal fin muscles. *n.* 1. First spinal or hypoglossal nerve. *n.* 1–5. Spinal nerves 1–5. *P.* Post-axial border of fin. *po. p.* Postcoracoid process. *pr. p.* Præcoracoid process. *r.* First formed radial. *r. d.* Distal radial. *r. m.* Mesial radial. *r. p.* Proximal radial. *sc.* Scapula or scapular region. *sc'.* Scapular process. *sp. c.* Spinal cord. *st.* Stomach. *t. m.* Trunk muscle.

Figs. 1—7, 9—11 were drawn from wax models reconstructed from serial sections.

FIGS. 1—8.—Pectoral skeleton of Salmo salar.

FIG. 1.—Stage 1. 15·5 mm. long. × 68. External view.

Fig. 2.—Ditto. Internal view.

Fig. 3.—Stage II. 19·0 mm. long. × 37. External view.

Fig. 3A.—Stage II. Diagrammatic vertical section.

Fig. 4.—Stage III. 22·0 mm. long. × 32. External view.

Fig. 5.—Ditto. Internal view.

Fig. 6.—Stage IV. 25·0 mm. long. × 30. Internal view.

Fig. 7.—Ditto. Ventro-lateral view.

Fig. 8.—Adult. Internal view.

Figs. 9—12.—Pectoral skeleton of Gasterosteus aculeatus.

Fig. 9.—Stage I. 3·6 mm. long. × 157. External view.

Fig. 10.—Stage II. 4·0 mm. long. × 157. External view.

Fig. 11.—Stage III. 5·4 mm. long. × 118. External view.

Fig. 12.—Stage IV. 11·0 mm. long. × 52. External view.

Fig. 13.—Gasterosteus aculeatus, Stage IV. Transverse section through the post-pectoral region. × 30.

Fig. 14.—Ditto. Portion of the same section, as in Fig. 13. × 150.

Fig. 15.—Acipenser sturio. Pectoral girdle and associated nerves posterior view. × $\frac{1}{2}$.

Observations on Hæmatozoa in Ceylon.

By

Aldo Castellani, M.D.,
Director of the Bacteriological Institute, Colombo,

and

Arthur Willey, F.R.S.,
Director of the Colombo Museum.

With Plate 24.

The observations recorded in this paper were made primarily with the object of ascertaining the distribution, frequency, and forms of infection of the parasites of the blood of vertebrates in Ceylon.

Since the publication of our preliminary note ('Spolia Zeylanica,' vol. ii, part 6, August, 1904, pp. 78–92) a few more cases have come under our notice, and as there is no immediate prospect of amplifying our investigations owing to the pressure of other duties, we deem it advisable to publish our results in full without further delay. The discoveries of Ross, Grassi, Schaudinn, and others, have rendered it clear that researches on blood parasites must be undertaken with a single aim, and with undivided attention, in order that they may be cyclically complete.

It seems that the experimental difficulties which beset the investigation of the life-cycle of the hæmogregarines and trypanosomes of cold-blooded animals are great; and the ignorance as to the means of transmission of these parasites, and with regard to the order of succession of their different

phases, leaves abundant room for future discoveries of biological importance.[1]

Schaudinn's memorable discovery, that the well-known hæmatozoon of birds called Halteridium is a phase in the life-history of species of Trypanosoma, has considerably modified the views as to the generic autonomy of the various protozoon parasites of the blood, besides apparently demonstrating a close affinity between the Sporozoa and the Flagellata. The name Halteridium will, however, doubtless survive to characterise the particular phase referred to, and the same use may probably be made of the term Hæmocystidium, which we have introduced to denote a well-marked endoglobular parasite observed by us in a gecko, an analogous phase having been previously described by Dr. Simond from a Trionyx.

I. On some Free, Unpigmented Gregariniform Bodies observed in the Blood of Man and Birds.

In the course of our examination of numerous samples of blood collected in different parts of the island, very many negative results, as was to be anticipated, have been obtained. So far as our observations have extended, they seem to point to the conclusion that the percentage of infected cases of all kinds is greater in the neighbourhood of centres of population than in outlying districts. This fact may indicate that the conditions of nutrition of the Hæmatozoa are more suitable under the circumstances of sanitation peculiar to towns, and that the incidence of blood-parasitism may be directly or indirectly assignable to the far-reaching influences of domestication.

There seems to be a marked contrast between the distribution of the Hæmatozoa and that of the majority of Entozoa or parasites of the digestive tract, the latter being at least

[1] There is, however, a paper by Dr. Siegel on "Die geschlechtliche Entwicklung von Hæmogregarina stepanovi im Rüsselegel, Placobdella catenigera," 'Arch. Protist,' vol. ii, 1903, pp. 339–342.

equally frequent in regions remote from human habitations as in areas of cultivation.

From the point of view suggested by these remarks, it is interesting to record the occurrence of peculiar parasitic bodies in the blood of birds and man, for which, so far as we can ascertain, no provision has been made in any of the published accounts of the life-histories of the Protozoa of the blood.

In the case of birds these bodies occur in company with Halteridium, and in man associated with the symptoms of malaria. They are crescentic organisms, rather exceeding the length or diameter of the blood-corpuscles, characterised by the absence of pigment and by the presence of vacuoles, and they are very rare in such preparations as we have examined. Their form and size relatively to the blood-cells are shown on Pl. 24, figs. 1—3.

In the blood of a bird, the common babbler, Crateropus striatus (Swainson), taken on July 20th, 1904, Halteridium was present in the films, though scarce, and, in addition, there were some rare parasites of an elongated shape, with two or three vacuoles, free in the plasma. In fresh preparations they appeared to be non-motile, though a slight change of shape could be detected, the parasite becoming slightly broader after a time.

Halteridium only was found in the spleen. After staining by Leishmann's method, the bodies were coloured deep blue, and did not show chromatin. Sometimes there is the appearance of a clear halo surrounding the body (Pl. 24, fig. 2). In a preparation which had become partly decolorised through lapse of time there appears to be a definite nucleus in the centre of the body.

In the blood of an Indian crow (Corvus splendens) similar bodies were found, but of smaller size, some of them appearing granular without vacuoles (Pl. 24, fig. 3); these would seem to be specifically distinct from the parasites of the babbler.

Finally, analogous bodies, exceedingly rare, have been

observed by us in the finger-blood of native fever patients. Their general outline and size might seem to suggest some relation, whether of growth or degeneration, with the ordinary crescents of tropical malaria; but the absence of pigment, the presence of vacuoles, and the deeply staining protoplasm exclude such an assumption, especially in view of their similarity to the bodies observed in birds. In the true crescents, as is well known, there is an aggregation of pigment granules interspersed with chromatin at the centre of the body; the protoplasm stains uniform pale blue, there are no vacuoles, and there are frequently traces of the original blood-cell in which the crescent developed adhering to it.

Unfortunately, the literature at our disposal does not permit us to say whether the bodies we have described are, or are not, identical with, or related to, the pseudo-vermicules described from certain birds by Danilewsky, Kruse, L. Pfeiffer, and MacCallum.[1]

II. Pigmented Endoglobular Parasites of a Gecko.

In the 'Annales de l'Institut Pasteur,' tome xv, 1901, p. 338, Dr. P. L. Simond described, under the name Hæmamœba metschnikovi, a parasite of the blood of the freshwater tortoise, Chitra indica (= Trionyx indicus), which appears at a certain phase of its life-history in the form of an amœboid and eventually rounded organism containing pigment granules. He says that the chelonian host is very common in the rivers of India, especially in the Ganges and its tributaries, and that it plays the part of a scavenger in the waters of the Ganges, devouring, in addition to its natural diet of fishes and other aquatic animals, the remains of the corpses of Hindus which are thrown into the river after an incomplete cremation owing to the paucity of firewood in certain regions. On this account it is greatly venerated by the Hindus.

[1] Cf. P. L. Simond, "Contribution à l'étude des Hématozoaires endoglobulaires des Reptiles," 'Ann. Inst. Pasteur,' vol. xv, 1901, see p. 347.

In all the adults of this species examined by Dr. Simond, more than twenty in number, he observed the pigmented stages of the Hæmatozoon, and, in addition, he found non-pigmented parasites of the Hæmogregarine type in constant association with the pigmented Hæmamœboids.

On this account, as well as from analogy and in view of the known fact that it is impossible to recognise sexual differences among true Hæmogregarines, whereas such differences are clear among the Hæmamœboids, Dr. Simond inclined to the opinion that the two forms of endoglobular parasites observed by him belonged to one cycle of development.

With regard to the life-history and mode of transmission of these parasites, the author points out that experiments in inoculation have hitherto proved futile, and the only method of dealing with them at present is the morphological method.

The scavenging activity of the tortoise Chitra indica[1] is of some interest in view of the remarks which we have made above and of some which are to follow.

Referring to Dr. Simond's discoveries, Professor E. A. Minchin makes the following comment in his work on the Sporozoa in Professor Lankester's 'Treatise on Zoology,' 1903, p. 270:

Hæmamœba metschnikovi "occurs as a minute pigmented endoglobular amœbula resembling the malarial parasites of birds and mammals. Its presence in a cold-blooded animal is, therefore, remarkable and quite exceptional. . . . Further investigations of this interesting form are required, and Laveran admits it only with some reserve to rank in his genus Hæmamœba."

In the blood of a tree-dwelling gecko, Hemidactylus leschenaultii, taken at the village of Mamadu, near Vavuniya, in the Northern Province of Ceylon in April, 1904, we observed a pigmented endoglobular parasite which did not appear to fall within the definition of any hitherto described

[1] This is the name by which the tortoise is designated by Mr. G. A. Boulenger in the volume on "Reptilia and Batrachia" in the 'Fauna of Brit. India,' 1890, p. 16.

genus of Hæmamœbidæ. We accordingly named it Hæmocystidium simondi.[1] No Hæmogregarine forms were found accompanying it. At the earliest stage observed it consists of a small irregular body with a belt of pigment granules across the centre, occasioning a slight displacement of the nucleus of the corpuscle.

With increase of size of the parasite the nucleus of the host-cell becomes pushed to one end of the corpuscle (Pl. 24, fig. 4).

The parasite was not observed in the fresh state, but in preparations stained by Leishmann's modification of Romanovsky's method two distinct kinds are found, resembling one another in size and form, but differing in their reactions to the stain.

These, no doubt, represent sexual differences, as in Halteridium. In the male type the body is seen to be faintly granular, the protoplasm is stained a delicate pale-blue, with numerous small pigment granules scattered round the periphery and in the substance of the oval or discoidal organism. In the female type the body is stained dark-blue, and the pigment granules are numerous, though appearing on the whole rather larger than in the male trophozoite. The female trophozoite differs further from the male by the constant presence of vacuoles, varying in number and size (Pl. 24, fig. 4).

Sometimes the contour of the Hæmocystidium makes an almost perfect circle, and in accordance with the shape of the blood-corpuscle it is probable that the parasite is not spherical but rather shaped like a biconvex lens. Its diameter nearly equals the shorter diameter of the corpuscle, while the elongated forms nearly fill the latter.

In one case of a double infection the growth of the two male trophozoites had caused a deep constriction of the blood-corpuscle, nearly cutting it in two.

The species described by Dr. Simond, which should now be

[1] Castellani and Willey, in 'Spolia Zeylanica,' vol. ii, 1904, p. 84.

named Hæmocystidium metschnikovi (Simond) resembles the parasite of the gecko in general features but differs markedly from it in point of size, rarely exceeding the half of the corpuscle in diameter, in the smaller number of pigment granules, and in the fact that it does not cause a displacement of the nucleus of the corpuscle, according to Dr. Simond's figures. Double or even treble infections of a corpuscle by H. metschnikovi do not affect the outline of the latter. It is, therefore, clearly a smaller species than H. simondi.

The natures of the hosts are also widely different, the one an aquatic chelonian, the other a terrestrial gecko living in the driest province of Ceylon.

The same species of gecko whose blood is infected with Hæmocystidium simondi harbours a nematode parasite in its intestine, which has been described by Dr. von Linstow as a new species of Oxyuris, O. megaloon.[1]

III. Hæmogregarine of Nicoria trijuga.

The hæmogregarines as a whole appear to favour foul-feeding animals, and there are few fouler feeders than Nicoria trijuga, a tortoise with amphibious habits commonly met with in the ditches and marshy lands round Colombo and in the Colombo Lake.

The larger specimens of this tortoise are generally found to be infected with a hæmogregarine which does not show any particularly striking properties distinguishing it from other similar species. It is, however, important to establish the fact of its almost constant occurrence in a host which derives its nourishment largely from offal.

The tortoise also harbours Nematode parasites in its intestine which have been kindly named and described by Dr. von Linstow as a new species of Oxysoma, O. falcatum.[2]

[1] The description of this species will shortly appear in 'Spolia Zeylanica.'

[2] For early publication in 'Spolia Zeylanica.'

When the blood is examined in the fresh condition, the crescent-shaped or reniform body of the endoglobular parasite presents a clear pole, one granular pole and a clear but sharply defined central tract, which in stained preparations proves to contain the nucleus.

Frequently the clear pole is directed towards the displaced nucleus of the blood-corpuscle, but there is no constant orientation.

The granular pole is the growing end of the organism, which becomes bent round upon itself in the manner characteristic of the genus Hæmogregarina.

The doubling of the parasite usually takes place by a very narrow bend, but occasionally a wider bight is produced. Young stages before the bending also came under our observation both in fresh and in stained preparations, and we have seen a double infection of a corpuscle, though this appears to be rare.

The nucleus consists of a more or less diffuse aggregation of chromatin or cyanophil granules which sometimes extend to the recurved limb of the parasite. The length of the bent organism is 0·01 mm. (10 μ). In one corpuscle the parasite had unbent itself and appeared as a long "vermicule," the corpuscle being enlarged and somewhat distorted.

In a hanging-drop prepared from the blood of a specimen which had been killed some hours previously we have once only observed a motile parasite free in the blood-plasma. It resembled the one which was found unrolled in the corpuscle. The movements consisted of slow revolutions in the direction of the arc of the parasite and also of movements of flexion. The granular pole was directed forwards, while the other pole remained more or less fixed and appeared to be adhesive. Finally, the parasite was attracted by an irresistible chemotaxis to a neighbouring phagocyte, by which it was gradually absorbed.

It may be noted here that we have hitherto not succeeded in finding any parasite in the blood of the herbivorous land tortoise of Ceylon, Testudo elegans.

The unrolling of the parasites would seem to precede its liberation from the corpuscle. This is obviously a critical moment in its life-history, but it is not known what happens next.

Occasionally the bent forms are seen free in the plasma, but this we attribute to accidental rupture or liquefaction of the corpuscle. When the parasite normally becomes free it is probable that the corpuscle undergoes disintegration.

We have named this species Hæmogregarina nicoriæ.[1] In the present stage of knowledge of the hæmogregarines the specific differences are largely a matter of host, or, in other words, of environment. All authorities seem to be agreed that there are different species, and that it would be wrong to call all hæmogregarines H. stepanovi, following the example of Halteridium, all the forms of which are called H. danilewskyi. It will be seen below that there are, so far as we can tell, extraordinary differences in the life-histories of hæmogregarines infesting different hosts.

The fact seems to be that the parasites are hard to distinguish in the immature, asexual, or hæmogregarine phase.

Finally, with regard to H. nicoriæ, it may be mentioned that although the bent parasite is not more than about one half the length and one third the width of the corpuscle, yet the nucleus of the latter is displaced towards one end of the cell.

In one or two cases the nucleus of the parasite has presented the appearance of having divided into four daughter nuclei, two of which occur at one end and two at the opposite end of the body. We have observed no further indications of sporulation.

IV. The Hæmogregarine of Tropidonotus piscator.

The freshwater snakes of the Colombo district seem to be frequently the unconscious hosts of a blood-parasite which in

[1] Castellani and Willey, op. cit., p. 85.

its endoglobular phase offers few characters of distinction. In snakes of this and other species obtained in places remote from Colombo we have searched in vain for Hæmatozoa, although Entozoa are common in all parts of the country.

A young snake of the above-named species, upwards of two feet long, was found to be infected with an endoglobular hæmogregarine of the usual type, all the trophozoites being approximately at the same stage of growth, more or less bent into a U shape. They show slight differences from Hæmogregarina nicoriæ. Their size is larger, 12 microns or ·012 mm. in length, the protoplasm stains uniform blue, the reddish-blue stained nucleus is denser, and placed near the pole opposite to the bent portion. The greater density of the nucleus is evidenced, not only by the closer aggregation of its chromatin material, but also by a greater resistance to the staining reagent; in many instances it has remained unstained. If the parasite is artificially liberated from the blood-corpuscle on the slide, its nucleus becomes well stained.

The parasites were kept fresh under observation in a hanging drop for several days, but no change was observed to take place.

It is not known what stimulus provokes the endoglobular hæmogregarines to enter upon a new course of development, and it appears that they may live for several months without undergoing much appreciable change within the blood-cells of their host.

It seems clear, however, that a point is reached eventually when something must happen, and a great change take place analogous to the change which Schaudinn had described in the life-history of Halteridium.

A few days after making the observations described above we were able to examine the blood of a large snake of the same species, nearly four feet in length. This examination revealed the presence of great numbers of hæmogregarines free in the blood-plasma in which they performed active movements, displacing the corpuscles as they glided along. Several hanging drops were prepared as usual, but on the

following morning the parasites had all undergone dissolution after the manner of Trypanosoma.

The movements consist of slow gliding and turning, the "vermicule" sometimes actually doubling upon itself. Sometimes it will become fixed by its attenuated hinder end, and will revolve by a slow screw-like motion after the manner of the spores of Sarcocystis. Then may ensue a rapid whirling of the body, causing a disturbance among the neighbouring corpuscles.

Many films of the snake's blood were prepared and stained, and in most of them we found that, besides the free parasites, many of the corpuscles contained bodies of relatively large size, more or less crescentic in shape. These endoglobular organisms were not at all like the ordinary hæmogregarines, but consisted of a delicate protoplasmic membrane with red-stained granules surrounding a pale-blue stained central body with a densely staining nucleus. The discovery of various stages of development within certain narrow limits enabled us to recognise the enveloping body as the mother-cell of the contained body, and we accordingly described it as a cytocyst.

The single organism or monozoite which the cytocyst produces eventually escapes from the membrane and from the corpuscle, very much as a young Nemertine worm escapes from its pilidium, and becomes the free motile parasite described above. We find the monozoites at all stages of emergence, and it is probable that some of the rapid oscillations of the parasite which were observed in the fresh preparations represented violent efforts to free their hinder extremities from the corpuscles.

The nucleus of the monozoite lies behind the centre of the body both before and after its birth. When fully formed within the cytocyst the hinder end of the monozoite is slightly bent, indicating that some pressure or tension is being exerted within. In the next stage the anterior end is found to have perforated the wall of the cytocyst, and the monozoite begins to push its way out through the opening thus produced. Here and there a corpuscle contains an empty cytocyst from which

the monozoite has escaped. In such cases the orifice of exit can still be discerned.

In some cases at the moment of fixation of the blood-film on the slide the monozoite had extended its body as far as the level of the nucleus, which appears constricted in the narrow orifice as if it were being squeezed through. Sometimes only the hinder end remains within the cytocyst and corpuscle, the rest of the body being free.

Occasionally, instead of emerging from the corpuscle the monozoite issues from its mother-cell again into the substance of the corpuscle. This is probably a miscarriage.

Sometimes the cytocyst is difficult to distinguish, and the monozoite appears to lie in the corpuscle without a sheath. In such cases the membrane can often be identified on close inspection, but not always.

The monozoites which emerge from the cytocysts are all of one kind and of one size within the limits of a slight variation.

Among the free-living forms in the plasma of the blood some are found to be about twice as bulky as others, indicating that they continue to grow after becoming free. The staining reactions of all are the same, namely, pale-blue cytoplasm and dense reddish-blue nucleus.

We have been fortunate enough to find stages in the formation of the monozoite within the cytocyst, the substance of the former being only partly differentiated and merging imperceptibly into the protoplasm of its mother-cell, the cytocyst. Such differentiation as has taken place is indicated by the pale-blue staining reaction, but chiefly by the structure of the nucleus, in which the chromatin loops or threads are clearly visible, showing unmistakable signs of formative activity.

With regard to the red-staining granules which we have mentioned above in association with the membrane of the cytocyst, we have interpreted these as belonging to a thin layer of residual protoplasm which is left round the periphery of the mother-cell after the formation of the axial monozoite. The membrane which is left empty within the corpuscle after

the birth of the monozite sometimes show a slight crumpling, due to collapse; it clearly belongs to the category of plasmatic membranes.

The provisional assumption of a direct genetic connection between the endoglobular hæmogregarines of the first snake and the cytocysts of the second is not supported by observation, but is based upon analogy, upon the specific identity of the hosts, and upon the proximity of the localities. If it is true, and if Simond's analogous assumption in the case of Hæmocystidium metschnikovi is also true, the life-history of the Hæmogregarines must be highly varied and complicated, and cannot be interpreted until some of the many gaps are filled up.

Another large Tropidonotus piscator was dug up, together with a clump of eggs, from a garden in Colombo, in February, 1905. Its blood was found to be in the same condition as in the previous specimen which was captured in July, 1904—that is to say, infested with cytocysts and free monozoites. No further information was obtained beyond a confirmation of results. The eggs contained young in an advanced stage of development, united to the yolk-sac by a narrow umbilical cord. The examination of the blood of these young incubants was negative in respect to Hæmatozoa.

Since the publication of our preliminary note we have become acquainted with a paper by N. Berestneff ("Über einen neuen Blutparasiten der indischen Frösche," 'Archiv. f. Protistenkunde,' vol. ii, 1903, pp. 343–348), in which a closely similar parasite was described from the blood of Indian frogs. The illustrations in the plate accompanying the paper were executed by the method of micro-photography, with the result that the points are not very clearly brought out. Micro-photography appears to have the advantage of absolute fidelity, counterbalanced by considerable obscurity. However, the text makes amends for the shortcomings of the plate.

Dr. Berestneff examined the blood of cold-blooded fresh-water vertebrates in the neighbourhood of a plague laboratory

and a leper asylum in Bombay. He found Trypanosoma rare in tortoises, no hæmatozoa in lizards, but only exceptionally did he find a frog free from blood-parasites. The Bombay frogs, of which he examined 372, belonged to two species—Rana tigrina and Rana limnocharis, both of which also occur in Ceylon, where, however, we have not yet established the presence of Hæmatozoa.

Besides Drepanidium monilis, Danilewskya krusei, and Trypanosoma, Dr. Berestneff found a parasite which he believed rightly had not been previously described. He gives numerical details, which we regret we cannot clearly understand. There is one table, from which it appears that out of the 372 frogs 47 contained the new parasite; but he mentions other frogs examined in June and July, 1900, which are not included in the table, so that we are unable to calculate the percentage of infected cases. Dr. Berestneff described the parasite as follows:—In the red blood-corpuscles there is to be seen a strongly refringent colourless capsule like a cylindrical tube, with one end enlarged in a club-shaped manner. The parasite lies within the capsule; the attenuated end of the capsule is empty, both ends are rounded and curved, the whole embracing the displaced nucleus of the corpuscle after the manner of Halteridium.

The author states that when the red corpuscle becomes disintegrated or dissolved the capsule with its contained parasite issues into the blood-plasma and straightens itself out, and under these conditions the parasite only occupies a portion of the capsule and eventually begins to move about inside the capsule, gliding from the wider portion into the narrower portion of the latter. Thereupon the motile parasite perforates the capsule at its narrower end and emerges into the plasma actively moving.

Dr. Berestneff says that the parasite closely resembles the free Danilewskya krusei. It is blunt at one end, acuminate at the other; it moves with the blunt wider end directed forwards and the nucleus lies near to the anterior end. The movements are much slower than those of the Drepanidia.

After a few minutes, while under observation, the movements cease, the parasite becomes hyaline, and finally dissolves in the plasma.

He observed simultaneous infection of a corpuscle by the new parasite and by a Drepanidium. In his modification of the Romanowsky method the nucleus of the parasite stained intensely reddish violet, the protoplasm blue, the capsule reddish-violet.

While encapsuled inside the corpuscle the acuminate posterior end of the parasite lies in the widened end of the capsule.

Dr. Berestneff says that the parasite which he discovered in the Bombay frogs belongs by its structure, form, and movements, to the Hæmogregarinidæ, and shows close affinity to Danilewskya krusei, Labbé (Drepanidium magnum = Hæmogregarina magna, Grassi and Feletti).

Attempts to transmit the parasite from infected to non-infected frogs by injection into the dorsal lymph sinus gave no result.

From the above description, which we have thought well to quote at some length, it seems certain that Dr. Berestneff's parasite differs at least specifically from that described by us, as shown, to mention only two points, by the relation of the monozoite to its capsule, and by the position of the nucleus. For facility of future reference we propose to name Berestneff's parasite Hæmogregarina berestneffi. It will probably not enjoy this name long, since the genus Hæmogregarina will most likely be found to have as little stability as Halteridium.

V. Trypanosoma and Halteridium.

In the light of the discoveries which Schaudinn has recorded these two parasitic forms should be considered together, although we have not yet found them associated in the same host.

Trypanosoma lewisi (Sav. Kent) is extremely common in the house rats (Mus decumanus) of Colombo. Some-

times the blood is literally seething with them, and it is known that they may equal or surpass the corpuscles of the blood in number. Like Hæmogregarina, the Trypanosoma is chiefly met with in domesticated or semi-domesticated animals. We have not found it in birds, but have met with it in several species of freshwater fishes occurring in the Colombo Lake, a sheet of water in the heart of the town. One of the principal hosts is the siluroid fish, Saccobranchus fossilis; most of the individuals of this species in the Colombo Lake, from seven to about twelve inches in length, appeared to be infected with a species of Trypanosoma, which we will name, in accordance with the method adopted by MM. Laveran and Mesnil, T. saccobranchi. Here, as in the rat, the parasites vary greatly in number in different individuals, from very rare to very numerous. In a fresh hanging drop the parasites appeared two or three in the field of the microscope; about two hours later those near the edges of the drop had begun to slow down, so that the movements of the undulating membrane could be observed. These consisted of a rapid shivering along one side of the body. The movements of the membrane determine the serpentine convolutions of the body, which are perpetual. Sometimes the body rests momentarily, only the flagellum and anterior portion keeping up their movements.

Four or five hours later bacteria had penetrated the entire drop, but in a portion of the latter where the bacteria were particularly dense the Trypanosomes had collected together, as many as twenty being visible at a time in the field of the microscope, a few only being found elsewhere in the drop.

In their recent monograph on 'Trypanosomes et Trypanosomiases,' Paris, 1904, MM. Laveran and Mesnil have shown how difficult, and in some cases impossible, it is to distinguish one species of Trypanosoma from another on morphological grounds. There is the greatest possible similarity of structure and proportions between the Trypanosoma of rats and those of fishes. Such points as the position

of the nucleus and the length of the flagellum can hardly be regarded as sufficiently constant to be diagnostic. The length of the tail or portion of the body behind the centrosome or blepharoplast is rather characteristic, but this, again, fluctuates.

It seems hardly worth while to attempt a detailed differentiation of species from different hosts until some method of culture can be devised which will reveal the true properties of each. There is at present no basis of comparison, and it seems that none is afforded by the mere retention of Trypanosoma under observation for several days in citrated blood.

In T. saccobranchi the caudal prolongation is obsolescent, the centrosome being placed very far backwards, much as in T. danilewskyi, Lav. Mesn., from Cyprinus carpio.

Dr. Lingard has recorded the presence of Trypanosoma in the blood of several species of freshwater fishes in India, namely, Trichogaster fasciatus, Ophiocephalus striatus, Macrones seenghala, and Macrones tengara.[1] He noted that the fishes that live in mud are more frequently infected than others, and that the parasites appeared in greatest number during the months of May and June.

We have not seen Dr. Lingard's original Report in which the above observations were published, but it is to be presumed that he obtained his material in Northern India, since only one of the above-named fishes ranges so far south as Ceylon, namely the Ophiocephalus striatus, known locally as the "lulla." The examination of the blood of one individual of this species from a tank at Mamadu, in the Northern Province of Ceylon, gave negative results. It would be interesting to examine the blood of a large number of specimens from different tanks, an investigation which, merely from the standpoint of distribution and environment, might yield important information.

With regard to Trypanosoma saccobranchi from the polluted Colombo Lake we have found it abundantly in the months of August and September. On the other hand, the

[1] Laveran et Mesnil, op. cit., 1904, p. 379.

examination of the blood of an Ophiocephalus striatus from the Colombo Lake in May, 1905, gave negative results.

We have also found Trypanosoma in the blood of Macrones cavasius (Siluridæ) and in Gobius giuris.

As a mere matter of fact we may mention that we have never observed Trypanosoma associated with endoglobular parasites in the same host, nor have we found Trypanosoma in any species of animals in which we have found endoglobular forms.

Dr. Hanna has described a Trypanosoma in the blood of the Indian Crow (Corvus splendens), and we have seen Halteridium in this species as well as in the Black Crow (Corvus macrorhynchus), both examined in the month of August, also, as mentioned above, in the common babbler, Crateropus striatus, examined in July, and in the common Scops owl, Scops bakkamœna, var. malabaricus in July. All these hosts are more or less open to the charge of foul-feeding, and all frequent the neighbourhood, sometimes even the precincts, of human habitations.

MM. Laveran and Mesnil have described Trypanosoma soleæ, from Solea vulgaris, in association with Hæmogregarina simondi.[1]

VI. Filaria.

Besides Filaria immitis in the dog and Filaria vivipara, v. Linstow, in the Indian crow, we found a Filaria in the blood of the Brahminy lizard, Mabuia carinata, and described it in our preliminary note under the name of Filaria mansoni.[2] Dr. von Linstow has since pointed out, in a paper which will shortly be published in 'Spolia Zeylanica,' that this name had already been applied by Cobbold in 1880 to a species of Filaria from the orbit of Gallus gallinaceus, and he accordingly proposes the new name Filaria tuberosa, so that the species now reads F. man-

[1] Laveran et Mesnil. 'Trypanosomes et Trypanosomiases,' Paris, 1904, p. 389, and 'C. R. Ac. Sci. Paris,' cxxxiii, Oct. 14th, 1901.

[2] 'Spolia Zeylanica,' Part 6, 1904, pp. 79, 80.

soni, Castellani and Willey (not Cobbold) = F. tuberosa, v. Linstow. Two adult females were found imbedded in the musculature of the body-wall, one in the ventro-lateral abdominal region, showing through the peritoneum, the other in the dorsal wall of the body cavity.[1]

The blood-filariæ of Sauropsida are the embryos of adult Nematoda, which are to be found in the peritoneum of the same host which harbours them. We have never found embryos in the blood without adults in the peritoneum; the females are always ovoviviparous and the males appear to be rare. Both in the Indian crow and in the Brahminy lizard only females were found.

Filariæ occurred also in the blood of a lizard, Calotes versicolor, examined in August, 1904. These were about six times the length of a blood-corpuscle and offered no very striking characters. Two adults, a smaller male and a female about three times larger, were found in the mesentery below the aorta at the level of the testes. Both of them were characterised by the bright lemon-yellow colour of the intestine, and we accordingly proposed the name Filaria flavescens, subsequently forwarding the specimens to Dr. von Linstow, who was good enough to confirm the identification, adding further details, which will be duly published.

In the blood of a Scops owl (Scops bakkamœna, var. malabarica) which died in Colombo in September, 1904, numerous filariæ of an unusually large size occurred, measuring 0·22 mm. in length, the anterior end rounded and sometimes, in the dried film, somewhat withdrawn from the cuticle, the tail acuminate. We name this Filaria scopsiana. Three adults were found in the peritoneum, the largest of which measured 13·25 mm. in length, the smallest less than half this size. The œsophagus was rather indistinct, but appeared to measure between $\frac{1}{10}$th and $\frac{1}{12}$th of the total length.

[1] In front of the caudal tuberosity which we described and figured there was an appearance of a vent, but Dr. v. Linstow says there is no anus. Instead he describes in front of the swelling a pair of papillæ.

EXPLANATION OF PLATE 24.

Fig. 1.—Non-pigmented parasite ("pseudovermicule") in the blood of a fever case from the Police Hospital, Colombo [malarial parasites absent; vidal reaction negative]. April 24th, 1905.

Fig. 2.—Another parasite of the same kind; the two dark spots are chromatoid granules. In this case a few ring-forms of tropical malaria were observed, but no crescents. Feb. 17th, 1905.

Fig. 3.—A third example of the same parasite. Stained by Jenner's method, dark blue near the terminal vacuoles, paler blue in the centre. March 16th, 1905.

Fig. 4.—Analogous "pseudovermicules" in the blood of a common babbler, Crateropus striatus. Sometimes the free parasite appears to be surrounded by a clear halo. Two blood-corpuscles are shown with Halteridium. July 21st, 1904.

Fig. 5.—"Pseudovermicules" in the blood of the Indian crow, Corvus splendens. Colombo, Aug. 9th, 1904.

Fig. 6.—Hæmocystidium simondi, Castellani and Willey, in the blood of Hemidactylus leschenaulti.

Fig. 7.—Hæmogregarina nicoriæ, from Nicoria trijuga; one of the corpuscles shows a double infection.

Fig. 8.—Hæmogregarina mirabilis, Castellani and Willey, from Tropidonotus piscator. (*a*) Normal corpuscle; (*b*) the pale blue monozoite inside its red sheath; (*c*) monozoite commencing to issue from the sheath (cytocyst) and corpuscle; (*d*) monozoite still further emergent, and in this case taking a reddish tinge as though it were coated with a thin film of mucus or residual protoplasm; (*e*) monozoite nearly free; (*f*) monozoite free; (*g*) corpuscle containing an empty cytocyst from which the monozoite has been discharged.

Stained by Leishmann's modification of Romanowsky's method.

Fig. 9.—Trypanosoma saccobranchi n. sp. from Saccobranchus fossilis.

Figs. 10 and 11.—Two embryos of Filaria tuberosa from the blood of Mabuia carinata. The dried blood-film was fixed in absolute alcohol and stained with hæmatoxylin and eosin. The body of the organism is seen to be contracted within its cuticular sheath.

Fig. 12.—Embryo of Filaria flavescens n. sp., from the blood of Calotes versicolor.

Fig. 13.—Embryo of Filaria scopsiana n. sp., from the blood of Scops bakkamœna var. malabarica (Colombo).

The Gastrulation of the Vertebrates.[1]

By

A. A. W. Hubrecht.

On p. 945 of the first volume of his 'Handbuch der Entwickelungslehre' Oscar Hertwig refers (in a postscript to his theory of the germinal layers) to a theoretical view concerning the process of gastrulation in mammals (and also in vertebrates generally) which I have attempted to establish in my article on 'Keimblattbildung und Furchung bei Tarsius spectrum' (1902, Verhandel. Kön. Akad. v. Wetensch. Amsterdam). He points out that I have "considerably changed my views," and that I have "now been induced to look upon matters in a way which differs considerably from what he (Hertwig) and many other embryologists are ready to uphold."

This paper is meant to establish that the "divergence" of views which Hertwig accentuates will, in all probability, be only a temporary one. An explanation, or rather a rational interpretation, of what we understand by gastrulation as it has been introduced into science by Haeckel and Ray

[1] Since this article was first published in German in the 'Anat. Anzeiger,' vol. xxvi, p. 353, several months have elapsed, and papers have appeared in that same periodical by Assheton (vol. xxvii, p. 167), and by Brachet (vol. xxvii, p. 212) concerning the same subject and referring to the original article. I must refrain from entering into any discussion in this translation, but will do so on another occasion. I only wish to point out that Assheton has quite misunderstood my German version in so far as he believes that I hold the Vertebrate mouth to be in any way derived from the stomodæum of an Actinia-like animal. My article "Keimblattbildung bei Tarsius," cited above, leaves no doubt on this head.

Lankester can only contribute to eliminate certain difficulties which ever and again present themselves when the different stages of the ontogeny of vertebrates are analysed and compared.

Turning back to the very earliest publications in which Haeckel and Ray Lankester have formulated their ideas, we are immediately struck by a difference in their views which has only too often been undervalued, and which concerns the earliest phylogenetic origin of the two-layered embryonic stage. Haeckel looks upon the process of invagination, Ray Lankester upon that of delamination, as the more primitive. I must confess that to me Ray Lankester's view is more sympathetic, because I find it easier to imagine that a one-layered, hollow, spherical blastula—the starting-point of the Metazoa—has, by division of labour, become two-layered in consequence of the differentiation in each cell of an ectodermal half (principally sensitive and integumentary) and an entodermal portion (principally digestive) than that I could be satisfied with the proposition that the two halves of the hollow sphere have prepared themselves (independently of the natural process of division of labour above noted) to bring about an invagination. The result of this invagination will, then, also be a defensive layer (ectoderm) which encloses a digestive layer (entoderm), both together constituting a two-layered stage that has originated by invagination, with an opening, the primitive mouth (Urmund).

A two-layered gastrula is thus evolved out of a one-layered blastula either by delamination[1] or by invagination. In the second case the primitive mouth is the natural consequence of the process of invagination itself: in the first case an opening has to become established at a given point in the course of time, to which Ray Lankester[2] has conferred, in 1875, the name of "blastopore" (opening, that is, present in the blastoderm).

As a matter of course the names "blastopore" and "primitive

[1] In this case the blastocoel gives rise quite naturally to the archenteron.

[2] "On the Invaginate Planula," etc., 'Quart. Journ. of Micr. Sci.,' vol. xv, 1875, p. 163.

mouth" have been looked upon from the beginning as synonyms, the more so as in the phylum of the Cœlenterates (Polyps, Medusæ, Corals, Sea Anemones, etc.), in which we find duplication of the layers by delamination in one genus, by invagination in others, the primitive mouth or blastopore gradually becomes the so-called mouth of the Medusa or Hydra, or the oral slit of the full-grown Coral or Anemone.

It should at the same time be noticed that the oral slit of the Anemone (Actinia) undergoes a further complication by the formation of a stomodæum. In consequence of the formation of this ectodermal involution, below the level of the oral disk, the primitive mouth is displaced to the lower

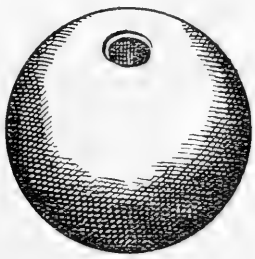

FIG. 1.—A gastrula with open blastopore.

level at which the stomodæum passes into the entoderm. It is all the more important to keep this in view as this complication of the full-grown Cœlenterate has its echo in the phylogeny of the higher animals. We thus find occasion to insist, already now, on the inadequateness of confusing the oral slit of an Actinia with a primitive mouth.

We shall be able to approach the question of gastrulation, as also that of the formation of the germ-layers in Vertebrates, with a more open mind if we can provisionally disinterest ourselves in the question as to whether delamination or invagination was the more primitive process by which a one-layered blastula became converted into a two-layered gastrula. Those who hold with Haeckel that invagination has been the prototype of all gastrulation are biassed and for

them even the definition of what gastrulation really is cannot then be free from prejudice. Such a definition should be applicable both to Invertebrates and to Vertebrates, and should formulate the origin of the two-layered out of the one-layered stage.

When we look round for the definition which the most trustworthy investigators have given of the gastrulation of the Vertebrates we immediately observe how heavy a penalty has been incurred by not leaving the crucial question as to whether delamination or invagination has been the origin of the didermic stage of the vertebrate blastocyst provisionally unanswered. As a rule gastrulation and invagination have been looked upon as synonymous.

All this can be fully appreciated when we open Keibel's very complete and most conscientious 'Referat' on "Gastrulation und Keimblattbildung der Wirbelthiere" in Vol. X of the Ergebnisse der Anatomie und Entwickelungsgeschichte (1901). On p. 1111 we read:

"Three definitions of gastrulation stand out conspicuously when among the number that have been given we eliminate certain erroneous elements.

"The first would be: Gastrulation is a process during which the cells that will form the intestinal lining find their way into the interior of the embryo.

"The second: Gastrulation is the process during which the material for notochord and mesoderm finds its way into the interior of the embryo.

"The third one, given above, would be: Gastrulation is the process by which the material for entoderm, mesoderm, and notochord finds its way into the interior of the embryo."

The citation here given shows clearly that the definitions 2 and 3 cannot hold good for the Invertebrata, because the latter do not possess a notochord. The conception of gastrulation is, however, only then of importance when by it we are enabled to hold in one grasp developmental phenomena from Hydra up to man.

Keibel, who rejects 1 and 2 and accepts 3, tries to circumvent

this difficulty by emphatically stating (loc. cit., p. 1113) that he refers to gastrulation in Vertebrates, etc. He appears not to have sufficiently realised that by this limitation to the Vertebrates the important generalisation that in the ontogenesis of all Metazoa a didermic phase occurs, which we term "gastrula," is mutilated and loses its value as a generalisation.

As to the second definition Keibel rejects it definitely; but with respect to the first he feels inclined to be more lenient. He holds this definition to be quite acceptable for a zoologist who takes into account all the existing Metazoa. This first

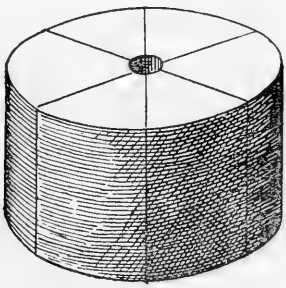

Fig. 2.—A radially symmetrical actinia-like Cœlenterate.

definition, which was especially patronised by Lwoff (who has given the first energetic impulse towards the clarifying of the conception of gastrulation)[1] suffers, however, by the fact that here again Haeckel's view concerning the primary significance of invagination is tacitly admitted.[2] Gastrulation is said to be the process during which the in-

[1] Lwoff, "Die Bildung der primären Keimblätter und die Entstehung der Chorda," etc., 'Bull Soc. imp. der naturalistes de Moscou,' 1894.

[2] I must emphatically assert that the reproach of having included the invagination process in the definition of gastrulation does not apply to Keibel himself, however much this might seem to be the case if we consider his definition No. 1. He has, however, on pp. 1109–1110 of the 'Referat' above alluded to, most distinctly stated that he wished this definition to apply to a delamination-gastrula as well.

testinal entoderm finds its way into the interior of the embryo. This implies that the intestinal epithelium here referred to is originally situated elsewhere, and must now undergo a transportation by which it is transferred to the interior. This petitio principii cannot be allowed to stand, and the definition should be formulated so as to cover delamination as well. It ought, then, to be as follows:

Gastrulation is a process during which an intestinal entoderm is differentiated as against an integumentary ectoderm, and by which a monodermic blastos is changed into a didermic.

This definition is applicable to Invertebrates as well as to Vertebrates.[1]

We should, however, be very strict in not allowing our conception of gastrulation to be further extended to ulterior processes that give rise to different organs, and during which an undeniable invagination takes place. For that very reason those processes have up to now been erroneously looked upon as gastrulation. By having persisted in considering gastrulation to be necessarily linked to invagination, the actual purpose of gastrulation, viz. bringing about the didermic stage, has been thrust into the background, and undue weight has been attached to the invaginating process.

We would, however, be quite as little justified to look upon the origin of the medullary canal or of the lens and the auditory vesicle by local invaginations as a further continued gastrulation as we are when we assert that the process by which the notochord and the mesoblastic somites are brought about is a gastrulation-process.

During this process true invagination, which has even been

[1] In Haeckel's 'Anthropogenie' (4th ed., 1891, p. 156), which may also be considered as decisive on this question, we read: "[The cleavage cells] arrange themselves in two separate layers, the two primary germinal layers. These surround a digestive cavity, the 'Urdarm' (primitive enteron), with an opening, the 'Urmund' (primitive mouth). The important embryonic stage which possesses these oldest primitive organs we call the gastrula, the process by which it originates gastrulation."

cinematographed by Kopsch, undeniably takes place, and is all the more misleading because in Amphioxus it is in direct continuity with the invagination of the gastrula.

However, when we inquire whether the invagination here alluded to contributes towards the origin of the didermic phase of development, viz. towards what we have termed gastrulation, our answer must be emphatically negative. Gastrulation, i. e., the duplication of the germinal layers, is terminated before the commencement of the process of in-

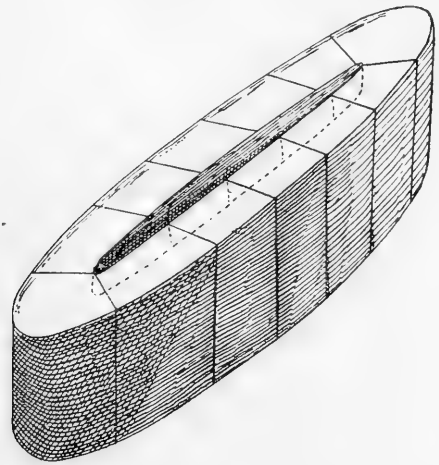

Fig. 3.—A bilaterally symmetrical, elongated, vermiform, actinia-like animal with stomodæum and enteric compartments.

vagination, by which the bilateral, symmetrical and metamerical vertebrate is completed. This process of invagination can be looked upon as a kind of budding (notogenesis), which is consecutive upon the formation of the didermic apical portion (cephalogenesis). It gives rise to the trunk.

Moreover the process by which the didermic stage is reached in the higher vertebrates, more especially in mammals, can be traced with such perfect clearness, that we may wonder that in the face of these facts the invagination hypothesis has been so long upheld. The entoderm in mammals makes its appearance by a very striking delamina-

tion out of a complex of cells which is comparable to the monodermic blastula. And similarly in Sauropsids and Elasmobranchs delamination is the process by which entoderm and ectoderm are respectively differentiated from one another. This takes place in a horizontal plane in consequence of the accumulation of yolk substance. In Amphibia, Cyclostomes, many Ganoids and Dipnoi, which have been looked upon as important supports for the view that gastrulation is effected by invagination, it has appeared more and more evident to successive investigators (Bellonci, Graham Kerr, Brauer, etc.) that here, too, the primary separation between ectoderm and entoderm is brought about by a process of delamination. As soon as the so-called blastopore (Rusconian anus) appears, which travels a certain distance over the surface of the egg, and which, in many cases, is turned into the definite anus, we have no longer before us the gastrulation process, but a process by which the metamerical and bilaterally symmetrical dorsal organs and the notochord are budded into existence.

Thus the naked facts force the conclusion upon us that in the Acrania (Amphioxus) the gastrula arises by invagination, in the Craniata (all other Vertebrates) by delamination. Once the didermic gastrula-stage reached, a second phase of ontogenetic development is inaugurated which is also of high phylogenetic importance. In this phase the bilaterally symmetric metameric animal gradually appears which we have to compare with possible phylogenetic transition forms that have connected the Vertebrates with radially symmetrical ancestors. This attempt at a plausible and rational reconstruction of the Vertebrate ancestry is, of course, hampered by the circumstance that no trace of those forms is any longer in existence. Still, an actinia-like, vermiform being, elongated in the direction of the mouth-slit, imposes itself upon our imagination, such as has served for the theoretical speculations of Sedgwick on this same subject, and has once been accepted by van Beneden for the precursors of the Chordata.

We have already above considered that the processes of growth by which a Cœlenterate gastrula becomes fixed and

gradually changes into a sessile Actinian can hardly be looked upon as protracted phases of gastrulation. This will be more difficult yet when the animal has already acquired a higher degree of complication than that of the Cœlenterates, and swims about in the shape of a worm-like, lower chordate animal. We know of Polygordius and of other primitive worm types that to the radial, didermic larval stage—the Trochophora—another developmental phase succeeds, during which we observe proliferation in the anal region, leading to an increase in the distance between the anus and the apex of

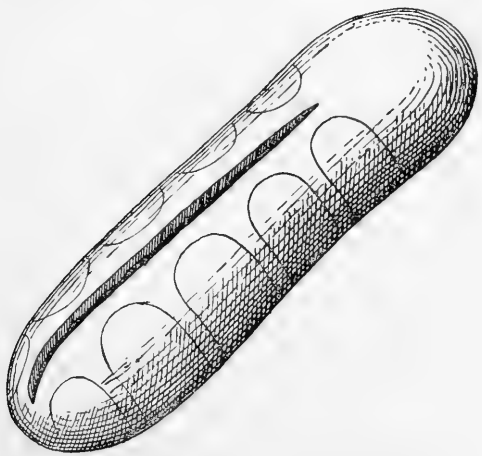

FIG. 4.—A vermiform protochordate with earliest differentiation of head, trunk, notochord, and incipient metamerism.

the metamerical worm, the latter budding off, so to say, from the radial trochophora.

We find similar processes in the Vertebrates, but without a free trochophora larva, and to this latter radial and didermic primitive stage corresponds in the Craniata the rapidly passing earliest phase in which delamination calls forth two germinal layers. Both in Elasmobranchs and in mammals we notice that the cellular material which is present in those very earliest stages contributes especially—as it does in the trochophora—towards the formation of the anterior part, the head, and that, following upon this, a proliferation-process is in-

augurated (comparable to the origin of the metamerical worm out of the trochophora larva) by which the notochord and the somites, i. e. the bilaterally symmetrical metameric animal are called into existence.

And so this latter process must necessarily correspond—if we go back into phylogeny only far enough—to the transition of the gastrula larva into the longitudinally stretched Actinia-like animal. We have already demonstrated above that it is irrational to continue to use the term "gastrulation" for this phase of development. The cœlomic diverticula of the Actinia that are yet in continuity with the intestine are the predecessors of the somites, the nerve-ring on the oral disk that of the medulla, the stomodæum stands for the notochord and the oval slit of the Actinia (no primitive mouth or blastopore!) is reflected in the primitive groove which is in so natural a continuity with the notochord.

In a former publication[1] I have distinguished simultaneously with Keibel,[2] but independently of him, two phases in the gastrulation process, of which the second was erroneously termed "the palingenetic phase of gastrulation." It is identical with the second process above described, which has only certain superficial analogies with gastrulation by invagination, and which, nevertheless, is bound up quite as closely to the real gastrulation as is the growth of the elongated Actinia to that of the gastrula-larva.

The primitive mouth of the Actinia-gastrula is gradually elongated into the mouth-slit of the Actinia, which in its turn leads into the stomodæum. The blastoporus of the Erinaceus-gastrula, which very soon closes up again, lengthens out posteriorly in the primitive groove, the floor of which—the primitive streak—produces the material for the notochord.

There is, then, during ontogeny an unbroken continuity between the blastopore of the Actinian and its oral slit,

[1] 'Anat. Anzeiger,' iii, 1888, p. 911; 'Quart. Journ. of Micr. Science,' vol. xxxi, 1890, p. 552.

[2] "Zur Entwickelungsgeschichte der Chorda, etc.," 'Archiv f. Anat. u. Physiol., Anat. Abth.,' 1889, p. 376.

between the blastopore of the Vertebrate (often only potential in mammals[1] and not identical with the opening that is called by that name in Sauropsids) and its primitive groove. A phylogenetic continuity has to be statuated between this oral slit of the Actinia and the peculiar spot (behind the so-called anterior lip of the blastopore) which on the Vertebrate embryonic shield gradually moves backwards and establishes in many cases an open communication between a portion of the Vertebrate intestine and the exterior. The primitive streak, however, the solid material that proliferates downwards from the ectoderm, coalesces with the entoderm, and brings forth the notochord from its median (though really paired) portion and the somites from its lateral wings—this

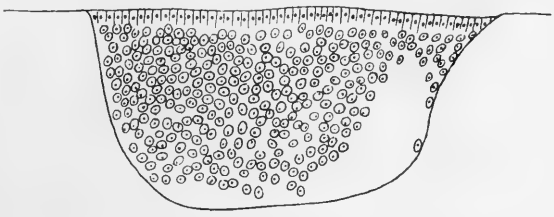

Fig. 5.—Gastrula stage of Torpedo.

primitive streak can never be identified with a blastopore or primitive mouth, neither with the lips of the blastopore. For we have above attempted to demonstrate that in this primitive streak we encounter the material which, also in the Actinia, (1) proliferates downwards from the ectoderm and produces the stomodæum, (2) coalesces with the entoderm, (3) is in direct continuity with those parts which are preparing to give rise to cœlomic pouches but are yet continuous with the primitive enteron.

Certain prudent changes of nomenclature would perhaps

[1] An open blastopore has up to now only rarely been noticed in Mammals. In Erinaceus I have observed it quite clearly (' Furchung und Keimblattbildung bei Tarsius,' Pl. XII, figs. 8, 9). Keibel (l.c. Taf. 24, figs. 46, 47) is less positive as far as the rabbit goes, so are Selenka (Taf. 17 and 18) for the opossum, and Bonnet (Anat. Hefte, Bd. 9, Taf. 32) for the dog.

recommend themselves. For that portion of the vertebrate embryonic disk which I have proposed to compare to the Cœlenterate mouth-slit and stomodæum, the name of dorsal mouth might be chosen. The difference between the phyla of Annelids and Molluscs as compared to Vertebrates is thereby all the better marked.

Provisionally we are not yet enabled to enter into a detailed comparison between ontogenetic and phylogenetic processes. The possibility of instituting such comparisons will probably have disappeared in the graves of the numerous transition forms that now rest among the fossils. But the chief outlines of the evolutionary process of notochord and somites stand out boldly enough and correspond to what Sedgwick has first hinted at and what van Beneden has later extended but never fully worked out. At the same time not only the Diplo- and Hemichordata but also the Cephalochordata are relegated to a more modest lateral situation in the pedigree of Vertebrates.

The distinction between the head-segment of Polygordius and the trunk of the same animal is thus of the same order and belongs to the same category as that between the very foremost portion of the body of a Vertebrate and the segments that extend behind it. In an earlier publication[1] I have accentuated this distinction by the use of the terms "cephalogenesis" and "notogenesis." I wish to adhere to this, and yet to observe that the distinction here intended between "kephale" and "notos" is not identical with that between head and trunk (trunk-segments having been ascertained to enter into the composition of the head), but that on one side should be ranged the very foremost portion of the head to which the ophthalmic and optic nerves belong, whereas on the other we place the further subdivisions of the brain with their cephalic nerves, as also the basis of the skull with the remains of the notochord it contains, the visceral arches and the whole of the trunk.

[1] 'Furchung und Keimblattbildung bei Tarsius,' Amsterdam, 1902, K. Akademie v. Wetensch.

We are in no way justified in including the whole process of the formation of the notochord in the gastrulation phenomenon, nor either that of the cœlom and the somites. In Echinoderms the formation of enterocœl, hydrocœl, etc., is only inaugurated after gastrulation has been completed; in worms the appearance of the somites is synchronic with the increase in length which has been noted above and which follows upon gastrulation. How the process of cœlomogenesis in the higher vertebrates is derived from that of their invertebrate ancestors (which are unknown to us) cannot for the present be said to be sufficiently elucidated. Certain peculiarities in the development of Balanoglossus should here be considered,

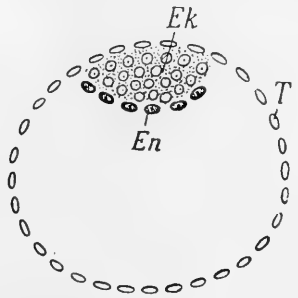

FIG. 6.—Gastrula stage of a Mammal. *T*, Trophoblast. *Ek*, Ectoderm. *En*, Entoderm.

and may to some extent be comparable with what occurs in Vertebrates, but up to now we have not sufficient data. Bateson has shown that Balanoglossus possesses an unpaired anterior cœlomic sac (cavity of the gland), that paired sacs of collar-cœlom follow upon this and that an unpaired hindermost cœlom provides the trunk and soon becomes paired.[1]

To explain the fact that the mutual relation between the earlier gastrulation phases of vertebrates and their later pro-

[1] Whether these arrangements are repeated in higher Vertebrates has not yet been decisively shown. It should, however, be noticed that the mode of formation of the pericardium which was two years ago described by me for Tarsius (l. c., p. 3, Pl. VIII, fig. 70 *a*, *b*), is in no way isolated, but has since been also observed by me in Sciurus and Tupaja. For Tupaja the gradual transition of an evagination of the entoderm that becomes constricted off into the

cesses of growth and organisation have been so hopelessly mixed up and misunderstood, we have to keep in view that an exaggerated importance has been ascribed to Amphioxus.

I wish to insist upon this somewhat more fully. It is quite natural that, in the literature of the preceding century, a most particular and isolated position has been allotted to Amphioxus, as the very lowest fish-like being in the system of the Vertebrates, and we can neither wonder that in the second half of that century, in which the theory of evolution made its way, this position has considerably increased in importance, and that there arose a tendency to look upon Amphioxus as the real ancestral Vertebrate, out of which all the other fishes and Vertebrates had originated. Haeckel has from the first accepted this view in his phylogenetic papers, and when then Kowalewsky had elucidated in such a masterly manner the ontogenesis of Amphioxus and of the Ascidians, the theory of the descent of the Vertebrates out of Invertebrates viâ the Ascidians and Amphioxus seemed to be definitely settled. This, however, was not the case, but then the beautiful and detailed researches of Hatschek on the development of the organs of Amphioxus have shown this animal to be such a perfect model of clear histogenesis in its early development, that it was only natural that all subsequent observers, who occupied themselves with the embryonic development of higher Vertebrates, should have attempted to start from the data furnished by Amphioxus.

anterior median portion of the pericardium is demonstrated in more than one preparation. In Sciurus it has not been followed out in full, but an early stage was noticed. Bats, too, seem promising in this respect. At all events, if this should be further confirmed, our whole interpretation of the vertebrate cœlom would have to be recast. That in such a case Balanoglossus among Invertebrates would have to be considered as an object of comparison would not be astonishing if we remember how a certain comparability between the branchial arrangement of Balanoglossus and Amphioxus has long been known. The so-called notochord of Balanoglossus I would be inclined with Spengel to regard as a delusion. Gegenbaur in the latest edition of his comparative anatomy (Bd. 1, p. 185) has allotted to Rhabdopleura, which is related to Balanoglossus, a decided significance in the pedigree of Vertebrates.

This position as a central and archaic form in Vertebrate phylogeny, confirmed as it seemed by embryology, is decidedly a usurped position.

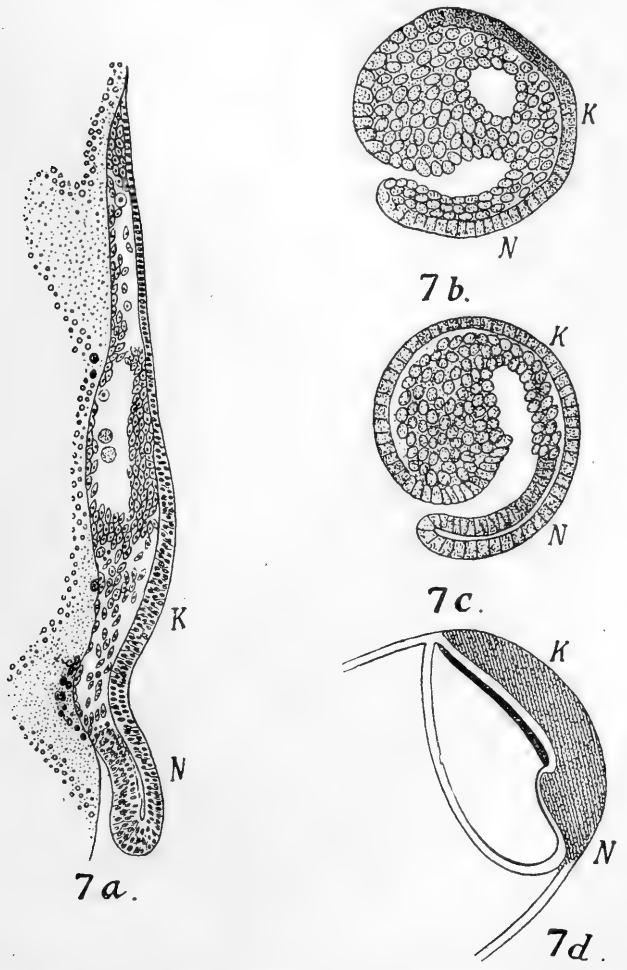

Fig. 7.—Earliest stages of cephalogenesis (K) and notogenesis (N) in longitudinal median sections. *a*, Elasmobranchs (Torpedo, after Rückert). *b* and *c* Cyclostomes (after Götte), two successive stages of Petromyzon. *d*, Mammals (Tarsius). 'Akad. v. Wetensch.,' Amsterdam, vol. viii, No. 6, 1902.

For as soon as we also consider the anatomy of Amphioxus

it becomes evident that the Craniota have not sprung from this acraniate animal; that the relation between the anterior extremity of the notochord and the brain must have been different in the real ancestral Vertebrates; that eyes and static organs (otoliths) cannot have been absent in those ancestors as they are in Amphioxus—in short, that Dohrn was quite right when he proclaimed Amphioxus to be a distantly related, partly degenerated, relict, which could not furnish us with important conclusions concerning the phylogeny of Vertebrates.

We find in Amphioxus when we try to compare it with the higher Vertebrates the same difficulties which we encounter when comparing the Cyclostomes with the other fishes or the Ornithodelphia with the higher mammals. All three are representatives of old and early stems that have branched off sideways at a very early stage, and that thus represent lateral lines of development. They have no considerable significance for comparative phylogeny, and may on no account be looked upon as transitional forms that occupy a place on the line of descent between the Cœlenterates and the Primates.

Once we have fixed upon this position for Amphioxus in the system, there will be no difficulty in looking upon the subdivisions Acrania and Craniota as being equivalent to a new subdivision, Invaginata and Delaminata. The process of invagination, which is noticed in so many Vertebrates as accompanying the origin of the notochord and the somites, will not, then, any longer be indicated by the name gastrulation.

Together with this the significance of the primitive streak as coalesced lips of the blastopore will have to be abandoned; the streak and the groove represent the oral slit of the Actinia with its dependent stomodæum, which, when compressed into the notochord, would thus be homologous to that organ in Vertebrates.

I feel very hopeful that on this basis a mutual understanding will soon be arrived at, by which many embryological results which up to now seem hopelessly confused will fall into line.

The numerous and important contributions to Vertebrate embryology which we owe to so many contemporary investigators, who for the present yet adhere to earlier theoretical propositions, retain their full importance as far as the actual observations are concerned. The incorporation of those observations in the new theoretical interpretations will come about by itself.

The Gastrulation Question.

By

Franz Keibel.

In consequence of the frequent discussions that took place in the course of the summer between Professor Hubrecht and myself concerning the gastrulation of the Vertebrates, he kindly sent me his paper on this subject for inspection before its publication. I would like to add a short explanation to his argumentation.

(1) Hubrecht is undoubtedly justified in demanding that the definition of gastrulation should be so formulated that it can apply to all the Metazoa. I have already acknowledged that this was admissible in my paper: "Ueber die Gastrulation und die Keimblattbildung der Wirbelthiere," p. 1112, with special reference to Lwoff,[1] but then, starting from Amphioxus, I gave a definition which could only apply to Vertebrates (p. 1113). I now hold that this compromise with the current views is no longer justified, and on this point I entirely agree with Hubrecht.

(2) I am, further, in perfect agreement with Hubrecht that in the definition of the phenomenon of gastrulation the pro-

[1] We read there: "Dagegen ist die Definition, dass die Gastrulation ein Vorgang ist, bei dem die dem Darm oder genauer das Darmepithel bildenden Zellen, in das Innere des Eies gelangen nicht so ohne Wieteres abzulehnen. Lwoff der diese Definition vertritt, ist durchaus consequent und da wir bei den Wirbellosen Organismen kennen welche dem Mesoderm und der Chorda der Wirbelthiere ohne weiteres zu vergleichende Bildungen nicht besitzen, ist seine Definition vom Standpunkt der die Gesammtheit der Metazoa betrachtenden Zoologen durchaus berechtigt."

cess of invagination should in no way be involved. My arguments have been ventilated before (l. c.,[1] p. 1109 and 1110), and I had come to the conclusion that in formulating the phenomenon of gastrulation the final result should be kept in view, and the special mode in which it is brought about should not be considered. I would then define as follows: Gastrulation is the process by which the cells of the Metazoan embryo are differentiated into ecto- and entoderm. By entoderm should only be meant those cells that will form the gut.[2]

(3) Hubrecht is, in my opinion, also justified—and it is a logical consequence of the definition—in emphasising that the primitive streak of the Amniota cannot be homologous with the primitive mouth (Urmund) or with the border of the blastopore. The primitive streak is a formation which has close and important connections with the blastopore, but which may not be without reservation homologised with it.

(4) The difference which Hubrecht establishes between kephalogenesis and notogenesis should decidedly be acknowledged. The publications of Kopsch[3] (1896) and Jablonowski[4] (1898), to which I have already referred in a similar sense in 1901 (l. c., p. 1055–1061) should here be kept in view, and it should be specially insisted upon that the formation of the primitive streak stands in intimate relation to the phenomenon of notogenesis, and should thus for this

[1] Keibel, F., "Die Gastrulation und die Keimblattbildung der Wirbelthiere," 'Ergebnisse der Anatomie und Entwickelungsgeschichte,' Bd. x, 1900. Wiesbaden, 1901.

[2] In his article "On Growth-centres in Vertebrate Embryos," 'Anat. Anz.,' T. xxvii, p. 167, Assheton has misunderstood the sense of my definition of gastrulation, as will be found out by what I have said here. I do not think it necessary to treat the question with more detail in this paper.

[3] F. Kopsch, "Experimentelle Untersuchungen über den Keimhautrand der Salmoniden," 'Verhandl. Anat. Gesellsch.,' 1898.

[4] Jablonowski, J., "Ueber einige Vorgänge in der Entwickelung des Salmonidenembryos nebst Bemerkungen über ihre Bedeutung für die Beurtheilung der Bildung des Wirbelthierkörpers," 'Anat. Anzeiger,' Bd. 14 pp. 532–57.

additional reason not be homologised unreasonably with the blastopore.

(5) I agree with Hubrecht in this, that I look upon Amphioxus as a form which occupies a position far away from the direct line of descent of the Vertebrates. I would, however, for the present, reserve my opinion as to the advisability of any comparison with Balanoglossus, notwithstanding the observations which Hubrecht mentions concerning the formation of the pericardium in embryos of Tarsius, Tupaja and Sciurus.

(6) It is my opinion, as it is Hubrecht's, that the facts which have up to now come to light concerning the development of Vertebrates can, without difficulty, be brought into line with Hubrecht's interpretation of the gastrulation process. The way to effect this has already been indicated by the hypothesis of the gastrulation in two phases, simultaneously formulated by Hubrecht and by myself, which I have later worked out more fully, and which has since been fully accepted also by Oscar Hertwig. However, in future we will only be allowed to call gastrulation that which I have defined as the first phase of gastrulation. The so-called second phase of gastrulation may no longer be looked upon as such. It is the process of formation of notochord and mesoderm, and is peculiar to Vertebrates. It should be borne in mind that in many cases it may often prove difficult to draw a strict line between the two processes, because, as in so many other chapters of embryology, processes may overlap which have phylogenetically been originally separated.

In conclusion, I can say that in all the important points I agree with Hubrecht. In detail, several points of difference may be noted. Thus I believe that in the gastrulation of Vertebrates a more considerable part is played by invagination than Hubrecht would be willing to concede. I am also in doubt as yet concerning the comparability between the development of the pericardium in mammals with certain features in Balanoglossus. Still, these and other points about which we have not come to an entire agreement while inspect-

ing and discussing the preparations have no direct connection with the chief question concerning the definition of gastrulation and the interpretation of the primitive streak in Amniota. They will be solved in the course of time by special researches, some of them of a more complicated nature.

Here I only wanted to express, and to establish to a certain extent, that it would be well to follow Hubrecht in the definition of gastrulation and that we should introduce certain corresponding alterations in our nomenclature.

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MEMOIRS:

Studies on the Turbellaria.

By
W. A. Haswell, M.A., D.Sc., F.R.S.,
Challis Professor of Biology, University of Sydney.

PARTS I AND II.

With Plates 25—27.

Part I.—On Heterochærus, an "Acœlous" Turbellarian.

Introductory—Occurrence—External Features—Movements.—The genus Amphichærus was founded by von Graff (**15**) for a species previously (**13**) named by him Convoluta cinerea. A nearly allied North American form was described in 1892 by E. L. Mark (**20**), and further details of its structure, with an account of its development, were subsequently published by Gardiner (**9** and **10**).

The feature which was originally supposed to distinguish Amphichærus from all other members of the family (Aphanostomida) to which it belongs, was the presence of a bursa seminalis containing two chitinous "mouth-pieces," but forms nearly related to A. cinereus, and regarded as referable to the same genus, have been found by von Graff (**16**) to possess a number of such chitinous parts. Polychærus, the genus described by Mark, has many chitinous mouth-pieces in the bursa, but it differs from all the known species of Amphichærus in having distinct vitellaria. In this latter point the form to be now dealt with differs from Polychærus, and agrees with Amphichærus, but in the

absence of a "frontal organ," as well as, probably, in other points, differs so widely from the other members of the group that it requires to be designated by a new generic name. I propose to call it Heterochærus, and the species H. australis.

It occurs in shallow rock-pools between tidal limits in Port Jackson towards high-water mark, and is most abundant in places in which it is exposed all day, without shelter of any kind, to the full glare of the sun. The breeding period extends throughout the summer and autumn months—November to May—specimens collected in October were sexually immature, while in June the sexual apparatus apparently undergoes degeneration, and soon practically disappears.

The worms were best fixed by running the water off them, and subjecting them in an almost dry condition to the action of the vapour of osmic acid. This kills most of them very soon without producing much contraction. They may then be treated with Flemming's strong chromosmic-acetic solution or with Hermann's solution, or with a picro-acetic-osmic solution. Hermann's solution, followed by Merkel's, gave some good results, but on the whole the picro-acetic-osmic combination was the most successful. Solutions containing sublimate (including Lang's and Gibson's formulæ) did not yield satisfactory results. Sabussow's (**24**) modification of Kultschitzky's photoxylin-paraffin method, with some alterations, proved entirely satisfactory for this brittle class of objects.

The largest specimens are about 4 mm. in length, 1·5 mm. in greatest breadth. The body (fig. 1) is compressed dorso-ventrally, thickish towards the middle line, thin at the sides, tapering to a blunt point in front, deeply excavated behind, and frequently unsymmetrical. Only in one case, among the very numerous specimens examined, have I found the thin lateral margins folded back on the dorsal surface after the fashion which prevails in Convoluta.

Locomotion may be effected by steady gliding through the water, usually in close contact with the solid substratum,

except that the head end is very frequently raised, sometimes in mid-water, by the agency of the cilia. When the movement is more rapid it is brought about by rhythmical undulations, in which the thin lateral flanges and the pointed anterior regions take the leading part; by means of similar movements the animal is able to swim rapidly in mid-water. As the animal moves along the anterior region is turned about actively in all directions, probing and testing, and seems to be by far the most sensitive part, responding with great rapidity to contact with foreign bodies—contact wth other individuals of the same species causing a particularly active recoil often followed by what appears like a rapid aggressive movement. When at rest the anterior and lateral parts, together with the posterior processes, alone adhere to the surface, the body being somewhat arched in such a way that there is a wide space below into which the mouth and reproductive apertures open—the space communicating with the exterior freely behind between the posterior processes.

There is no frontal organ, such as occurs in most other Acœla.[1]

The mouth, a short longitudinal slit, is situated towards the middle of the ventral surface. Behind it are the two median reproductive apertures, the female in front and the male behind, separated from one another by a space which is considerably less than that separating the anterior from the mouth, the posterior separated from the posterior border by an interval slightly longer than that which intervenes between the female aperture and the mouth. There is a pair of minute eyes towards the anterior end, and an otocyst situated between them. In a mature animal the vesicula seminalis projects as a rounded prominence on the dorsal surface near the posterior border, and in front of this, also on the dorsal surface, is a well-marked depression marking the position of the aperture of the supposed Laurer's canal.

The colour, which varies greatly, depends partly on the symbiotic Algæ present, partly on pigment in the epidermis.

[1] Not in Haplodiscus ussowii, according to Sabussow (**24**).

The pigment is arranged in the form of a network of glistening narrow threads which lie immediately below the surface.

In some full-grown specimens it occurs in relatively small amount, and such specimens may appear of a uniform green or brownish-green colour. Usually the largest specimens are the darkest in colour.

The general coloration produced by the presence of the symbiotic Algæ and of pigment of various hues is a mottling of green, brown, and yellowish white, but the pattern which results is subject to endless variation. Very commonly there is a light (yellowish) spot over the region in front of the eyes and otocyst, and one on each side just behind, but this arrangement is by no means invariable. The whole animal is usually very opaque, and it is in most instances impossible to make out any part of the internal organisation of an entire living adult specimen. The opacity is due partly to the pigment and the symbiotic Algæ, partly to the presence of numerous drops of oily matter in the protoplasm of the cells. When the animals are looked at under incident light with a simple lens or a low power of the microscope the pigment presents a glittering metallic appearance, giving the surface a lustre like that of frosted silver. Viewed by transmitted light it appears dark and opaque. The pigment is scanty or absent in immature individuals.

Integument and Muscular Layers.—I have found no indication either in sections of specimens fixed by various reagents, or of macerated and teased specimens, of the division of the epidermal layer into cells.[1] Nuclei occur at irregular intervals; in diameter they average ·004 mm. Externally the epidermis (fig. 3, *ep.*) in some series of sections appears bounded by a definite layer, which becomes more strongly affected by staining agents than the rest. This has been looked upon as a cuticle, but is regarded by von Graff as formed by the bases of the cilia. I do not think that it is

[1] In Convoluta von Graff (**14**) succeeded by maceration in isolating epidermal cells.

an independent layer, but that it is to be looked upon as a somewhat modified portion of the epidermis.

The cilia, which cover both surfaces, have an average length of about ·008 mm. Here and there are longer flagella, which appear to be non-vibratile, but are often passively moved by the cilia at their bases. Each cilium consists of two segments, a proximal, straighter and stiffer, and a distal, more flexible and whip-like. Vertical lines in the protoplasm of the epidermis have the appearance of continuations of the cilia inwards.

In A. cinereus von Graff (**15**, p. 4) describes the epidermal cells as resting on the underlying muscular layer by a base which is often branched, the presence of the branches with the spaces between them giving an alveolar appearance to the inner zone. This appearance was not observed in Heterochærus.

In the case of A. cinereus von Graff states that, in addition to the true epidermal cells, and the gland cells with their ducts, the epidermal layer also comprises a number of cells which he terms interstitial cells. In Heterochærus these do not occur, at least in the same form; but stellate pigment cells (fig. 4) extending over the dorsal surface in mature specimens, and often spreading to some extent over the ventral, may represent them. The processes of these stellate cells are usually completely united into a network, and in most parts, at least in mature specimens, their cellular nature becomes obscured, especially since nuclei are very difficult of detection among the dense pigment, and it is, besides, very hard to distinguish them with certainty from the ordinary nuclei of the epidermis. In some cases, however, more particularly in immature specimens, it becomes manifest that the pigment is contained in stellate cells, the processes of which anastomose to form a network, extending through the epidermal layer towards its deeper surface.

Though the occurrence of epidermal pigment appears to be somewhat exceptional in the Turbellaria, yet bodies of the same nature as those here regarded by me as stellate pigment

cells combining to form a network, occur in other Acœla. Von Graff, who re-directs attention to these in a recently published work (**17**) on the Turbellaria as parasites and hosts, does not express any definite opinion as to their nature, though he rejects his previous supposition that they might have some connection with the so-called "crystalloids" (really spores of a Sporozoan) occurring in certain fresh-water Rhabdocœles. But the regular distribution of the bodies in question, to form a bilaterally symmetrical pattern, and their restriction, or virtual restriction, to the dorsal surface, seem, with the other points already referred to, to render it evident that we have here to do with a normal constituent of the epidermal layer, and one which may be best regarded as a peculiar variety of pigment cell.

Here and there a little process or papilla projects on the surface of the epidermis; each of these is a mass of rhabdites projecting through an aperture in the epidermal layer—the opening of the duct of a rhabdite-forming gland. The rhabdites (fig. 5) are of two kinds,—shorter, fusiform, and longer, very slender. They often collect in packets in the ducts near their extremities. The ducts are sometimes longer, sometimes shorter—the glands being sometimes near the surface, sometimes more deeply sunk. When the rhabdites are discharged a sticky gelatinous fluid is poured out with them, and by means of this secretion the animal is able to adhere with surprising firmness to a smooth surface, quite a strong jet of water being necessary in order to displace it. In this the whole ventral surface shares; but the posterior processes maintain their hold most tenaciously. Rhabdites are given off freely on the dorsal as well as on the ventral surface. When there is some irritant present in the water, a rapid discharge takes place, and the animal becomes enclosed in a whitish covering, which soon stiffens to form a protecting cocoon. This is, in all probability, the main function performed by these integmentary glands. But it is quite likely that the secretion may have some effect in entangling, and

perhaps poisoning, the relatively large and often active animals on which this Turbellarian preys.

The muscular layers present no features calling for special remark. As in all the Acœla hitherto described, there are three layers—an outer circular, a middle diagonal,[1] and an inner longitudinal. In addition, there is an abundantly-developed system of parenchyma-muscle, chiefly composed of dorso-ventral fibres.

The Algæ.—The symbiotic Algæ, in the full-grown animal, form two dense strata—one dorsal, the other ventral—just within the muscular layers. But they are by no means confined to these situations, occurring, often in abundance, all through the cortical parenchyma, and between the cells of the ovaries and the lobes of the testes. They also occur, though rarely, in the digestive parenchyma (fig. 7). In the smallest specimens, just escaped from the egg, only one or two of the Algæ are to be found; but they appear to multiply rapidly by fission, as somewhat larger specimens of the worm contain large numbers. They are, apparently, simply embedded in the parenchyma, and are not contained in the interior of cells. Sometimes they are isolated, more commonly they adhere together in irregular strings; but in the latter case the adhesion is very slight, and, when the host is crushed or broken up, the algal cells become detached from one another.

The Algæ (fig. 6) have an average diameter of ·025 mm. They have a very definite shape—oval, with the broader end rounded and entire, and the other end usually with a distinct incision or notch, which may be double.

An important feature distinguishing this Algæ from that which occurs in Convoluta[2] is the presence of a distinct, though somewhat thin, non-protoplasmic cell-wall. This becomes very clear in stained cells, particularly when plasmolysed. It does not respond to the iodine and sulphuric acid

[1] In the figures of sections these diagonal fibres are not represented as distinct from the circular.

[2] Haberlandt (in **15**), Gamble and Keeble (**8**).

test; but there can be little doubt, from its appearance and behaviour in other respects, that it is composed of cellulose.

The colour of the chromatophore, which occupies a large part of the interior, is somewhat variable. It is rarely grass-green, but always contains a brownish ingredient, and in some cases would be best described as brown with a tinge of green—the appearance presented closely resembling that of the chromatophores of Diatoms or Dinoflagellates. All the colouring matter is soluble in alcohol.

The nucleus always occupies the same position, in a mass of uncoloured protoplasm at the broader end of the cell. It contains a very regular chromatin network without nucleoli or other similar bodies. Towards the middle of the cell is a single spherical pyrenoid, very rarely a couple.

A large proportion of the Acœla seem to lodge "symbiotic" Algæ. In many cases these are bright green in colour, containing no other colouring matter than chlorophyll. But in Haplodiscus, and in some species of Convoluta and Amphichærus, they are yellow or brown. Whether the presence of a cell-wall is a special feature of the Zooxanthella of the Australian form remains to be determined.

In the case of Convoluta, Gamble and Keeble (**8**) conclude that the green cells are not of paramount importance in the nutrition of the worm, which freely ingests various organisms. In the Australian form there is still less need for any symbiotic nutrition, since recently-captured specimens are almost invariably found to contain relatively large animals—Rotifers, Polychæts, Crustacea, etc., in process of digestion.

Further details with regard to these Algæ are reserved for the present until more complete data have been obtained.

Digestive System and Parenchyma.—As in most Acœla, a pharynx is absent. To apply the term, as von Graff, Pereyaslawzewa (**22**), and others do, to what is neither more nor less than an integumentary pit with the epidermal layer somewhat thickened, appears to me to be misleading.

In Heterochærus, as will be rendered clear by fig. 8, representing a section passing through the mouth, the latter is an opening leading directly inwards—the integument and muscular layers being involuted round its margin to a very slight extent to form a very short passage, but not becoming modified in any way, except that the integumentary glands are slightly more numerous.

There is no intestinal epithelium—the food being received into a mass of nucleated protoplasmic material, which is devoid of definite arrangement, and is not distinguishable into cells. This is fibrillated, and consists of strands which have for the most part a radial arrangement, with irregular vacuoles between them. In sections of specimens in which no food is present the appearance represented in figs. 9—11 is fairly constant. There is always a relatively large, well-defined space (*ent.*) situated towards the middle, and representing the intestinal lumen. The mouth usually, as in the specimen represented in fig. 9, leads directly into the central space, but sometimes a mass of the plasmodium intervenes.

Surrounding this central space in a fairly regular manner are a number of smaller spaces, usually narrow and elongated, and having a general radiating arrangement. The partitions between these are composed of the same material as that surrounding the central space, but reinforced by a good many muscular fibres.

An extension of the central parenchyma in the form of a vacuolated horizontal plate runs out to the marginal region of the body on each side, and also forwards and backwards, and passes distally into the superficial layer. It is perforated by numerous dorso-ventral muscular fibres. Both above and below it is a series of vertically elongated spaces, like the cells of a honeycomb, with thin perforated walls. The substance of which the partitions are composed is of a more hyaline appearance than the central plasmodium, and is not fibrillated. Running in the partitions, but never through the spaces, are many dorso-ventral muscular fibres. The partitions end in the superficial layer of parenchyma. The

result of this arrangement of the parenchyma and its vacuoles is that in a transverse section the region between the central or digestive parenchyma and the superficial layer appears divided by numerous vertical lines, while in a horizontal section it has, more or less, the appearance of a cross-section of a honeycomb. This appears in figs. 9 and 10.

In the genital region, which occupies the greater part of the length of the body, the cells of the testis of each side lie in the series of spaces to the dorsal side of the horizontal plate, while the ovarian cells occupy the corresponding spaces on the ventral side, the plate thus acting as a partition between the two. The latter must act also as the main support for the digestive plasmodium.

Immediately within the body-wall the parenchyma forms a superficial layer, embedded in which are the greater number of the Algæ and all the rhabdite-forming glands, and in which lie also the main nerve-cords and the plexuses to which they give rise.

The whole structure and arrangement of the parenchyma in Heterochærus closely resemble those of that tissue in Haplodiscus ussowii, as described by Sabussow (**24**), particularly with regard to the horizontal plate and the sets of spaces above and below it.

The food consists of a variety of organisms, many of them comparatively high in the scale—Rotifers, small Polychæts, Entomostraca, small Amphipoda, etc. These are found in the large central space, or, if of minute dimensions, may be contained in the interior of the smaller vacuoles.

In the case of Amphichærus cinereus von Graff (**15**, p. 15, Taf. I, fig. 12) describes and figures special cells which he looks upon as concerned in the formation of a digestive secretion. In the case of Heterochærus the only elements at all resembling in appearance and position those represented are deep-lying Algæ; and nothing of the nature of special secretory cells is to be found.

From the above account of the digestive system of Heterochærus it follows that, in this form at least, the

subordinal name of Acœla is not strictly applicable, since an enteric cavity, of a sort, is undoubtedly present, and though liable to modification, fairly constant in form and position. To the alternative name proposed by Mdlle. Pereyaslawzewa, viz. Pseudo-acœla, there is the objection, which appears to me formidable, that its proposal was founded on the erroneous conception that the apparent absence of an epithelium is due to the action of reagents. Perhaps the term Adelocœla might be adopted, if the conditions obtaining in Heterochærus should prove, on further investigation, to prevail in the other members of the group.

Nervous System.—There are three pairs of longitudinal nerve trunks—two dorsal, viz. inner and outer, and one lateral. The last are much the largest and most important. They extend throughout the length of the body, near the lateral margins, and nearer the ventral than the dorsal surface. From these main lateral trunks branches are given off, some of which break up to form a plexus in the shape of a network (fig. 13), extending over the entire ventral surface below the muscular layers. Anteriorly the two lateral trunks converge, and, about midway between the dorsal and ventral surfaces, enter the brain.

The two dorsal pairs of nerve-trunks unite to form, on each side, a common root, which is little more than a dorsally-directed process from the brain.

The brain (figs. 12 and 14) is a commissure of finely-fibrillated material which, on its upper (dorsal) aspect, gives off the roots of the dorsal trunks, while on a lower plane the lateral trunks pass out from it. Between the roots of the latter is a rounded median recess, open posteriorly, in the brain substance. This is partly covered over dorsally by brain substance, but is not so bounded either ventrally or posteriorly: it is almost completely filled by the otocyst (*ot.*). At the sides are the two small and simple eyes (*e.*), consisting merely of masses of pigment embedded in the substance of the ventral portion of the brain, which, owing to the presence of the otocyst recess, is divided into two lobes, each giving

off the corresponding lateral nerve. Nerve-cells surround the fibrillated material of the brain, and are most abundant over the dorsal portion.

The dorsal nerve trunks, four or, more rarely, six in number, run backwards beneath the muscular layers, and give off numerous branches, which branch and anastomose to form a plexus extending over the entire dorsal surface.

From the same part of the brain (the ventral portion) which gives origin to the lateral nerves are given off several nerves, which run forwards in the region in front of the brain, breaking up into numerous branches, forming a rich plexus, which extends to the anterior extremity.

Von Graff (**15**, p. 31) describes the brain of A. cinereus, but did not succeed in making out the arrangement of the longitudinal nerve-cords. The brain is, in its upper part, a transversely-elongated four-sided mass, bearing anteriorly two swellings, from which arise two nerves that unite to form the "anterior commissure." Below the brain increases in bulk, mainly owing to the accumulation of nerve-cells at its posterior angles giving rise to the dilatations from which the longitudinal nerves are given off, and to the presence of a pair of lateral swellings from which the "posterior roots" of the middle longitudinal nerves arise dorsally. The arrangement of the longitudinal nerves is supposed to correspond to that observable in C. roscoffensis as described originally by Delage (**5**). In this form there are six longitudinal nerves, which run parallel with one another to the posterior extremity of the body, where they lose themselves in a network of anastomoses. Delage terms these inner, middle, and outer pairs of longitudinal nerves. The last lie almost at equal distances from the dorsal and the ventral surfaces, while the other two are distinctly dorsal, lying immediately below the stratum of Zoochlorellæ. The two inner arise from the posterior angles of the more dorsally-situated part of the brain, while the others originate on either side from a common root which arises from the anterior (ventral) part. In addition the middle longitudinal nerve is connected with

the dorsal (posterior) ganglia of the brain by a transverse anastomosis going off from the outer angle, so that it has a double origin. Neighbouring longitudinal nerves are connected together by anastomoses between their branches, the arrangement of which is subject to variation in different individuals.

On the whole there is a very close correspondence between the nervous system of Heterochærus and that of allied forms. Owing to the absence of a frontal organ it is not possible to distinguish the "anterior and posterior commissures" described by v. Graff, and there is thus an appearance of greater simplicity in the brain. All the nerve cords, except the lateral and those giving rise to the frontal plexus, arise from the dorsal part of the brain, and this appears also to be a distinguishing feature.

The two little masses of pigment which represent eyes in the Acœla, instead of being situated in the epidermis as they are stated to be in other members of the group, are embedded in the substance of the brain. In this point Heterochærus resembles C. schultzii (Delage, **5**, p. 131).

Reproductive Apparatus.—The male reproductive apparatus of Heterochærus does not differ in any essential respects from that of allied forms. The testes (fig. 2, *t.*) are composed of numerous minute lobes, which extend throughout the greater part of the length of the body towards its dorsal aspect. The two vasa deferentia have no definite walls, but are merely of the nature of channels in the parenchyma, which run backwards some little distance from the lateral margins, increasing in width as they receive additional accessions of spermatozoa from the testicular lobes. At its posterior end each vas deferens expands into a wide vesicula seminalis (*v.*), and these bend inwards to meet in a median reservoir at the base of the penis. Into this open the ducts of numerous unicellular glands, the secretion of which consists of, or contains, minute rounded granules. These glands appear to correspond to the prostate- or granule-glands of other Turbellarians. From the median reservoir a very short

and wide ejaculatory duct leads to the male aperture at the extremity of the penis. The penis is devoid of any chitinous parts. It is in the form of a truncated cone with muscular walls and an epithelium of cylindrical cells.

There are two very distinct kinds of spermatozoa—a larger and a smaller. The larger, or "giant" form (fig. 15) is ·35 mm. in length—about eight or ten times the length of the smaller. It is a long, cylindrical filament. Of this the posterior portion, which tapers very finely, represents the tail, the greater part of the filament representing the middle-piece. Along the length of the middle-piece and tail runs a very inconspicuous, contractile, spiral flange. The head is represented by the anterior portion of the filament, which is not modified in any marked way, but is distinguishable from the middle-piece merely by being usually bent at an acute angle on the latter, and by being devoid of the spiral flange. No chromatin elements were brought into view in any part by the action of methylene-blue or gentian, unless two rows of minute granules which run throughout the length of the middle-piece are of this nature.

The smaller sperm (fig. 14) has likewise no definite head, but the anterior end is slightly enlarged, and terminates in an abrupt, nearly transverse, face. Some distance behind this the sperm becomes distinctly compressed, and this compression is continued to near the posterior extremity, where a short, uncompressed, filamentous portion represents the tail. Staining with gentian-violet brings out a series of granules in the axis of the middle-piece increasing in bulk towards its posterior end. The smaller sperm moves actively with a wriggling motion, during which it is thrown into a spiral. The movement is intermittent, and, when it ceases, the sperm usually becomes straightened out, but sometimes assumes various definite curves. The giant sperm moves with a gliding motion when a number are in contact, unless they are wedged very close. But it also sometimes executes wriggling movements of the tail part, which are much less active than those of the smaller sperm.

In connection with the subject of the spermatozoa it may be worth while to mention that a remarkable micro-organism, presumably a Schizomycete, which is extremely common as a parasite of Heterochærus, particularly in the winter and spring, bears a very considerable resemblance to a spermatozoon both in form and movements. It occurs in the interior of vesicles which are apparently degenerating cells, sometimes in the interior of degenerating Algæ, proved to be such by vestiges of the chromatophore. They are filaments thickened at one end, and they execute wriggling movements. Each vesicle or cell may contain only one or several of these bodies. They are only about ·025 mm. in length, or a good deal smaller than the smaller sperms. They are doubtless similar in character to the "parasitische Gebilde," referred to by Böhmig (**2**)[1] and Sabussow (**24**, p. 377), and supposed by Monticelli to be spermatozoa.

The female reproductive aperture leads into a thick-walled passage (figs. 2 and 17, *ant.*), which may be termed the antrum femininum. This is lined with columnar ciliated epithelium, outside of which is a thick muscular coat, composed of external circular and internal longitudinal layers. Perforating the wall and opening into the lumen of the antrum are the ducts of a great number of unicellular glands (*gl.*), which produce a secretion precisely similar in appearance to that of the granule-glands of the male apparatus. The ducts are dilated just before they open to form reservoirs for the secretion, so that a great quantity can be discharged very rapidly. When they are discharged the granules become disintegrated, and give rise to a nearly homogeneous mass with irregular granulations.

From the antrum proper a passage runs upwards and forwards, and its upper extremity lies immediately below the integument of the dorsal surface. During the breeding season, but not at other times, the dorsal wall of the body in

[1] I only know this paper through abstracts in the 'Zool. Jahresbericht' and 'Zool. Centralblatt.'

this position always presents a colourless spot, owing to the absence, in the area directly over the antrum and female aperture, of the parasitic Algæ, and also of pigment. The area is always depressed, and in it there is frequently to be recognised an appearance like that of a closed cleft or fissure. In only a few cases was an actual opening observed. But the study of sections through this region shows that there is always, in sexually mature animals, an actual or potential passage, leading from the dorsal surface into the antrum. This appears most distinctly in sagittal sections, such as those represented in figs. 21 and 22, which are accurate copies of photographs. In all series of sections of specimens with ripe ova this cleft or passage is to be detected. This observation must have an important bearing on the question of the functions of the various parts of the reproductive apparatus, and will be referred to later. In the meantime I will point out that this passage, if not homologous, is at least closely comparable with the "canal of Laurer" of Trematodes.

Near its dorsal extremity the antrum, or, more correctly, the passage which continues it forwards and dorsad, gives off, nearly at right angles, the two ducts of the bursæ. These are lined externally with a continuation of the muscular layer of the antrum; but they have no regular epithelium, containing instead a somewhat irregular layer of cells, which does not bound a definite lumen, but has running through it several narrow passages. Those passages, and the lumen of the vagina, often appear blocked with the secretion of the unicellular glands.

Each of the lateral ducts into which the antrum divides terminates in the corresponding bursa seminalis (*b. s.*). The wall of this is composed of the same kind of material as the wall of the duct—a thin layer of muscle (fig. 18, *b. s.'*) surrounding a mass of cells in which the remaining parts of the bursa are enclosed. Outside this is a thin layer of cells, the nuclei of which resemble those of the unicellular glands that surround the antrum. The bursa encloses the chitinous

mouth-pieces and a series of rounded bodies, which I will term bulbs, with one of which each of the chitinous pieces is connected.

From the irregular channels into which the lumen of the duct is divided a narrow canal (fig. 17, *d.*) runs to the base of each bulb, and, passing through it, enters the corresponding mouth-piece, through the axis of which it is continued to the free extremity. The canal is dilated (*d.'*) immediately below the base of the mouth-piece, and sometimes, though rarely, there is a second dilatation further back.

The structure of the bulb (fig. 18) is exceedingly difficult of determination, owing to its being nearly always packed full of sperms. In the few cases in which this was found not to be the case, it had a shrivelled appearance. It must have a bounding wall preventing the escape of the sperms and maintaining its regular form, and internally it appears to be of the nature of a sponge. Mark's view that it is formed of greatly vacuolated and fused cells seems to me to be highly probable. The chitinous mouth-piece is attached to it by a number of fibres, which run from the basal part of the mouth-piece into the substance of the bulb. The spaces in the bulb must communicate freely with the canal as it passes through, to enable the sperms to be received, and to be afterwards discharged.

The chitinous mouth-pieces (fig. 18, *ch.*) are, on the whole, very similar to those of Amphichærus and Polychærus, as described by von Graff and Mark. Each is a slender cylindrical rod, tapering towards the extremity, and more or less strongly curved. Its chitinous wall consists of a large number of minute pieces (figs. 19 and 20), which are not rings, or perforated discs, as they are stated by von Graff to be in the species which he examined, but form each a little less than half a ring.

As the mouth-piece tapers towards its free extremity the chitinous segments undergo a corresponding reduction in size. Besides being enclosed by these chitinous bodies the fine canal has a wall of its own, and at the free extremity

this usually extends some distance beyond the last of the chitinous bodies.

From its base, which is slightly embedded in the bulb, to its apex the mouth-piece is enclosed in a sheath of cells (fig. 18, *ch. c.*) which are continuous with those of the wall of the bursa, and not widely different from them in appearance. These are arranged in a single layer with their long axes at right angles to that of the mouth-piece, and each is produced into a narrow lamellar process, which becomes connected with one of the chitinous bodies. These cells have nuclei similar to the nuclei of the wall of the bursa, situated towards their outer ends, but, in the fresh condition, these are barely visible without the addition of acetic acid.

In each cell, however, nearly always on the inner side of the nucleus, is, in most specimens, a rounded globule which, on account of its high refractive power, is very much brighter and more conspicuous than the nucleus itself. Like the cells themselves these globules gradually diminish in size towards the fine extremity of the mouth-piece. Each of them is of about the same volume as one of the chitinous bodies in its vicinity. It seems to me that these appearances point to the conclusion that the cellular sheath of the mouth-piece is composed of cells by whose action the chitinous matter is formed, and that the globules occurring in the interior of the cells are actually chitinous pieces in process of formation.

Where the canal enters the bulb from the base of the mouth-piece it becomes dilated, as already mentioned, into a rounded cavity (fig. 17, *d'*) in the substance of the bulb.[1] In the interior of this are a number of crescent-shaped bodies, ·008 mm. in length, narrower at one end than at the other, apparently composed of the same material as the parts of the mouth-piece. In sections these appear irregularly arranged, but in their natural relations they are, in all probability, disposed in a regular way around the lumen with their concavities in-

[1] In some specimens a second enlargement, presenting very similar appearances, occurs further back.

wards, like the bodies in the wall of the mouth-piece itself. It is these crescent-shaped bodies, I apprehend, that are referred to by von Graff (**15**) as gland-cells, and that are also noticed by Mark (**20**) in Polychærus. One end of each of these, the broader, becomes much more strongly stained than the rest, and this appearance may have led to the supposition that the bodies in question are of the nature of cells. The narrower end is continuous with a fibre or fibres, which pass outwards into the substance of the bulb. It seems to me that the presence of these chitinous bodies in the interior of the bulb, with the fibre-like strands passing out from them, points directly to the conclusion that the bulb is composed of cells which, originally, like those forming the investment of the mouth-piece, have lost, for the most part, their secreting power, and have coalesced and become vacuolated to receive the sperms.

The free extremities of the mouth-pieces extend far beyond the wall of the bursa. Towards the extreme point, where the chitinous bodies become discontinued, the slender canal loses all traces of sheath, and terminates in the parenchyma, near the ventral surface of the body, in close proximity to the posterior extremity of the ovary and the ripe ova. In several specimens I was able to see sperms issuing from the end of the canal, and wandering out into the surrounding parenchyma.

In number and arrangement the chitinous mouth-pieces are very irregular. Most commonly there are four in each bursa; but in some cases there were only two, and in others as many as nine. Very often the number in the two bursæ is unequal. In one specimen the entire bursa was duplicated on one side, though not on the other. Often a bulb, or more than one, has no mouth-piece. Nearly always two of the bulbs are partially fused. Very frequently one or more of the mouth-pieces are incomplete—the basal part, usually, being absent,—as if in course of absorption or regeneration.

The parts just described, namely, those known as bursæ seminales, with their chitinous mouth-pieces, are very im-

perfectly understood; and various conflicting statements have been put forward as to their structure and functions.

The account of them given by von Graff (**15**), in the case of A. cinereus, is assuredly incorrect, unless that species differs in a very thorough and radical manner from the Australian form. He represents the female genital aperture as leading into a sort of vestibule, from which, in turn, lead three passages—a median and two lateral. The former is a short and wide passage leading backwards into a cavity—the cavity of the bursa seminalis,—which becomes filled with a mass of spermatozoa as the result of an act of copulation. The two lateral passages are much narrower than the median; they curve outwards and backwards and open into the bursa seminalis. In the posterior part of each of them is lodged the corresponding chitinous end-piece, the base of which is supported on the mass of spermatozoa, while the apex is directed towards the external opening. Each mouth-piece is described as a curved, tapering rod, made up of a series of perforated chitinous discs, the central canal being bounded by a longitudinally striated membrana intima. Surrounding the chitinous tube is a layer of ring-shaped cells, each of which is the matrix cell, or formative cell, of one of the chitinous discs.

Von Graff regards the mouth-pieces as organs capable of eversion, and of acting as organs, by means of which spermatozoa are transferred to another individual, the lateral passages, with their chitinous mouth-pieces, being regarded by him as the parts by means of which the ova are fertilised when discharged. But in Amphichærus and Polychærus, as in Convoluta, it has been shown by Pereslawzewa (**21**), Repiachoff (**23**), Mark (**20**), Gardiner (**10**), and others, that the eggs are internally fertilised.

A deeper insight into the structure and mode of action of these organs was attained by Mark (**20**), who studied them in his Polychærus caudatus, in which there are a number of mouth-pieces on each side, instead of merely one as is usually the case in A. cinereus. "Each chitinous structure consists

of a slightly tapering, conical, central portion, which is traversed by a narrow axial canal, and from which diverge numerous thin, close-set lamellæ, which are directed obliquely outward and towards the narrower end of the central piece. . . . From the arrangement of the nuclei at the periphery of the ventral portion of each such organ, I conclude that the chitinous structures result from the secretion activity of cells whose nuclei occupy their peripheral ends, while the axial blade-like prolongations of the cells extend as far as the conical chitinous axis, and separate the successive chitinous lamellæ which go off from the latter. The basal end of the mouth-piece is surrounded by enlargements, which are probably identical with the "Drusenkranz" figured by von Graff (Taf. II, figs. 1 and 2) for Amphichærus. . . . Cross-sections of the chitinous mouth-pieces and their surrounding mantle of cells show that both are circular, and that the lumen of the former is likewise cylindrical and very narrow. Lying in a vacuole near the basal or larger end of the chitinous cone—sometimes in contact with it—there is almost invariably a small ball of tangled spermatozoa. . . . The exact histological nature of the deep or dorsal half of these cell-masses—the ventral portions of which secrete the chitinous mouth-pieces—is not easily determined. The substance of adjacent masses seems to be more or less confluent into a finely granular pale substance, in which are scattered a few faintly coloured nuclei. The appearance is as though the cells of the deep half of each cluster had become distended into enormous gland cells, and then, becoming confluent with each other, had finally become vacuolated, and lost to a great extent their cell boundaries. . . . There are small lacunar passages, in the parenchyma between the ventral ends of the ovoid masses and the ventral wall of the body, and I imagine that these serve in some way to transmit the spermatozoa to the ova, but I have not yet found spermatozoa in these passages nor even satisfactory evidence that they pass through the narrow lumen of the chitinous mouth-pieces" (pp. 307 and 308).

Gardiner (**10**) states that he has frequently seen one specimen of Polychærus caudatus pursuing and trying to get on the back of another, which showed symptoms of restlessness and discomfort, and endeavoured to escape. Under the dorsal integument of the second individual, in such a case, were found numerous spermatozoa, and in the vicinity abrasions were observed. From these facts he concludes that we have here an example of hypodermic impregnation, and that the function of the chitinous mouth-pieces is to bring about this result.

This appears to me not to be a tenable view of the function of the parts. There is no direct evidence that the bursæ are capable of being everted in such a way as to be brought into play as organs for effecting hypodermic impregnation, and it appears very improbable, from their structure, that they are capable of being so used. Moreover, such a theory involves a supposition which bears great improbability on its face. We should have to suppose that in these animals we have a process of fertilisation without parallel in the animal kingdom —a process in which one individual, having received into a part of its female apparatus a mass of spermatozoa from another, uses them to impregnate a third!

The conclusions to which I have come with regard to the functions of these parts have already been partly indicated. The observation of the passage of a stream of spermatozoa from the apex of the mouth-pieces into the tissues in the near neighbourhood of the mature ova (previously also recorded by Repiachoff [**23**] with reference to another member of the group) seems to prove the function of the bursæ to be the internal fertilisation, one by one, of the ova as they become mature. The structure of the parts seems to render it unlikely that the spermatozoa so utilised can be derived from the same individual, so that a process of copulation, though not actually observed, most probably occurs. This may take place, not through the ventral female aperture, but through the dorsal passage (Laurer's canal) the existence of which is otherwise not easy of explanation, and, if so,

in all probability, the eggs pass out through the antrum femininum.

Like the testes the ovaries (fig. 2, *ov.*) are devoid of any investment. Each simply consists of a number of cells which extend on a more ventrally-situated plane than that on which the testes lie, from near the anterior end of the body to the neighbourhood of the oviducts. There is no differentiation of vitellogen cells or shell-gland cells. The most anteriorly-placed cells are the smallest, and their size gradually increases as we pass backwards until we reach the ripe ova. The nuclei of all the unmatured cells are of the same character. In the larger ova they are ·05 mm. in diameter. There is a loose chromatin network and a large nucleolus, ·02 mm. in diameter, within which are to be detected either one or two spherical bodies, having the appearance of vacuoles. The protoplasm of the ripe ova is very mobile, and, if it does not undergo active amœboid movements, readily flows backwards and forwards through the spaces in the parenchyma in which the ovaries are situated. Usually the full-grown ova are drawn out in the direction of the long axis of the body and they may attain a length of ·4 mm.

In the posterior part of their extent the two ovaries become more or less completely united behind the digestive cavity, and in this position during the breeding season are to be found a varying number (sometimes as many as eighteen) of completed and fertilised eggs. These (fig. 2, *oo*) are usually subspherical. With few exceptions all the many eggs examined were in precisely the same stage, viz. that of the first segmentation spindle. It would appear, therefore, that at this stage there is an arrest in the development, and that the process only goes on further when the egg is discharged.

Maturation-stages were very rarely met with—a circumstance that would seem to point to the conclusion that the process takes place very rapidly. In the stage immediately following, the female pronucleus (fig. 24) has the peculiar character described and figured by Gardiner in Polychærus,

having the appearance of a rounded group of vesicles each enclosing a minute spherical mass of chromatin. This appearance it still retains after the sperm has entered the cytoplasm, the actual union of the male and female nuclei was not observed. The polar bodies (fig. 23, *pl.*) were embedded in the cytoplasm not far from the surface in all cases in which they were detected, and if they ever become actually separated out, the separation evidently takes places at a later stage.

The material of the thin egg-shells in which the fertilised ova are enclosed is secreted by the ova themselves, and not derived from any special glands. In some eosinated preparations this is rendered very obvious, some of the ova being surrounded by a discontinuous investment of droplets or granules, others having a complete shell, while droplets or granules of what appears to be the same material are discernible in the substance of the ova, both fertilised and unfertilised, and in the spaces between them.

The mode of passage of the fertilised eggs to the exterior remains doubtful. The spaces in which they lie have no definite outlet. Gardiner gives a diagram of the parts in Polychærus in which the "vitelline glands" enclosing the eggs are represented as opening on the exterior by means of the female aperture. This is not in accord with Mark's observations on that genus. In Heterochærus such a communication does not exist. I can merely conjecture that when a number of completed eggs have accumulated a temporary passage is formed between the space in which they lie, and the lumen of the antrum. A short, blind, anterior diverticulum of the latter, which occurs in some specimens, and is shown in fig. 17 (*k*) may be a vestige of such a temporary outlet.[1]

During the breeding season, when a number of specimens were kept for a day or two, many of them deposited their

[1] Gamble and Keeble (**8**) state that in Convoluta roscoffensis the eggs may be laid singly without disintegration of the parent, or a number are discharged at once with rupture of the parent, which sometimes breaks into two parts.

eggs on the bottom of the vessel. I have never witnessed the act of oviposition, and it probably takes place during the night. Solitary eggs are occasionally found; but, as a rule, a varying number—sometimes as many as thirty—are found enclosed together in a transparent capsule adhering to the glass. The capsule is structureless with, frequently, included foreign bodies, such as unicellular Algæ. Often spermatozoa of the animal are embedded in it. The individuals from which those eggs were discharged were found not to be ruptured in any way, and there seems to me to be little doubt, that the eggs pass out through the female aperture, and that the substance of the capsule consists of the secretion of the unicellular glands opening into the antrum femininum.

A remarkable point is that the reproductive apparatus is entirely absent during the winter. In specimens collected in April it was found to be intact, and in many there were fertilised ova. In the latter symptoms of degeneration showed themselves, however, most containing large vacuoles in the cytoplasm, though the nuclear spindles were still intact. But in June, July, and August none of the specimens examined showed any trace of reproductive apertures, and the examination of sections of a number showed not only that the apertures were completely absent, but that there was no vestige of reproductive apparatus, either gonads or ducts. These were full-grown specimens, resembling in every other respect those found two months before to contain mature sexual organs. Whether this means that the reproductive system becomes completely absorbed in the winter, to be regenerated in the spring, or whether it is that the generation which was sexually mature in April had perished, and a new generation had become fully grown without showing any rudiments of the gonads or their ducts, still remains to be determined.

Part II. On Anomalocœlus cæcus, a new type of Rhabdocœle.

The Rhabdocœle Turbellarian, of which the following is a description, was found among the mud at the bottom of dams at Glanmire Hall, near Bathurst, in New South Wales. Its eyeless condition is, doubtless, correlated with the fact that it was never found on the surface, but always burrowing more or less deeply in the mud. Nothing is known of its range; it does not occur in any of the other dams in the neighbourhood that were examined for it, and has not been found elsewhere.

The largest specimens were about 5 mm. in length when alive and fully extended: the breadth under the same conditions was about one-sixth of the length. The living animal is semi-transparent, of a reddish colour, which varies greatly in intensity. The region in front of the pharynx is always bright red, the colour being usually most intense in an axial streak in front of the brain. In young living specimens the intestine is clearly distinguishable as a cylindrical, rather opaque, band behind the rounded pharynx: in older specimens this becomes hidden by the reproductive apparatus. The vitelline glands appear as a glistening network extending over the entire post-pharyngeal region. The mouth is a rounded aperture situated anteriorly about an eighth of the total length from the anterior extremity. There is a single reproductive aperture situated somewhat in front of the middle of the ventral surface.

Integument.—The most important feature of the integument is the presence of a system of canals between the epidermal cells. The epidermal cells are flat polyhedral plates, like those of the majority of Rhabdocœles. They vary greatly in size, but are on an average about ·04 mm. in length and ·012 mm. in thickness. Each contains a nucleus (about ·005 mm. in diameter), which is very rarely spherical, but nearly always lobed or mulberry-like. The cell-proto-

plasm is vertically fibrillated. Each cell is perforated by the ducts of a number of rhabdite-forming glands. When these are empty they are clearly to be recognised in stained sections as perforations passing through the cell; when secretion has been passing through them at the time of fixation it becomes strongly stained, and the effect in horizontal sections is the appearance of a number of dark spots in the body of the cell, and in vertical sections of a number of narrow, vertical, deeply-stained strands passing right through the cell, and sometimes projecting slightly on the surface. A thin layer on the surface of each cell marked by innumerable fine vertical lines, corresponding apparently to the bases of the cilia, is probably of the nature of a cuticle, but it is not very sharply differentiated, and there are no indications that it is capable of being detached from the underlying layer. The epidermis is supported on a distinct, but thin, basement-membrane.

The system of channels above alluded to is not equally distinct in all specimens, owing to the channels being sometimes more, sometimes less, dilated. In a favourable specimen the entire dorsal surface, and, in some cases, the ventral also, of the living worm is covered with a network of clear bands of varying width. When the surface is examined under a moderately high power of the microscope, it is found that these bands lie in the intervals between the cells of the epidermis, and represent branching vessels of varying calibre. In one or two of the specimens which I fixed and mounted whole these vessels are almost as distinct as they were in the living condition. In fig. 29 I have represented a portion of this network as seen in a surface view of one of these entire preserved specimens. Examination of the living specimen showed clearly that the appearance in question is not due to the presence of open clefts between the epidermal cells. In an uninjured animal no such clefts exist, the ciliated surface being smoothly continued without interruption from one cell to another across the clear interval. But in the majority of my series of sections, in cases in which the system is well

developed a rupture has taken place, so that what were in the natural state, closed tubes, assume the character of open clefts between the cells. In many cases, however, the closed character is retained. Such sections as those represented in figs. 26 and 28, which are facsimile copies of photographs, show that we have to do with a system of fine vessels which run in the intervals between the cells of the epidermis. They are superficially placed, and, while their lateral walls are formed by the edges of the cells, their outer wall, formed, apparently, by an extension of the protoplasm of both the contiguous cells, with the "cuticle," is exceedingly thin, and is readily ruptured during the processes of fixing and sectioning. The deeper portions of the cells are united underneath the vessels by numerous fine protoplasmic filaments.

In several instances in living specimens I thought I was able to trace a connection between this network of capillaries [1] and small vessels of the excretory system, and it appears very likely that such a communication exists, and that the network is to be looked upon as an extension, perhaps respiratory, of the water-vascular system.[2] That this plexus should vary greatly in different individuals as regards the degree of distinctness with which it appears, accords well with the character of the excretory system in general, in which the vessels frequently collapse and vanish completely, and, when they are in a contracted state, are quite indistinguishable.

It is unlikely that the occurrence of this remarkable network of channels forming an inter-cellular plexus of capillaries in the epidermal layer is a peculiarity of Anomalocœlus. In fact, there seems to be some evidence, if only in certain of the figures of von Graff's 'Monograph,' that the same thing occurs in some other groups of Rhabdo-

[1] I use this term as a convenient one, though the varying calibre of the vessels in question renders it not strictly appropriate.

[2] The only reference which I have discovered to excretory vessels in the epidermal layer is Vejdovsky's statement (**28**, p. 183) that in Bothrioplana blind branches extend into that layer.

cœles. This, however, is a subject for further investigation. I will merely at present direct attention to the figures of the epidermis of Mesostomum Ehrenbergii (Taf. v, fig. 12), M. tetragonum (Taf. iv, fig. 19), and M. lingua (Taf. vi. fig. 1), Microstomum lineare (Taf. xv, fig. 4), and Vortex viridis (Taf. xii, figs. 4 and 5). As regards the last-named species, fig. 4 represents a vertical section in which there appear between the cells rounded spaces having very much the same appearance as those occurring in the case of Anomalocœlus; in the explanation, however, the author refers to them as "Bindegewebeslücke." In the general part of the text (p. 45) the author remarks on this figure:—"Dass auf dem Querschnitte (fig. 4, *v*) so grosse Hohlräume zwischen den einzelnen Zellen übrig bleiben, erkläre ich mir aus der durch die conservirende Flüssigkeit hervorgerufenen Schrumpfung, wodurch die Fortsätze benachbarter Zellen sich von einander zurückzogen." [1]

The only definite statement I have succeeded in finding concerning such a system of intercellular channels in the epidermis is a brief one by M. Braun (**4**, p. 35). In his account of Mesostoma platycephalum he has the following:—"Die haut besteht aus grossen, platten, am Rande mit Zacken besetzten Zellen, deren freie Fläche etwas grösser ist als die Basis; der Querschnitt einer solchen Zelle erscheint dan trapezformig mit eingebogenen kurzen Seiten. In Folge dieser Gestalt sieht Manzwischen zwei Zellen immer einen rundlichen leeren Raum auf dem Schnitt, der den schon von Graff abgebildeten Gängen zwischen den Epithelzellen entspricht; falls das Ganze nicht auf Rechnung der Reagentien zu setzen ist, findet sich hier, wie bei vielen anderen Rhabdocœliden, ein System von Kanälen, welche

[1] In connection with this subject it may perhaps be as well to point out here that the "wasserklare Räume," to which particular attention has been given by Böhmig (**2**), are intra-cellular, and that, whatever be their significance, and that of the pore-canals that run inwards from them, they have nothing to do with the capillary network. The latter manifestly does not occur in the genera (of Rhabdocœles), in which Böhmig made a special study of the integument.

um die Epithelzellen verlaufen, nach aussen und seitlich von den letzteren, nach innen noch zum Theil vom Hautmuskelschlauch begrenzt werden." That these canals are not, as it is here suggested they may be, formed as the result of the action of reagents is rendered evident by their conspicuousness in living specimens. But even if we had not that evidence, their arrangement in such a preserved specimen as that from which fig. 29 was drawn, following as it does a regular system, appears to me to be quite convincing. In such a view as that represented, which is a small part of what extends over the entire surface, main channels are traceable, giving off branches, the size of which is, on the whole, very uniformly regulated by that of the vessels from which they are derived. To bring this about, cells of a variety of sizes and of innumerable shapes are fitted in together in a highly-complicated manner.

In Schneider's 'Lehrbuch der vergleichenden Histologie der Thiere' there is a figure (fig. 317) of the epidermis of Planocera folium, which is given, rather strangely, to illustrate the structure of Dendrocœlum lacteum. In this is shown an intercellular space, which is not unlike those we are considering, but is made to perforate the basement membrane. In the text the author says:

"Zwischen den Deckzellen finden sich oft deutlich hervortretende Intercellularlücken; auch wurden in den Zellen vieler Turbellarien (Sekera, Böhmig u. a.) helle aufsteigende Kanälchen beobachtet die einerseits die Grenzlamelle durchsetzen und mit dem Enchym zusammesshängen andererseits auch nach aussen ausmünden Können. Die Kanäle nehmen oft den character weiter vacuolen an. Sie sind wohl als Lymphkanälchen zu betrachten" (p. 296).

Here it is to be observed that it is the first clause of the first sentence only that refers to intercellular spaces. The rest deals with Sekera and Böhmig's "wasserklare Räume," which have never been shown to have anything to do with the intercellular spaces. The figure, unfortunately, seems to combine two quite distinct spaces—an intercellular space and

one of Böhmig's "Porenkanälchen"—which perforate the basement-membrane and the cells. The appearances represented, however, correspond neither to the Triclad Dendrocœlum nor to the Polyclad Planocera.

Digestive system.—The pharynx belongs to the type of von Graff's "pharynx rosulatus," which is characteristic of the Mesostomida and Proboscida. It is of rounded shape, its oral opening situated near the anterior end of its ventral wall, and its œsophageal near the posterior end of its dorsal wall. A thin, external, circular layer of muscular fibres lies outside the longitudinal layer. The latter consists of a single layer of broad flat fibres, whose edges are in close apposition. In the dorsal and ventral, but not the lateral, walls the radial muscles are arranged in regular rows. Between them are the gland cells, the secretion of these is very fluid, and, in the living specimens, is to be observed to run freely backwards and forwards in the spaces between the radial muscles. The internal circular layer of muscular fibres is much the thickest. The internal lining membrane has lost its epithelial character, and presents no trace of division into cells, though a nucleus occurs here and there at wide intervals. A number of unicellular glands lie around the junction of the pharynx with the intestine.

Retractor and protractor muscles pass from the pharynx through the parenchyma to the muscular layers of the body wall. One strong bundle passes straight forwards in the middle line from the anterior wall of the pharynx to the anterior extremity of the body, causing the brain to be slightly indented on its lower surface as it passes it. It is accompanied by red granular matter which renders the whole structure somewhat conspicuous in the living animal.

The intestine has an irregular space representing the lumen bounded by a masss of cells, which does not assume the character of an epithelium, and is not bounded externally by any muscular or fibrous layer. The enclosed cavity, though irregular in shape, must be fairly constant in position, since the dorso-ventral muscular fibres which traverse its wall in

great numbers, never pass through the cavity itself. This is very similar to the form of intestine characterising Gyrator, as described by Hallez (**18**, p. 568), and Macrorhynchus, Acrorhynchus, and other Proboscida according to von Graff (**14**, p. 94). A lumen is said to be absent in these in the adult condition, but by this is probably meant a definitely-bounded cavity.

Behind the intestine proper, between it and the posterior border, there was frequently to be observed in living specimens a space, the walls of which occasionally contracted; this was often observed to contain various foreign bodies, and, in some instances, living spermatozoa. A very slight pressure caused rupture and the discharge of the foreign bodies. In sections of some specimens this posterior diverticulum of the intestine is to be recognised as a space enclosing such objects as the setæ and other remnants of aquatic Oligochæta. This is a feature which I have not observed in any other Rhabdocœles, and one which appears to be of considerable importance.

Excretory System.—The excretory system was not traced out with adequate thoroughness. It was not in every specimen that vessels were visible at all in the living state; when contracted they seemed absolutely to vanish. The absence of specialised walls which this seems to imply is also indicated by the fact that of the numerous specimens sectioned (fixed mainly by strong Flemming or by Lang's solution), there is not one in which it is possible to follow with certainty the course even of the largest vessels.[1]

There are two main longitudinal vessels on each side, running more or less parallel with one another, the one on the dorsal, the other on the ventral side of the vitelline glands. Of these, the more dorsally situated bifurcates anteriorly close to the anterior margin of the pharynx, one of the branches running transversely inwards just behind

[1] In all probability young specimens would prove very much more favourable for the study of the vessels than the adults; but hitherto none but fully-mature animals have been observed.

the brain to join the corresponding vessel from the opposite side, while the other runs forwards to join the anterior prolongation of the ventral vessel.

Posteriorly both vessels usually become wider. In some specimens they united behind some distance from the middle line, and there was no evidence of any approximation of the right and left vessels, so that there would appear to be two separate excretory apertures at or near the posterior extremity.

Both of these longitudinal vessels were sometimes observed to contract peristaltically, sometimes with an approach to regularity.

A small number of ciliary flames were observed in very close relation to the main vessels—so close in fact that they moved with the movements of the vessel in such a way that they actually seemed to lie in the interior of the cells, forming its wall, or to be contained in extremely short branches.

In view of the meagreness of the above statement, it would scarcely be profitable to institute a systematic comparison between the excretory system of Anomalocœlus and those of described genera of Rhabdocœles. Apparently, so far as the arrangement of the main trunks and their mode of opening is concerned, the nearest alliance is with the Vorticida. The statements of O. Schmidt (**25**), Hallez (**18**, p. 21), and von Graff lead to the conclusion that, whereas in Vortex the main vessels open in the neighbourhood of the pharynx, if not actually into the pharyngeal sac, in Derostoma they open on the exterior by two apertures situated towards the posterior extremity. More recently Fuhrmann (**7**) has stated that in this respect Vortex and Derostoma are alike—the apertures in both genera being situated far back.

Reproductive Apparatus.—The testis is diffuse, its lobes extending throughout the part of the body situated behind the pharynx. Definite ducts are not developed, but by some means the sperms filter through channels in the parenchyma to the vesicula seminalis, which is situated in the anterior part of the space enclosed by the penis sheath.

From this the median ejaculatory duct runs backwards to the penial opening. Laterally a pair of strands of granule (prostate) ducts pass within the penis sheath, and strings of granules accumulate on either side of the ejaculatory duct to form a granule reservoir. The unicellular granule or prostate glands are scattered through the surrounding region, extending outwards nearly as far as the lateral border, and nearly to the posterior end.

The muscular penis sheath encloses the vesicula seminalis, the ejaculatory duct, the granule reservoir, and the penis. Posteriorly it divides into two layers, one becoming continuous with the wall of the penis, the other with that of the genital atrium.

The penis is a wide tube with thin and flexible walls, armed with chitinous teeth. The entire inner surface is beset with these teeth, which, extremely minute in front, become of relatively large size towards the opening of the penis into the atrium. The largest teeth, ·08 mm. in length, vary in number in different individuals from three or four to twelve or more. The teeth are situated on the inner surface of the eversible penis when at rest, with their apices directed inwards, and must bristle outwards when protrusion takes place. Between the penis sac and the enclosed structures is a space filled with some uncolourable substance, through which extends in sections an irregular felt-work of fine filaments. This becomes closer towards the line along which penis sheath passes into penis, and, notwithstanding their extreme fineness, these fibres are doubtless muscular. It is the contraction of the muscular sheath that, acting through the contained fluid, must be the means of causing the eversion of the penis. Annular fibres constitute a sphincter of the sheath. The ejaculatory duct, traversing the penis, opens into a cavity of small size with muscular walls, into which the oviduct and bursa copulatrix open; this is the genital atrium.

The single ovary lies close to the penis sheath, on its right side anteriorly. It is of compact elliptical form, with its long

axis directed obliquely outwards and backwards. It is covered with a thin investing layer containing a few widely sundered nuclei. Towards the inner end (base) one or more of these nuclei may be observed sunk below the level of the investing layer. The inner end of the ovary contains numerous nuclei intermediate in character between those of the investing layer and those of the more mature ova. That the cells of the investing layer actually become converted into ova in the mature animal there is not sufficient evidence to establish definitely.

With the exception of its inner end the ovary consists throughout of ova which extend right through the organ from side to side.

Close to the ovary, and somewhat posterior as well as external to it, is the receptaculum seminis. It has a wall composed of a small number of cells with homogeneous protoplasm, usually not clearly distinguishable from one another. Internally this wall gives off irregularly arranged processes, which, though not forming actual partitions, give the outer part of the cavity a cellular character. In the interior is always a mass of spermatozoa.

The oviduct, continuous with the receptaculum seminis, and opening towards the ovary, with the investment of which its outer layers are continuous, runs backwards, and then curves inwards to open into the atrium. Its muscular layers, thin in front, become greatly increased in thickness posteriorly. On the same side of the atrium as the oviduct (i. e. the right) opens a sac which appears, in position at least, to represent the bursa copulatrix. It is an oval sac, of small size, with thinnish wall, devoid of chitinous parts, which opens by a narrow neck or duct into the atrium.

The atrium does not open directly on the exterior, but leads into a rounded chamber which acts as a uterus, and from this the common genital aperture leads outwards. The uterus is thus, as in most Proboscida and others (von Graff, **14**, p. 139), merely the outer part of the atrium. In the uterus an egg, fully or partly completed, was very frequently found. Into

the cavity of the uterus, and into that of the bursa copulatrix, open the ducts of numerous unicellular glands—the shell glands,—which lie all round this part of the reproductive apparatus, and reach nearly as far as the lateral margin of the body. When an egg is not present in the uterus, the latter contains a quantity of fibrillar matter, which is perhaps coagulated secretion from the shell glands. In one living specimen the bursa copulatrix was observed to contain a number of rounded yellow globules, somewhat smaller than the vitelline spherules. These perhaps consist of the secretion of the shell glands, of which this sac may perhaps act as a reservoir.

In many specimens an egg was found in the uterus. Its shell was always so thick and opaque as to prevent any satisfactory view of its contents being obtained, except in one instance in which the formation of the egg-shell was incomplete. Enclosed within the transparent egg-shell in this specimen was the unsegmented oosperm surrounded by a mass of yolk-corpuscles. The oosperm was ·06 mm. in diameter, the corpuscles, on an average, about ·04 mm.

The vitelline glands form a continuous network extending throughout all the post-pharyngeal region. In the cytoplasm of the cells two kinds of material are produced. One of these appears first as extremely fine spherical granules or droplets, which become stained with eosin. These become aggregated into larger masses. The other substance is not colourable; it forms larger irregular masses. These two sets of elements escape from the cell into the irregular lumen of the gland; there is no evidence that the cell itself breaks up to form a part of the vitelline substance, and no evidence of the active cell-multiplication which such a process would entail.[1]

Moreover, in the completed egg the very characteristic and readily-recognisable nuclei of the vitelline cells are not to be recognised.

[1] Von Graff (**14**) describes a breaking down of the vitelline cells in Turbellaria in general, and Hallez (**18**) speaks of a continuous multiplication by division.

The fully-formed egg is ·25 mm. in greatest diameter. It is not spherical, but more nearly of the form of a hemisphere, with one side strongly convex and the other flat. It is enclosed in a hard, opaque, chitinous shell.

After being formed the egg does not appear to remain very long in the uterus, but soon drops out to lie among the mud. A good many were picked out of the sediment at the bottom of the vessel, and were treated with a solution recommended by Hemming for softening chitin, but I was disappointed to find that, though some of them had been kept in water for days after being laid, none of those of which successful sections were made had developed, each containing the unaltered oosperm (in one or two cases two) with its enclosing mass of vitelline matter.

Affinities.—The combination of characters with which Anomalocœlus presents us places it outside the limits of all the known families of the Rhabdocœla. The arrangement of the male ducts (with the vesicula seminalis and prostate-reservoir both enclosed in the penis-sheath) is a very exceptional one, occurring among previously-known forms only in the Vorticida, from all of which the new form is distinguished (1) by having diffuse or follicular testes, and (2) by having a pharynx rosulatus instead of a pharynx doliiformis.

The possession of follicular testes is also a somewhat exceptional character, occurring only in the Alloiocœla and in the genera Mecynostoma and Alaurina, with none of which Anomalocœlus has any close alliance in other respects.

Von Graff (**14**, p. 217) enumerates those genera in which the ovary is single. Of these Prorhynchus, Microstoma, and Anoplodium may be at once left out of account as widely differing from the new form in many respects. We may also rule out, as manifestly not nearly related, Omalostoma, with its pharynx simplex and two reproductive apertures, Byrsophlebs, which has compact rounded testes and two reproductive apertures, Mesostoma and Castrada,

with their elongated compact testes, and Solenopharynx, with its compact testes and elongated tubular pharynx.

There remain the Vorticid genera Vortex, Jensenia, and Derostoma, and the Proboscidan genus Gyrator. Of the former group Vortex and Jensenia have a pharynx doliiformis, compact paired testes, and unbranched vitelline glands, while Derostoma, though it also has compact testes, has the vitelline glands reticulate, and has sometimes a pharynx variabilis, while, like the other Vorticid genera, as mentioned above, it resembles Anomalocœlus in the special arrangement of the male ducts. Gyrator, even if we were to regard the strand of fibres in front of the brain in Anomalocœlus as a rudiment or vestige of a proboscis, is pretty widely removed, owing to the possession of compact testes and two reproductive apertures; and of the other Proboscida, Pseudorhynchus, which is perhaps the nearest, has two ovaries and compact testes.

On the whole, perhaps the closest affinities of Anomalocœlus are with some of the Vorticida; but these affinities are not sufficiently close to allow of the new genus being included in that group. Thus, if we follow the general scheme of division adopted by von Graff, it will be necessary to form a new family for the reception of this genus. Following von Graff's phraseology, we should define the family Anomalocœlidæ in the following terms:—Rhabdocœla with one reproductive aperture, a single ovary, the uterus combined with the genital atrium, reticulate vitelline glands, diffuse testes; with the vesicula seminalis and prostate reservoir enclosed in the penis-sheath; the penis armed with numerous chitinous teeth; the pharynx a pharynx rosulatus; the intestine devoid of a definite layer of epithelium.

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EXPLANATION OF PLATES 25—27,[1]

Illustrating Prof. W. A. Haswell's paper, "Studies on the Turbellaria."

List of Reference Letters.

a. Atrium. *ant*. Antrum femininum. *b*. Bursa copulatrix. *bl*. Bulb of bursa seminalis. *b. m*. Basement membrane. *br*. Brain. *b. s*. Bursa seminalis. *b. s*^{1}. Outer layer of wall of bursa seminalis. *b. s*^{2}. Inner layer of wall of bursa seminalis. *b. s. d*. Duct leading from antrum to bursa seminalis. *c*. So-called cuticle. *ch*. Chitinous mouth-piece. *ch. c*. Cells secreting substance of chitinous mouth-pieces. *c. m*. Layer of circular muscular fibres of body-wall. *cp*. Capillary canals in epidermis. *c. w*. Cell-wall of Alga.

[1] Figures 7, 9, 10, 12, 13, 16, 21, 22, 26, 28, and 32 were drawn by Mr. A. Cronin, of the Physiological Department of Sydney University, from my photographs of sections, of which they are, to all intents and purposes, facsimiles.

d. Ducts leading to chitinous mouth-pieces. *d'.* Enlargements of ducts of mouth-pieces. *e.* Eye. *ent.* Enteric cavity. *ep.* Epidermis. *ex.* Main excretory vessels. *f. n.* Frontal nerves. *g. d.* Ducts of granule glands. *gl.* Unicellular glands opening into antrum. *g. r.* Granule reservoir. *i.* Intestine. *k.* Supposed passage by which the eggs are discharged. *l. m.* Longitudinal muscular fibres of body-wall. *l. n.* Lateral nerve. *mth.* Mouth. *n.* Nucleus. *od.* Oviduct. *oo.* Oosperm. *ot.* Cavity in which otocyst lies. *ov.* Ovary. *p.* Penis. *ph.* Pharynx. *pl.* Polar body. *pr.* Processes connecting epidermal cells. *p. s.* Penis sheath. *rh.* Rhabdite-forming or other integumentary glands. *rh. d.* Ducts of integumentary glands. *r. s.* Receptaculum seminis. *sh.* Egg-membrane. *t.* Testis. *u.* Uterus. *v.* Vesicula seminalis. *x.* Position of supposed Laurer's canal. *z.* Group of cells behind brain. ♂. Male reproductive aperture. ♀. Female reproductive aperture.

PLATE 25.

FIG. 1.—Outline of Heterochærus magnified 20 times to show the relative positions of the mouth and reproductive apertures.

FIG. 2.—Semidiagrammatic view of the general organisation. × 40.

FIG. 3.—Transverse section through the epidermis and underlying layers. × 1500.

FIG. 4.—Pigment cell of epidermis. × 1675.

FIG. 5.—Rhabdites. × 1500.

FIG. 6.—Symbiotic Zoochlorella. × 600.

FIG. 7.—Portion of a horizontal section passing through the digestive syncytium, showing large numbers of the Algæ in the more superficial layers of the latter. Facsimile of a photograph.

FIG. 8.—Portion of a transverse section passing through the mouth.

FIG. 9.—Horizontal section passing through the so-called pharynx and the main enteric cavity. Facsimile of photograph.

FIG. 10.—Horizontal section on a higher (more dorsally situated) plane than that represented in Fig. 8, showing the enteric cavity and the digestive plasmodium, with portions of the testes at the sides. Facsimile of photograph.

FIG. 11.—Central part of a similar section more highly magnified. The black dots are the nuclei of the plasmodium.

FIG. 12.—Anterior part of a horizontal section passing through the brain, the eyes, and the cavity in which the otocyst is enclosed. Facsimile of photograph.

PLATE 26.

Fig. 13.—Horizontal section of Heterochærus showing a considerable part of the ventral nerve-plexus. Facsimile of photograph.

Fig. 14.—Part of a similar section to that represented in Fig. 12, more highly magnified.

Fig. 15 *a*.—Small sperm. × 1500.

Fig. 15 *b*.—Head end of giant sperm. × 1500.

Fig. 16.—Horizontal section in the plane of the ovaries, the bursæ, and their ducts. Facsimile of photograph.

Fig. 17.—General view (semidiagrammatic) of the left bursa seminalis with its duct and the antrum femininum.

Fig. 18.—Section through one of the chitinous mouth-pieces with its bulb and the wall of the bursa seminalis. From a horizontal section of the animal. × 700.

Fig. 19.—Lateral view of some of the chitinous elements of the mouth-piece in their natural relations.

Fig. 20.—Isolated chitinous elements of the mouth-piece.

Fig. 21.—Approximately sagittal section, showing Laurer's canal (*x*). Facsimile of photograph.

Fig. 22.—Sagittal section, showing the relation of Laurer's canal in another specimen. Here the canal shows no distinct lumen, but its course is clearly indicated by the arrangement of the histological elements between the dorsal aperture (*x*) and the antrum femininum (*ant*).

Fig. 23.—Section through the fertilised ovum passing somewhat obliquely through the first cleavage spindle and through one of the polar bodies. × 330.

Fig. 24.—Portion of a section through unfertilised ovum, showing appearance of chromatin elements after formation of polar bodies. × 600.

PLATE 27.

Fig. 25.—General view of the organisation of Anomalocœlus, seen from the ventral aspect. The testes and the vitelline glands are not represented. × 45.

Fig. 26.—Vertical section of the integument, showing transverse sections of some of the epidermal capillaries. Facsimile of photograph.

Fig. 27.—Vertical section through the epidermis. × 1500.

Fig. 28.—Portion of a horizontal section of the epidermis. Facsimile of photograph.

FIG. 29.—Surface view of a part of an entire preserved specimen. × 1500.

FIG. 30.—General view of the reproductive apparatus. × 280.

FIG. 31.—Horizontal section passing through the ovary, receptaculum seminis, and uterus. × 280.

FIG. 32.—Similar section on a somewhat higher plane. Facsimile of photograph.

FIG. 33.—Horizontal section passing through the vesicula seminalis and prostate reservoir. × 280.

FIG. 34.—Teeth of penis. *a*, Larger; *b*, smaller.

Notes on the Segmentation and Phylogeny of the Arthropoda, with an Account of the Maxillæ in Polyxenus lagurus.

By

George H. Carpenter, B.Sc.Lond., M.R.I.A.,
Professor of Zoology in the Royal College of Science, Dublin.

With Plate 28.

In the introductory note appended to his article on "The Structure and Classification of the Arthropoda" (**19**), Prof. E. Ray Lankester mentions "two authors who, while recently writing general essays" on the same subject, unfortunately overlooked his article, with its companion article on the Arachnida (**20**), as originally published in the 'Encyclopædia Britannica.' These authors are the late Professor A. S. Packard, whose paper (**24**) was read before the American Philosophical Society in April, 1903, and the present writer, whose essay (**3**) was communicated to the Royal Irish Academy in the succeeding month. It is a pleasure to respond to Prof. Lankester's courteous invitation to discuss further in this Journal the questions raised, in the light of his latest invaluable contributions which, as he hopes, will now surely "not fail to come under the notice of zoologists."

The Unity of the Arthropodan type.

The two papers published almost simultaneously by Packard and myself form a striking object-lesson on the wide divergence of opinion among students of Arthropod relationships.

While he advocates the dismemberment of the Arthropoda into five separate phyla, I support strongly the older view that they must be regarded as a "natural" monophyletic group. It must be admitted that most modern zoologists who have written on the subject incline towards the polyphyletic origin of the Arthropoda, though they may not go so far as Packard in separating from each other the various classes [Hutton, etc. (**14**)]. All the more cheering, therefore, has been Lankester's vindication of the essential unity of the Arthropodan type, and in the 'Encyclopædia' articles his position is even stronger than in the earlier paper (**18**) quoted in my recent essay.

It may be objected that, with such differences of opinion as to the theoretical interpretation of well-known facts, further discussion must prove useless, and that fresh facts are needed before any secure conclusions can be reached. But, if the fundamental assumptions underlying all modern phylogenetic speculation be accepted, it seems to me that we have plenty of facts from which to arrive at a conclusion, and that our conclusion can only be Lankester's. Unless zoologists as a whole have "followed wandering fires," in believing that "community of descent is the bond which is partially revealed to us by our classifications," they must surely admit that the "remarkable and distinctive features of structure which hold the Arthropoda together render it impossible to conceive of them as having a polyphyletic origin."

Those zoologists who wish to divide the Arthropoda into several distinct phyla constantly refer to the most obvious external features of the group—the hard, segmented exoskeleton and jointed limbs—as conceivably due to "convergence" or "homoplasy." But such a superficial view overlooks the profoundly important internal characters which distinguish the Arthropoda: the reduced cœlom; the heart with paired openings, leading from a "pericardium" made up of greatly enlarged blood-spaces; the mesodermal excretory tubes; the apparent absence of true nephridia; the absence

of ciliated epithelium; the possession of striated muscular tissue. And if it be objected that any of these are the necessary accompaniments of a hard exoskeleton and jointed limbs, let it be remembered that all of them (except the two last-mentioned)[1] are displayed by that class of Arthropoda (the Malacopoda) whose members are not arthropodous. I must confess that to me belief in the polyphyletic origin of the Vertebrata, the Echinoderma, or the Mollusca might be maintained with as great—or as little—reason as belief in the multiple descent of the Arthropoda.

Numerical Correspondence in Segmentation.

In my own recent essay the endeavour was made to prove an exact numerical correspondence in segmentation between typical Crustacea, Insecta, and Arachnida. The establishment of such a fact would conclusively demonstrate that the Arthropoda are monophyletic, since the independent origin of three classes of similarly formed animals with exactly the same number of primitive segments is surely incredible. In trying some years ago to trace homologies between the appendages of the Crayfish, Cockroach and Scorpion, I was astonished at the apparent identity in the number of segments in those three animals, and it was with much gratification that I afterwards found the same correspondence to have been suggested by Huxley (**15**) nearly fifty years ago, when he wrote: "I venture to think it a matter of no small moment if it can be proved that a Lobster, a Cockroach and a Scorpion are composed of the same primitive number of somites."

The facts and arguments that can be brought forward in favour of this contention have been set forth in my recent paper with considerable detail. The appended table showing the correspondence in segmentation between members of the various arthropod classes will serve for the present occasion as a sufficient summary of the evidence, and the references will enable those interested to estimate the authority on

[1] Hewitt has, however, lately pointed out that the muscle-fibres which work the jaw-levers of Peripatus are striated ('Manchester Memoirs,' vol. l, p. 4).

Somites.	ARACHNIDA.			CRUSTACEA.	
	SCORPIONIDA.	MEROSTOMATA.	PYCNOGONIDA.	TRILOBITA.	LEPTOSTRACA.
1	Eyes	Eyes	Eyes	Eyes	Stalked eyes
2	Rostrum	—	—	Feelers (Triarthrus) Beecher (1)	Antennules
3	Cheliceræ	Cheliceræ	Cheliceræ	1st biramous head limbs	Antennæ
4	[Vestigial segment in Epeira]. Lendl (22)	—	Palpi	2nd „	Mandibles
5	Pedipalps	1st simple limbs	Ovigerous legs	3rd „	Maxillulæ
6	1st legs	2nd „	1st legs, ♀	4th „	Maxillæ
7	2nd „	3rd „	2nd „ ♀	1st trunk-limbs	1st thoracic limbs
8	3rd „	4th „	3rd „ ♂ ♀	2nd „	2nd „
9	4th „	Paddle-limbs	4th „ ♂ ♀	3rd „	3rd „
10	Sternum (?). Pregenital segment. Brauer (2)	Chilaria	(Abdomen reduced and condensed)	4th „	4th „
11	Genital operculum, ♂ ♀[1]	Genital operculum, ♂ ♀	—	5th „	5th „
12	Pectines	1st gill-plates	—	6th „	6th „ ♀
13	1st lung-books	2nd „	—	7th „	7th „
14	2nd „	3rd „	—	8th „	8th „ ♂
15	3rd „	4th „	—	9th „	1st pleopods
16	4th „	5th „	—	10th „	2nd „
17	7th abdominal segment	7th abdominal segment	—	11th „	3rd „
18	1st tail-segment	1st tail-segment	—	12th „	4th „
19	2nd „	2nd „	—	13th „	5th „
20	3rd „	3rd „	—	14th „	6th „
21	4th „	4th „	—	15th „ (Number of trunk segments highly variable)	Limbless segment
22	Anal segment Sting	Anal segment Telson	Anal segment (?)	Anal segment Telson	Anal segment Furca

[1] The signs ♂ ♀ indicate the

CRUSTACEA. Malacostraca (Astacus).	INSECTA.	SYMPHYLA.	DIPLOPODA (Polyxenus).	CHILOPODA (Lithobius).	Somites.
Stalked eyes	Eyes	—	Eyes	Eyes. [Pre-antennal rudiment in Scolopendra. Heymons (11)]	1
Antennules	Feelers	Feelers	Feelers	Feelers	2
Antennæ	Tritocerebral rudiments. Wheeler (30), Uzel (29)	—	—	Tritocerebral rudiments. Heymons (11)	3
Mandibles	Mandibles	Mandibles	Mandibles	Mandibles	4
Maxillulæ	Maxillulæ. Hansen (6), Folsom (4)	Maxillulæ. Hansen (7), Carpenter (3)	Maxillulæ [Figs. 1—5, *mxl*, of present paper]	1st maxillæ	5
Maxillæ	1st maxillæ	1st maxillæ	1st maxillæ	2nd „	6
1st maxillipeds	2nd „ (labium)	2nd „ (labium). Hansen (7)	2nd „ (labium) (Figs. 1—5, *la*, of present paper)	Poison-feet	7
2nd „	1st legs	1st legs	1st legs	1st legs	8
3rd „	2nd „	2nd „	2nd „	2nd „	9
Chelæ	3rd „	3rd „ ♂ ♀	3rd „ ♂ ♀	3rd „	10
1st walking legs	1st abdominal segment	4th „	4th „	4th „	11
2nd „ ♀	2nd „	5th „	5th „	5th „	12
3rd „	3rd „	6th „	6th „	6th „	13
4th „ ♂	4th „	7th „	7th „	7th „	14
1st pleopods	5th „	8th „	8th „	8th „	15
2nd „	6th „	9th „	9th „	9th „	16
3rd „	7th „	10th „	10th „	10th „	17
4th „	8th „ ♀	11th „	11th „	11th „	18
5th „	9th „ ♂	12th „	12th „	12th „	19
[Segment represented in Gnathophausia]. See Sars (26)	10th „	Reduced limbs	13th „	13th „	20
Uropods	Cercopods. Heymons (9)	Cercopods	Limbless segment	14th „	21
Anal segment	Anal segment	Anal segment	Anal segment	15th „	22
Telson	Median cercopod in Thysanura and Ephemerida			Genital limbs, ♂ ♀	23
				Anal segment	24

positions of the genital openings.

which rests the existence of each somite, not evidently present in the various animals.

The conclusion to be drawn from this numerical correspondence in segmentation between typical members of the leading Arthropod classes is not merely that there must have existed common ancestors of all, with distinctively arthropodous characters, but that these ancestors must have possessed the definite number of segments still traceable in their descendants. It follows from this that Arthropods with very numerous segments—Apus, Julus, or Geophilus, for example—must be regarded as, in that respect at least, specialised forms. "Rich segmentation" has been so freely assumed to be necessarily a primitive character, that my view will probably not find ready acceptance with many students. For the present I simply lay stress on the fact that the Symphyla (which combine so strikingly the characters of Insects, Centipedes and Millipedes) and Polyxenus (which belongs to the primitive diplopodan order Pselaphognatha) both exhibit the typical and definite number of segments characterising "a Lobster, a Cockroach, and a Scorpion."

Kinship between Insects and Crustaceans.

Perhaps the point which needs strongest enforcement in order to recall zoologists to a reasonable view of the Arthropoda as one phylum is the somewhat close relationship that exists between Insects and Crustaceans. I have already (**3**, pp. 343–7) discussed this relationship at some length, and the arguments in favour of it are strongly pressed in Lankester's article (**19**, p. 573). The existence in the Collembola and Thysanura of maxillulæ whose true nature as a pair of appendages between the mandibles and first maxillæ has been established by Hansen (**6**) and Folsom (**4**), shows the closest correspondence between the Insectan and the Crustacean head, with which the foremost trunk-segment undergoes more or less complete fusion, its appendages forming the first maxillipeds in the Malacostraca and the second maxillæ

(labium) in the Insects. Together with these correspondences we find (see Table, p. 472) an exact numerical agreement between the trunk-segments of an Insect and those of a Malacostracan. Heymons has shown (**9**) that the cercopods of an insect belong to the embryonic eleventh abdominal segment, which, during development, fuses with the tenth—the evident cercopod-bearing segment in the adult. That an exactly similar fusion of the hindmost trunk-segments has taken place in the Malacostraca is shown by the presence of an additional somite in the Leptostraca, and by the structure of the uropod-bearing segment—evidently composed of two fused somites—in certain Schizopods of the genus Gnathophausia described and figured by Sars (**26**).[1]

I rejoice to find myself in such close agreement with Lankester's main position, for if the kinship of Insects to Crustaceans be generally admitted, belief in the polyphyletic origin of Arthropods must be rapidly given up. He, indeed, considers the relationship between the two classes closer than I do, for he writes: "It seems probable that in the case of the Hexapoda, at any rate, the point of departure [from the Crustacean main stem] was subsequent to the attainment of the nomomeristic character presented by the higher grade of Crustacea." My view is that the most primitive Crustacea were nomomeristic, and that the ancestors of the Insects and their allies must be sought far down the Crustacean stem. This difference of opinion in details depends on the differences between our respective estimates of the relationship of Insects to the various classes of "Myriapods," and between our views as to the nature of the most primitive Crustaceans.

Relationships between Insecta, Chilopoda, and Diplopoda.

Turning, then, to the discussion of these details, I take first the question of the relationship between Insects and the other classes of tracheate Arthropods. It is satisfactory to

[1] I am indebted to my friend Dr. W. T. Calman for kindly bringing this fact to my notice.

find Lankester's support given to the necessary abolition of the "Class Myriapoda." It surely cannot be long before zoologists generally will agree that the Chilopoda stand nearer to the Insecta than to the Diplopoda. This relationship of Centipedes to Insects would, however, be distinctly minimised by the acceptance of Lankester's suggestion (**19**, p. 570) that the pre-antennal, vestigial appendages in the embryo of Scolopendra, as described by Heymons (**11**), correspond to the functional feelers of Insects, and that the feelers of Centipedes must therefore be compared with the trito-cerebral vestiges of Insects. Heymons refers the pre-antennal vestiges which he discovered to the hinder region of the protocerebron, and they may be most probably regarded as representing the eye-stalks of primitive Crustacean ancestors. Further, Heymons detected in Scolopendra the presence of a trito-cerebral segment, thus showing the closest possible agreement between the Chilopodan and the Insectan head. I regard the two pairs of maxillæ in a centipede as corresponding with the maxillulæ and first maxillæ of the Aptera; the foremost trunk-segment, whose appendages are the poison-feet, will then represent the second maxillary (labial) segment of Insects. This segment is incompletely fused with the head in many insects—the cervical sclerites of the Cockroach apparently belong to it; and there can be little doubt that it has become associated independently with the head in various Crustacean orders (such as the Amphipoda and the Isopoda) as well as in Insects and Centipedes.

But while the Chilopoda must be regarded as near allies of the Insecta, the Diplopoda are by Lankester removed far away from these classes. This is one of the most important of his interpretations with which I am unable to agree. He contrasts the mono-prosthomerous condition of the head in Diplopods with its triprosthomerous condition in Chilopods and Insects, and states that in the first-mentioned class "only one somite following the first post-oral or mandibular segment has its appendages modified as jaws" (**19**, p. 556).

Now, the head of a Diplopod cannot be regarded as monoprosthomerous. The ocular segment must be accounted for, and its presence makes the antennal segment deutocerebral as in Insects. As yet we have no embryological evidence of the tritocerebral segment of any millipede, but if the appendages of the diplopod head can be shown by comparative morphology to correspond with those of the insectan head, we may expect with confidence that the existence of the tritocerebral segment in an embryo Millipede will one day be demonstrated.

The Symphyla are regarded by Lankester (**19**, p. 567) as belonging to the Diplopoda. The forward position of their genital aperture shows, indeed, that they have affinity with that class, but it is impossible to accept Schmidt's contention (**28**) that they are specialised millipedes. The aspect of the head and feelers of Scolopendrella has struck all observers as distinctively thysanuran. It is certain that there are two evident pairs of maxillæ behind the mandibles, as shown by Hansen (**7**) and Grassi (**5**); and it occurred to me that if the characteristic maxillulæ of the Collembola and Thysanura could be demonstrated in Scolopendrella, its affinity with those lower insects could no longer be regarded as doubtful. I found the maxillulæ, and figured them in my recent paper (**3**, fig. 3); and while that paper was in the press a brief account of them, with figures, was published by Hansen (**7**).

Scolopendrella, then, has three pairs of mouth-appendages behind the mandibles; and when we find that in the number of its trunk-segments it agrees exactly with a typical insect, and when we remember that in many Thysanura nearly all the abdominal segments carry short appendages, we cannot deny the near relationship of the Symphyla to the Aptera. And admitting such an affinity, without denying the affinity of the Symphyla to the Diplopoda, we bring all the tracheate[1] classes near to one another, for a rather close kinship between the Insecta and the Chilopoda is admitted on all hands.

[1] I do not include the Arachnida among the "tracheate" classes.

The Maxillæ of Polyxenus.

I am now glad to be able to show that Polyxenus—an undoubted Diplopod—closely resembles the lowest Insecta and the Symphyla in the structure of its mouth-organs. The gnathochilarium of a millipede is generally believed, on embryological grounds, to consist of one pair only of maxillæ, although its structure strongly suggests the presence of two pairs, the anterior of which has come—as in Scolopendrella—to lie externally to the hinder. Latzel, in his description (**21**) of Polyxenus, states that the nature of its gnathochilarium is doubtful, a pair of reduced appendages with conspicuous palps being evident, and in front of these a flat plate, apparently comparable to the hypopharynx of ordinary millipedes.

The collection of Diplopoda in the Dublin Museum contains numerous specimens of Polyxenus lagurus, collected many years ago by the late Robert Templeton; my friend Dr. R. F. Scharff has kindly given me facilities for examining this material. By dissecting heads, partially cleared in potash, under a compound erecting microscope, with fine needles, one can determine accurately the arrangement of the parts. The palps, very imperfectly segmented, rugose, and spiny (figs. 1, 4 *mx. p.*), and the rounded lobes, also rugose and spiny (fig. 1, *mx. lo.*), each partially divided into a larger posterior and a smaller anterior section (figs. 4, 5, *mx. lo.*), are borne upon basal sclerites (figs. 1, 4, 5, *mx.*) which are attached proximally to the ventral head-skeleton, and fused distally and centrally with the labium (see figs. 1–4, *la*). Otherwise, however, they lie within (*i. e.* anteriorly to) the labium. This latter organ consists of a narrow, transverse basal membrane (fig. 1, *la'*) attached to the ventral head-skeleton, and bearing a pair of broadly trapezoidal sclerites (fig. 1, *la*) which meet in a central, longitudinal suture. This labium clearly corresponds with the internal stipites of the typical diplopodan gnathochilarium, and represents, as I believe, a reduced second pair of maxillæ.

The basal sclerite of the palp-bearing maxilla in Polyxenus agrees with the external stipes of the Julid gnathochilarium. These are the first pair of maxillæ, and in Polyxenus they lie for the most part in front of, not exteriorly to, the second maxillæ (labium). Thus we see the specialised diplopodan structure in process of formation from two pairs of jaws. In the relative positions of maxillæ and labium, Polyxenus is more primitive than Scolopendrella, in their partial fusion more specialised.

When the hind head skeleton of Polyxenus is viewed from within, we find that the flat plate seen and figured by Latzel, consists not of the tongue only but of that organ (figs. 1, 2, 4 *li*) together with a pair of maxillulæ (figs. 1–5, *mxl*). The outer edge of these jaws can be clearly seen from behind (fig. 1), and when the maxillula is viewed from within (fig. 2) or isolated (fig. 3) its likeness to the corresponding appendage in Scolopendrella or a Springtail is unmistakable. It is a delicate, transparent, chitinous plate with outer and inner lobes, each with two or three prominences and the inner with several bristles at the tip. On its posterior face, close to the tip, the maxillula bears a long flagellate process (figs. 1, 3, 4 *fl.*) which assumes various positions with reference to the other structures (cf. figs. 1, 3) in different individuals which I have studied. The inner lobe of the maxillula is in close contact with the tongue, and the lateral region of the tongue unites with the hinder face of the maxillula near its outer edge (figs. 1, 2 *li*).

When we remember that the maxillular segment has only once been detected in an embryo insect, it is not surprising that it has hitherto been overlooked by the few zoologists who have studied the development of millipedes. The general agreement of embryologists that the diplopodan gnathochilarium arises from one pair only of maxillary appendages is, however, a more serious objection to the interpretation of the structure which I, following Hansen (**6**), advocate here. But Heymons (**10**), in his account of the germ-band of Julus and Glomeris, describes a post-maxillary segment which he

states to be always limbless. I confidently anticipate future proof that the central portions of the gnathochilarium really belong to this post-maxillary segment. In any case, this embryonic segment supports the view that the head of a Diplopod agrees closely with that of an Insect—an agreement now shown more clearly by the presence of maxillulæ in Polyxenus. And considering that Polyxenus, which shows this agreement so unmistakably, has exactly the same number of trunk-segments as Scolopendrella or a Thysanuran, we cannot doubt the near relationship of Insects to Millipedes.

Isolated Position of the Malacopoda.

The Malacopoda (Peripatidæ) must certainly be separated widely from the tracheates, as a grade of Arthropoda far lower than any other class. Belief in the polyphyletic origin of Arthropods has been largely supported by a supposed close relationship between the Malacopoda, the Chilopoda, and the Insecta. But a study of the characters of the "Protarthropoda" and "Euarthropoda" as given by Lankester (**19**, pp. 564–6) must surely convince the reader that Insects and Centipedes are much more nearly related to Crustaceans than to Peripatids.

Relationship between Crustacea and Arachnida.

My views on the general subject of the relationship between Insecta and Crustacea agree, then, in the main with Lankester's, while we differ on some points of detail. It is even so with regard to our interpretation of the kinship between Crustacea and Arachnida. He suggests (**19**, p. 573) that the most primitive Crustaceans "were not very far removed from the aquatic ancestors (Trilobites) of the Arachnida, but differed essentially from them by the higher specialisation of the head," while in my essay (**3**, p. 349) occurs the statement that "there is no difficulty in tracing back the Merostomata, the Xiphosura, and the Trilobita to a common ancestry; and thus the Arachnida as a class, like the Insecta, have been

evolved from Crustaceans," I firmly believe that, in any rational system of Arthropod classification, the Merostomata and Xiphosura must be regarded as Arachnids. Lankester claims that the Trilobita also are Arachnids, while in my opinion their possession of feelers and biramous limbs should lead us to consider them as Crustaceans. This is, however, rather a question of terminology than of principle. Lankester admits that the Trilobita had a common ancestry with the Crustacea, and I suggest "that the Arachnida arose from the base of the Trilobitan branch rather than from the main Crustacean stem."

The particular question on which Lankester and I are not in agreement is the nature of the segmentation of the proto-Arachnida. Regarding the Arachnida as descended from the Trilobita, and laying stress on the indefinite and often rich segmentation exhibited by many members of the latter order, he suggests that the definite number of primitive somites characterising typical Arachnids has been reached by reduction from an originally anomomeristic condition. Believing, on the other hand, that the number of segments in an Arachnid agrees exactly with the number in a typical Crustacean or Insect, I hold to the view that the ancestors of all three classes possessed such a definite segmentation. The indefinite segmentation of the Trilobita presents no difficulty to this view. I have shown in my recent paper (**3**, p. 333) that the average number of trunk segments in the genera of Trilobites increases steadily as we trace their history from Cambrian to Carboniferous, and that among the most primitive members of the group known (Olenellus) were species with only sixteen trunk-segments. It is reasonable, therefore, to suppose that the richly-segmented Trilobites were developed, like the Centipedes and Millipedes, from ancestors with moderate and definite segmentation.

This agreement in the number of body-segments among the Crustacea, Insecta and Arachnida, was long ago suggested, as mentioned above, by Huxley (**15**). Lankester, in referring to Huxley's view (**19**, p. 545), rejects it on the ground that

the older authors did not consider the nature (i. e. whether primitively pre-oral or post-oral) of the cephalic somites, and that the head of an Arachnid is less complex than that of a Crustacean or an Insect. My own belief is that the older authors' concept of a primitive Arthropod head with five limb-bearing somites (six if the eyes be regarded as appendicular), all of which are now known to have been originally post-oral, will yet be recognised as sound. Such a head clearly characterised the trilobites, and among them one somite only—that bearing the simple feelers of Triarthrus—had, in addition to the ocular somite, become pre-oral. These feelers must be compared with the antennules, not with the eye-stalks, of a typical Crustacean, and the foremost pair of a trilobite's biramous head-limbs (on whose segment the mouth apparently opened) with the Crustacean antennæ. This latter pair of limbs—behind which the mouth has shifted in Arachnids as in all recent Crustacea—are represented by the Arachnid cheliceræ. Embryological evidence for this comparison—suggested by many students of Arthropod morphology—might be found in the vestigial feelers described by von Jaworowski (**16**) in the embryo of the spider Trochosa, but the appendicular nature of the structures seen by him is open to doubt. More probably the trilobitan antennules are represented by the paired rudiments whence the Arachnid rostrum arises. The nerves of the rostrum take their origin, according to Korschelt and Heider (**17**, vol. 3, p. 13), from a small unpaired section of the brain in front of the cheliceral ganglia and behind the large protocerebral lobes whence the optic nerves arise. Brauer's description and figures of the developing scorpion's brain (**2**) further confirm the view that the cheliceral ganglia are tritocerebral.

The presence of three free leg-bearing somites in the Solifugida, the Palpigradi, and the Pycnogonida suggests that the arachnid cephalothorax has been formed by the union of three trunk-segments with the primitive Arthropodan head. If this suggestion be accepted it is found that the Arachnida agree exactly with the typical Insecta and Crustacea in the

number of their trunk-segments (see Table, p. 472), but that there is a missing head-segment in all Arachnida except the Pycnogonida. The only embryological evidence for such a segment is afforded by the evanescent somite with vestigial limbs described by Lendl (**22**) in the embryo of the spider Epeira, between the somite of the cheliceræ and that of the pedipalps. Unfortunately, Lendl gives no figures, but his description is precise, and the late appearance of the head-segments in the embryonic development of Arachnids generally makes it easy to believe that a segment has disappeared from that region. The suggested homology between the appendages of this lost segment and the palps of a Pycnogon brings the four pairs of walking-legs among the Pycnogonida into correspondence with those of spiders. However, Hodgson's (**12**) recent discovery in the Antarctic seas of a Nymphonid, and his rediscovery of a Collosendeid (**13**) with five pairs of legs may be thought to render this comparison of little value; for if Pentanymphon and Decalopoda be primitive forms, we must consider the Pycnogonida as probably descended from ancestors with many pairs of similar legs, most of which have disappeared successively from behind forwards. But the fifth pair of legs in these genera may possibly represent a comparatively new development, and, in either case, if the view now advocated be accepted, we find that the head of a Solifugid, of a Pycnogon, of a Trilobite, and of a typical Crustacean correspond exactly. Our conclusion is, therefore, that the Arachnida must be traced back to proto-Trilobitan ancestors which possessed a head with five and a trunk with fifteen limb-bearing segments.

As to the relationships between the orders of Arachnida, my views already published agree in all essential points with those of Lankester and Pocock (**20**), except that I would assign to the Solifugida and the Palpigradi a more primitive position than they do in the scheme of arrangement. The free thoracic segments of those animals forbid us to derive them from a scorpionoid form in which the segments of the cephalothorax had already become aggregated. In their

tracheal respiration, however, as in many other characters, the Solifugida are highly specialised. I must confess myself unable to understand how some students of Arachnid morphology have concluded that tracheal tubes preceded lung-books in the evolution of the class.

The position assigned by Lankester to the Pycnogonida—within the Arachnid class, but at a lower grade than all the other orders from the Merostomata onwards—agrees closely with my own views as shown in the genealogical table which accompanies my recent paper (**3**, pl. vi). Pycnogons certainly differ from typical Arachnids so markedly that their separation in a distinct sub-class, as proposed by Lankester, is abundantly justified. But their division into three orders, as proposed several years ago by Sars (**27**), and now, under new ordinal names, by Pocock (**20**, pp. 224-5) is open to serious objection. Despite differences in points of detail, the genera of Pycnogonida show such fundamental unity of structure combined with such varying relationships among themselves that it is best to comprise them all within a single order. Certainly their separation into ordinal groups formed on the presence or absence of the cheliceræ or the palps must lead to many unnatural associations. For example, the relationship of the Pallenidæ to the Nymphonidæ which is expressed by the scheme of Sars and Pocock is undeniable. But within the Pallenidæ must be included forms—Phoxichilidium and allies—with cheliceræ variously developed and with palps vestigial or absent, which lead on towards Phoxichilus, a genus placed, however, by Sars and Pocock in a distinct "order" along with Pycnogonum. Beyond the total absence of cheliceræ and palps, Phoxichilus and Pycnogonum have little in common, and the loss of a particular pair of appendages might readily be sustained independently by two or more divergent genera of a degenerate group.

Ancestry of the Crustacea.

If, then, the common ancestors of Arachnids, Crustaceans, Insects, Centipedes and Millipedes, possessed a definite and

limited number of segments, and if these ancestors were essentially Crustacean in nature, we are precluded from considering Apus or any similar form as the representative of such an ancestral stock. And this suggests the last, and perhaps the most important question on which my recently-published views are in disagreement with Lankester's: What were the earliest Crustacea like, and what was their relationship to the Annelida?

On the assumption that Apus and its allies represent the most primitive of living Crustaceans, the foliaceous appendages of those animals are derived directly from Annelidan parapodia, and the ancestry of the Arthropoda is traced directly to richly-segmented Chætopods. The typical biramous Crustacean limb is, on this view, to be understood as a specialization of the Branchiopodan limb owing to the suppression of four of the endites, and the elongation of the remaining two, as explained by Lankester (**19**, pp. 551–9). But there is much reason for regarding the Branchiopoda as somewhat specialized animals. The extreme reduction of both pairs of feelers, the absence of a palp on either mandible or first maxilla (maxillula) and its vestigial condition on the second maxilla, the modification of certain endites of the first trunk limb as tactile organs—all these are undoubtedly specializations, and it is not unreasonable to suppose that the foliaceous condition of the other trunk limb may be regarded as a specialization. Such foliaceous development is most nearly matched, among other Crustacea, in the maxillæ and anterior maxillipeds of many genera belonging to different orders. It is a modification that accompanies excessive crowding of the appendages, and in Apus we may readily conceive that the secondary development of very numerous pairs of limbs has led to their crowding together and to the correlated development of the leaf-like endites and exites.

For limbs like those of Apus are quite exceptional among the Crustacea; while the typical biramous limb, with or without an exite (epipodite) occurs in members of every crustacean

order and is present in every Nauplius larva. Surely, therefore, the presumption is that the foliaceous limb has been elaborated from the biramous and not that the latter has been simplified from the former. Now, the Crustacean orders in which the biramous (naupliar) condition of the limbs is most strikingly preserved are the Copepoda, the Trilobita and the Leptostraca. The trunk-limbs of the last are often mentioned as phyllopodous in character, but the foliaceous structures on the thoracic leg of Nebalia are the exopodite and epipodite of a typical crustacean appendage, while in Paranebalia —see Sars (**25**)—the exopodite is slender and fringed, and the epipodite quite small. Similarly, the close comparison often made between the Branchiopoda and the Trilobita is misleading, for trilobitan limbs—according to Beecher (**1**)—are biramous, and their foliaceous condition in the tail-region is due to a flattening of the segments of a typical endopodite (**20**, p. 217, fig. 35), not to the presence of numerous endites like those of Apus. My ideal ancestral crustacean is not therefore a Branchiopodan, but a form combining the most primitive characters of Triarthrus, Nebalia and Calanus. In this view I am largely in agreement with Sars (**25**), who suggests that the Nebaliidæ sprang from Copepod-like ancestors, and that the Branchiopoda are a "considerably modified" offshoot (less primitive than the Leptostraca) from the same stem. I cannot follow Sars, however, in denying near relationship between the Leptostraca and the Malacostraca. He would derive the latter group independently from naupliiform ancestors, but the generally accepted view that the Leptostraca are to some extent ancestral to the Malacostraca has much evidence in its favour. I have already suggested (p. 475, supra) how the two hindmost abdominal segments have undergone fusion in the Malacostraca as in the Insecta.

If we accept the nauplius larva as representing the ancestral stock of the Crustacea, and therefore of all Arthropods, and if we follow many special students of the Crustacea in considering the Copepoda as the most primitive living order of

the class, we may be tempted to regard the small number of trunk-segments in a typical Copepod as indicating a stage between the naupliar condition and the segmentation of the common ancestors of Arachnida, Crustacea, and Insecta with their fifteen limb-bearing trunk-segments. But when we consider the shortening that has undoubtedly taken place in many Crustaceans—*e. g.* the Cladocera, the Ostracoda, the Cirripedia—we must admit the great probability that the Copepoda have also undergone reduction in their segmentation, and that the Leptostraca represent in this respect the primitive Arthropodous condition.

Arthropoda and Annelida.

The ancestral standing of the nauplius suggested nearly forty years ago by Müller (**23**) has not been abandoned by all modern students of the Crustacea, although the tendency of most present-day zoologists is to derive Arthropods directly from elongate Annelid worms. No student can deny some relationship between Arthropods and Annelids, but we must ask whether the Annelida were the direct ancestors of the Arthropoda or whether both phyla must be traced back to an immensely remote common ancestry?

The opinion that the Apodidæ are the most primitive of Crustacea is held by those zoologists who believe in the homology between the parts of a polychætan parapodium and those of a branchiopodan limb, and in the direct derivation of Arthropods from Annelids. This position, first suggested, I believe, by Hatschek (**8**), is virtually accepted by Lankester, who considers that the most primitive Arthropoda "arose by modification of parapodiate annulate worms not very unlike some of the existing Chætopods" (**19**, p. 527). I have always regarded with suspicion this modern hypothesis, because its acceptance has led to the present widespread disbelief in the monophyletic origin of the Arthropoda. And it seems that many recent morphological advances have tended to sharpen the distinction between all Annelids and all

Arthropods. The hæmocœlic body cavity and reduced cœlom of Arthropods offer the most marked contrast to the closed vascular system and extensive cœlom of Annelids. True nephridia, so characteristic of the Annelids, are apparently unknown among the Arthropoda. The only characters common to the two groups are the metameric segmentation, the presence of paired hollow limbs, and paired cœlomoducts arranged segmentally, and the general structure of the nervous system. This last-named character—on which especial stress has been laid as indicative of affinity—is a necessary accompaniment of metameric segmentation in animals whose brain—whether archicerebrum or syncerebrum—is connected, by a nerve-ring surrounding the gullet, with paired lateral or ventral cords, so soon as the nerve-cells become aggregated segmentally into ganglia. And that such aggregation has arisen independently among Annelids and among Arthropods is strongly suggested by the condition of the nervous system in the Malacopoda (Onychophora), which, though essentially Arthropoda, possess laterally-situated nerve-cords without distinct ganglia. Thus the most primitive of living Arthropods exhibit a condition of the nervous system more primitive than is to be found among those Annelids from which the Arthropoda are believed by most zoologists to have been evolved. The structure of the eye is, indeed, the only unequivocal annelidan character exhibited by a Peripatid.

The more probable conclusion therefore seems to be not that Arthropods and Polychæte Annelids stand to each other in the relation of descendants to ancestors, but that the two groups represent specialised collateral branches from a common stock. My own view is that these common ancestors were microscopic animals, unsegmented, or with comparatively few segments between a broad head-lobe and a narrow tail-somite. The occurrence of the nauplius larva in some members of all the great Crustacean groups justifies the phylogenetic importance attached to that form by Müller, whose views will probably, in the near future, again dominate zoological opinion on the subject. Comparison of a Nauplius,

a Rotifer like Pedalion, and a Trocophore helps us to conceive the hypothetical ancestral stock of Annelids and Arthropods. But the lines of evolution must, on that assumption, converge in a past so immensely remote that we are hardly justified in including both Annelids and Arthropods within the limits of a single phylum. Good reason might indeed be shown for deriving the Mollusca from the same common ancestry. We conclude, then, that the divergences between Annelids and Arthropods are profound, while their correspondences are comparatively superficial. The Arthropoda must stand, therefore, in our zoological systems, not only as a natural monophyletic group, but also sharply marked off from other phyla as a great primary division of the Animal Kingdom.

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EXPLANATION OF PLATE 28,

Illustrating Professor Carpenter's paper on "Notes on the Segmentation and Phylogeny of the Arthropoda, with an Account of the Maxillæ in Polyxenus lagurus."

Reference Letters.

li. Tongue or hypopharynx. *la.* Labium. *la'.* Basal membrane of labium. *mx.* Maxilla, basal sclerite. *mx. lob.* Maxilla, lobe. *mx. p.* Maxilla, palp. *mxl.* Maxillula. *fl.* Flagellate process. *m. add.* Adductor muscle. *m. abd.* Abductor muscle. *md.* Tip of mandible. *lbr.* Edge of labrum.

Fig. 1.—External (posterior) view of the maxillæ of Polyxenus lagurus. The left maxilla has been removed to show the maxillula and tongue.

Fig. 2.—Internal (anterior) view of left maxillula and tongue of Polyxenus lagurus. The central region of the tongue only is shown.

Fig. 3.—Left maxillula of Polyxenus isolated; external (posterior) view.

Fig. 4.—Right maxilla, maxillula and apex of tongue; ventral view.

Fig. 5.—Semi-diagrammatic lateral view, showing relations of maxillula, maxilla, and labium; the palp is displaced and truncated.

All figures are magnified 285 diameters.

Notes on the Maturation of the Ovum of Alcyonium digitatum.

By

M. D. Hill, M.A.Oxon.,

Assistant Master at Eton College.

As long ago as 1892 Professor Hickson[1] drew attention to certain recorded facts as to the fragmentation of the oosperm in the ova of various animals, and added thereto subsequently accounts of his own researches on Distichopora and Alcyonium. In these papers he showed that at least there was strong reason to believe that the early development of these ova was strikingly different to what had been described in a host of other animals, and to what was generally held to be necessary from a theoretical standpoint. It has always been a matter of surprise to me that these observations did not attract more notice from cytologists. In fact, they appear to have been almost entirely overlooked. Prof. E. B. Wilson in his widely known summary of our knowledge of the cell[2] makes no reference to them, nor does he include Hickson's papers in his bibliography of cytological literature. There could, however, be no doubt that if Hickson's observations were correct a new series of phenomena in cytology had to be reckoned with. Press of administrative and other work prevented Prof. Hickson from following up his discovery, and in

[1] S. J. Hickson, "Fragmentation of the Oosperm Nucleus in certain Ova," 'Proc. Camb. Phil. Soc.,' viii; "Development of Distichopora," 'Q. J. M. S.,' vol. xxxv; "Embryology of Alcyonium," 'Rep. Brit. Assoc., Bristol,' p. 585.

[2] E. B. Wilson, 'The Cell in Development and Inheritance,' March, 1902.

1895 he suggested that I should take the matter up. He most kindly handed over to me all his material, preparations, and the notes he had made thereon. I found, however, that certain important stages were missing, and if the work was to be completed, it would be necessary to obtain ova preserved after artificial fertilisation, in all stages up to the time of the beginning of segmentation. To gain this object proved far more difficult than either of us had imagined, and altogether we made five attempts to obtain ova in the right stages of development. I myself visited Plymouth in December, 1896, and January, 1897, and at the same season in 1902–3. In 1900–1 I sent my laboratory assistant. It was, however, only in the first week of January, 1903, that I was able to get anything approaching to satisfactory material, and I am much indebted to Dr. E. J. Allen, Director of the Plymouth Biological Station, for the zeal with which he did all that was possible to provide me with ripe colonies of Alcyonium.

Alcyonium digitatum breeds at Plymouth during the coldest and stormiest part of the year, from the middle of December to the middle of January. Colonies in sufficient numbers could only be caught in the nets of trawlers, and owing to storms and Christmas festivities I had to wait many days before material could be procured.

But even with the Plymouth tanks full of Alcyonium digitatum, the difficulties of the investigation had but begun. In 1896, for instance, I found that in a hundredweight of Alcyonium there was only one male colony with free spermatozoa, and that was discovered the day before I had to leave. Furthermore, the spermatozoa were but faintly mobile, so that the investigation had to be abandoned for that year. In 1902–3 I was more lucky, and was able to make several partially successful fertilisations. But at the best not more than 10 per cent. of the ova gave satisfactory results.

It is often very hard to tell whether eggs have really been fertilised until they are cut into sections, as in the early stages the blastomeres are vaguely defined, and hardly, if

at all, visible. This uncertainty made the work far more laborious than it would have been in the case of Echinoderm eggs, of which, under even the most unfavourable conditions, scarcely a single egg fails to be fertilised. The ova were highly sensible to temperature, and to the degree of salinity of the water. It was found necessary to make arrangements whereby the eggs could be kept cool by a stream of running water, with as little evaporation as possible. To one accustomed to the ease with which the eggs of Echinoderms can be dealt with the perverseness of the ovum of Alcyonium was a most unwelcome change. Nevertheless, I have reason to believe that the fault lay more with the spermatozoa than the ova, and that, had I been able to obtain really ripe male colonies, far fewer failures would have resulted.

In consideration, therefore, that it would be unwise to publish the results of an investigation only partially successful, I have waited until now in the hopes that some zoologist with more time at his disposal, and with more luck or skill than myself, might attack the problem and thoroughly bring to light what my own incomplete work has shown to be a strange process. I have, however, come across no published records of any such research. But I have at least been able to confirm Professor Hickson's observation as to the disappearance of the nucleus before fertilisation,[1] and thrown some light on previous and subsequent stages. Though we realise that much yet remains obscure, we feel justified in bringing our observations to the notice of zoologists, owing to the fact that C. W. Hargitt,[2] in his paper on the early development of Pennaria tiarella McCr., has shown that the results I have obtained are by no means without parallel as far as the disappearance of the female pronucleus (not oosperm) goes. Not only in other Cœlenterates[3] but

[1] Hickson has examined alone nearly fifty complete series of sections and found no trace of a nucleus.

[2] "The Early Development of Pennaria tiarella McCr.," 'Arch. für Entwick. mechanik der Org.,' Band xviii, Heft 4.

[3] V. Koch, "Die Gorgoniden des Golfes von Neapel," 'Fauna u Flora Mon.,' xv.

also in Arthropods[1] has this phenomena been observed. Hargitt cites many instances, and by his own work adds one more, for the case of Pennaria is in many ways strikingly like that of Alcyonium. He is at least convinced that an enucleate stage is always to be found in the early development of the egg of Pennaria. I shall again refer to Hargitt's paper in dealing with the phenomenon of fertilisation.

Method.

Stocks of Alcyonium digitatum were kept in tanks in the laboratory, and their ova fertilised, preserved, and cut into sections according to the usual methods. Various preserving fluids were tried; the best results I obtained by leaving the eggs for twenty minutes in a saturated solution of corrosive sublimate with a few drops of glacial acetic. With Fleming's and Perenyi's fluids I had less good results, especially with the latter fluid, so often recommended for eggs which contain a good deal of food-yolk. The extreme opacity of the eggs quite prevents any glimpse of the process of maturation and fertilisation going on in the living egg. In fact, for cytological studies the egg of Alcyonium proved a most unfavourable object. Partly owing to the difficulties described above I was unable to make accurate observations of the actual time that elapsed after the eggs and sperms were mixed before segmentation took place. I therefore was obliged to preserve batches (about 30) of ova at short intervals. In consequence, much material accumulated, of which by far the greater part had to be discarded, for, as the non-segmentation of the ova showed, no fertilisation had taken place.

Maturation.

Maturation usually occurs before the egg leaves the polyp, and the ova when expelled are ready for fertilisation. Nevertheless, if an egg be taken from the parent colony it will mature while floating in the water. The egg on emerging

[1] "Zur Entwick. der Dekapoden," 'Jenaische Zeitschrift,' xi.

appears to be of exactly the same specific gravity as the sea water, floating at any depth, but I found that unless an egg is fertilised it sinks to the bottom of the tank after some hours.

The nucleus of the immature ovum is a large, oval, eccentrically placed structure, resembling that described in many cœlenterates. The chromatin is aggregated into a single spherical vacuolated "nucleolus" (Fig. 1). The achromatic

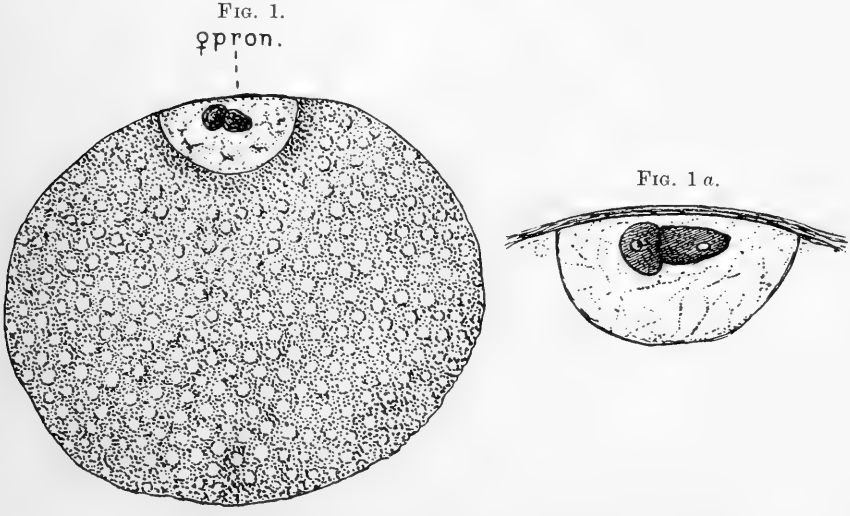

Fig. 1.—Section of a maturing ovum taken from. polyp. × 120 diams.
Fig. 1 *a*.—Nucleus of same drawn with Zeiss oil. imm. apoch.

network is scanty and wide-meshed, and is very sensitive to the action of acetic acid. With too strong a solution of corrosive acetic it almost entirely vanishes, leaving the mass of chromatin lying in a hollow vesicle.

The changes that now take place are quite unlike any that have hitherto been figured or described. Instead of the nuclear membrane dissolving and two mitoses taking place without an intermediary resting stage, nothing of the kind happens. Of this fact I feel convinced. Of the large number of eggs examined I have never seen the smallest

trace of anything that can be called a "polar body." Of what actually does take place in lieu thereof I am not equally sure, but if I interpret my preparations correctly it is somewhat as follows (Figs. 2 and 2 *a*): The lump of chromatin divides into halves amitotically. The nucleus becomes constricted into two, though there is no vestige of spindle or centrosome. A slight modification and absence of yolk in the protoplasm surrounding the nucleus may represent a modified archoplasm. I have never discovered the least trace of radiat-

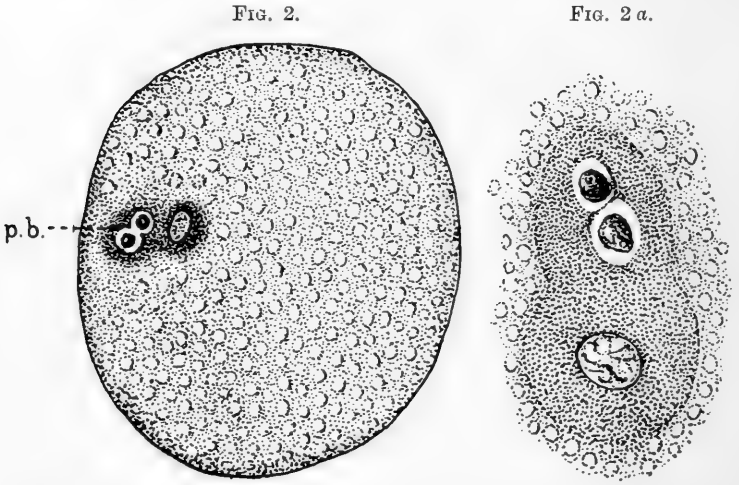

Fig. 2.—Section of an ovum showing direct division into polar bodies. × 120 diams.
Fig. 2 *a*.—Nuclei drawn with Zeiss oil. imm.

ing protoplasmic strands. One of the halves thus formed remains unaltered, except that the round chromatin mass breaks up into small bits, which soon dissolve, or at least no longer take the stain. The nuclear membrane is not re-formed. The remaining half divides again in the same way and almost immediately disappears. The chromatin seems to be rapidly disintegrated, becoming a faintly staining granular mass, and then all trace of this half of the original nucleus is lost. The other moiety (Fig. 3) is longer lived, and may now be called

the female pronucleus. It comes to lie on the extreme periphery of the cell, almost suggesting that it will be

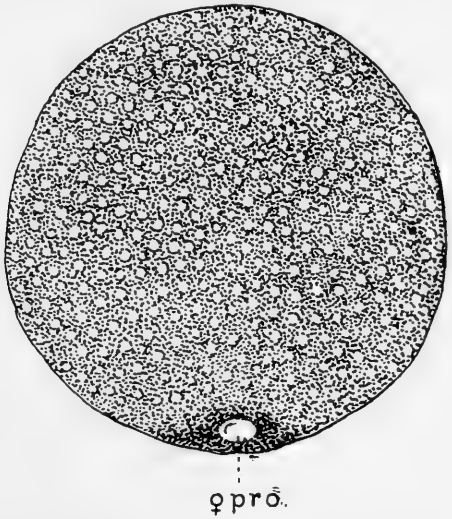

Fig. 3.—Section of an ovum showing female pronucleus with dissolved membrane, the chromatin having also disappeared.

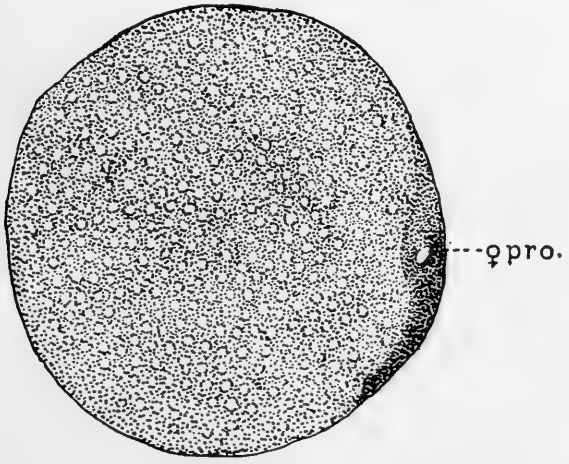

Fig. 4.—Further stage of × Fig. 3.

thrown out, but its fate is otherwise. It gradually dwindles to the smallest hollow vesicle, just discernible (Fig. 4),

and then disappears, leaving no trace of its presence. The deeply staining yolkless protoplasm, referred to as being possibly a modified archoplasm, also vanishes. The egg is now without any nucleus, and in this state leaves the polyp. Unless fertilised it remains "anucleate" until it dies. The death of the ovum is indicated by its sinking to the bottom, and assuming irregular shapes. Such an egg, if kept in a small glass jar on the laboratory table, will soon die (18–24 hours). In the tanks it will float for a day or two. In this

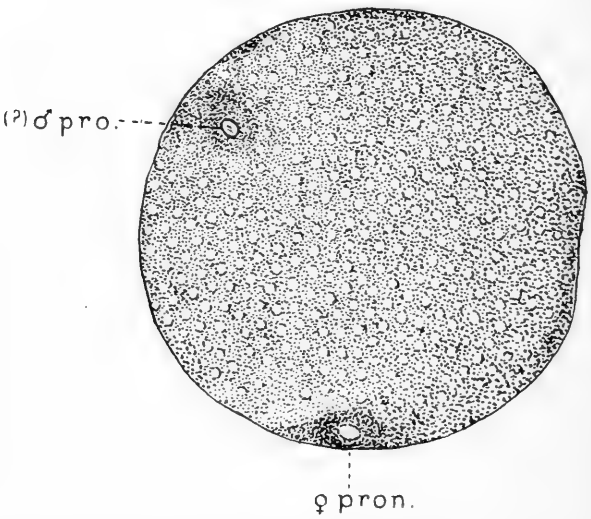

Fig. 5.—Female pronucleus nearly disappeared, (?) male pronucleus.

state were most of the eggs I examined, but owing to the difficulty of getting ripe male colonies, comparatively few eggs were fertilised.

Professor Hickson, however, was more successful than I, for in 1893–1894 and in 1897–1898 he succeeded in getting many eggs to segment. He found, however, a similar difficulty in obtaining male colonies, and believed that most of the eggs were fertilised by floating spermatozoa in the tank-water.

Fertilisation.

I tried to bring about artificial fertilisation in several ways, and without doubt a certain number of ova were impregnated, but only in two cases did I observe anything that one might reasonably suppose to be a male pronucleus. It was in both instances in the form of a small resting nucleus, lying in the centre of the egg, without centrosome or archoplasm (Fig. 5).

Fig. 6.

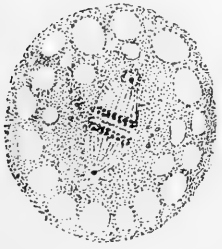

Fig. 7.

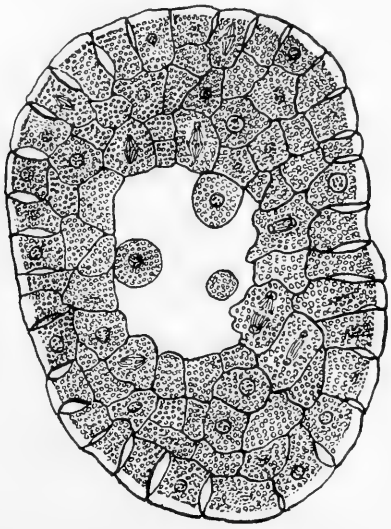

Fig. 6.—Section of a blastomere with nucleus dividing mitotically. (S. J. H., del.)
Fig. 7.—Section of an embryo showing mitotic nuclear figures. (S. J. H., del.)

The spermatozoa have spherical heads with conical points. It was not possible with the magnification (Zeiss apochrom. oil immersion) to assure myself of a middle piece, or to differentiate the "head" into any of its component parts, since I did not stain these bodies. It is quite possible that the nuclei (Fig. 5) are really first segmentation nuclei, though in what way formed I am quite unable to state. From their

small size, however, I suggest that they were male pronuclei, though it is difficult to imagine what, if any, of the disintegrated female pronucleus can eventually fuse with the male pronucleus.[1]

Segmentation.

The division of the egg into blastomeres is, as I have said, vague and ill defined. I cut several ova into sections, but failed to see in any of them anything like a mitotic figure. All that could be seen were small resting nuclei with ill-defined archoplasms around them. I am, however, enabled, through Professor Hickson's kindness, to reproduce two of his own figures (Figs. 6 and 7), showing that karyokinesis does take place, the archoplasms being very feebly developed though the centrosomes are well marked.

Summary and Conclusion.

Although the foregoing account is obviously incomplete, yet it is possible to draw some conclusions. I believe we have shown that:

(*a*) The egg of Alcyonium produces no polar bodies in the ordinary sense of the word.

(*b*) That the division of the female pronucleus before the entrance of the spermatozoon is irregular and amitotic.

(*c*) That no chromatin leaves the egg in the stage of ovocyte I, to use Boveri's nomenclature.

(*d*) That the female pronucleus completely disappears.

(*e*) That there are no bodies that can be termed chromosomes throughout the whole process.

(*f*) That the first segmentation nucleus is formed in a way (unknown) that must in any case be unlike anything hitherto described.

[1] It is interesting to note what Hargitt believes to be the fate of the sperm nucleus in Pennaria, whose course in the ovum he was unable to trace. "It seems," he says, "to lose more or less completely its chromatophilous properties soon after its entrance, nor does it appear to revive these characteristics in any appreciable degree, so far as I have been able to determine."

Furthermore I believe, though I cannot state so positively, that:

(*g*) A process takes place that may roughly be compared to the formation of polar bodies, but they disintegrate and do not leave the cell. So far as I have ascertained nothing in the shape of extrusion takes place. It is, however, curious to note that the nearest account that I have been able to find of like behaviour in an egg nucleus is that of Stoeckel.[1] This author found in a human ovary certain of the ova containing large nuclei, the membrane of which, as a rule, was well marked, but "oft geht diese scharfe Abgrenzung auch verloren. Die Konturen werden unregelmässeg Zackig, verschwommen." . . . Stoeckel believed that these changes in form were the beginning of amitotic nuclear divisions, giving rise to binucleated ova, of which he found several. I know of no other assumption of the direct division of an egg nucleus before fertilisation.

(*h*) The first segmentation nucleus is derived from the male pronucleus, though it is quite possible that chromatin equal in amount to that of the male may also be derived from the female pronucleus, though all trace of the latter has been lost.

If the foregoing statements be only partially true, it is obvious that a great gulf is fixed between the maturation processes in the egg of Alcyonium and all hitherto described cases. For this reason I am very loth to do more than state the bare conclusions to which I have come. To the best of my belief, no author has described amitotic nuclear divisions in the formation of polar bodies. We seem to have to deal with perfectly abnormal conditions. Nevertheless we are forced to admit that the maintenance of the individual parental chromosomes in a fertilised egg-cell is not universal. On the contrary, all trace of the chromatin of the original egg nucleus is lost. Furthermore, there are several instances among the protozoa of the breaking up and reorganisation

[1] W. Stoeckel, "Über Theilungs-vorgänge in Primordialeiern bei einer erwachsenen," 'Arch. f. mikr. Anat.,' Bd. liii, 1898.

of the nucleus. This occurs in many Ciliates. For instance: In 'Oxytricha and Lacrymaria' Gruber has shown that the meganucleus breaks up into minute fragments which become scattered through the protoplasm, but eventually reunite into a single body.

So much attractive speculation has been based on the ordinarily observed facts of maturation and fertilisation that we feel almost bound to assume that these processes are the same in all metazoa. But it is obvious that in certain cœlenterates we have facts before us that cannot be brought into line with what we feel we have a right to expect. At present the affair is a mystery. Pending further investigation it were unwise to speculate on the possible meaning of these phenomena, however much one may be tempted to do so. In case that what I have observed, and still more failed to observe, may induce some other zoologist to follow this cytological byeway, I can only hope that he will find this paper a path over which it may not be necessary to retrace his steps. There would seem to be four points to which attention should be specially directed:

(*a*) The nuclear history of the germ-cells from their earliest "Anlagen."

(*b*) The mode of formation of the polar bodies.

(*c*) The actual penetration of the spermatozoon.

(*d*) The way in which the first segmentation nucleus is built up.

Lastly, I have but to express my sincere thanks to Professor Hickson for his kindness in allowing me the use of his material, preparations, and notes, and for the many fruitful suggestions that I have received from him.

Addendum.

Since writing the above my attention has been drawn to another paper by C. W. Hargitt, viz. "Notes on the Hydromedusæ of the Bay of Naples," 'Mith. Zool. Stat. Neapel.,' xvi, 4, p. 562. Writing of Pachycordyle Weismanni, Hargitt

says: "I was unable to demonstrate any of the ordinary features of this process (i. e. maturation) either in living eggs or in those sectioned and stained. Intimately associated with these changes were nuclear modifications of a more or less remarkable character. Prominent among them is the dissociation of the nuclear membrane which occurs shortly before the birth of the medusæ and the discharge of the eggs. Following this there occurs a marked decrease in the mass of nuclear substance, probably due to the loss of nuclear sap or a dispersal of matter through the cytoplasm, so that the nucleus measures only about half that of the ovarian egg. Of still greater importance is the change which occurs in the chromatin network of the nucleus, which appears to wholly disintegrate and to disperse through the cytoplasm. Not the slightest traces of chromosomes or chromatin substance can be demonstrated in the nuclei or cytoplasm at the time of the liberation of the medusa. The nucleus itself, greatly reduced in size, may still be seen as a definite area of very homogeneous texture, but indefinitely merging into the surrounding cytoplasm, there being no trace of nuclear membrane."

It is unnecessary to insist on the great similarity of Hargitt's observations to what I have described in the case of Alcyonium digitatum.

M. D. Hill.

Eton,
May 18th, 1905.

On Some Points in the Anatomy of the Platydesmidæ.

By

F. G. Sinclair, F.L.S.

With Plate 29.

In 1901 I received a small amount of material which enabled me to make out a little of the anatomy of the species of Platydesmus, which I described in my paper on the Myriapods of the Skeat Expedition as Platydesmus kelantanicus.

The material was not enough to enable me to make out many points, and so was laid aside till 1903 when Dr. H. Gadow brought me back a quantity of P. mexicanus from Mexico, sufficient to allow me to proceed with my investigations. I am glad to have this opportunity of thanking him for his kindness.

The general appearance of P. mexicanus is given in Humbert and Saussure's work, "Études sur les Myriapodes," and of P. kelantanicus in my own paper, so it will not be necessary to describe them again here.

The first point that strikes one in examining this animal is the extreme smallness of the head in comparison with the rest of the body. It is almost concealed beneath the tergal plate of the neck. In the next place the similarity of the individual segments attract one's notice; there does not seem to be the same amount of specialisation of regions in the neighbourhood of the thorax that one finds in most Myriapods. This appearance is, however, to a great extent, deceptive, as closer

examination shows that the legs of the thoracic region are single pairs as in other Myriapods.

The external appearance of the head of the two species that I have worked at differs considerably owing to the presence in P. mexicanus of a peculiar sense-organ mentioned by Humbert and Saussure in their description, in which they mention their resemblance to visual organs. "Les deux points stematiform leur sont specieux et quoique nous n'ayons pas réussis a en distinguer nettement la nature, ils nous semblent cependant former des organes visuels, car ils sont révêtus d'une cornée tres distinct."

On a first examination one can hardly help coming to the opinion that the organ in question is visual in function. Seen from the outside (figs. 8, 16) one sees that the external chitin is thickened so as to form a projecting boss, and further that the chitin of which this boss is formed is clear and transparent, so that the whole has a white appearance. One can also make out that the pigment layer that lines the internal side of the hypodermis is thickened just below the boss, and its colour is much darker, so that it forms a sort of iris. It is not, however, an eye, for there is no division into separate eye spots, and the external surface of the boss is not smooth, but when viewed under magnification is beset with fine hairs. The internal features of this organ will be described later in connection with the brain.

P. mexicanus has two small light-coloured patches in the frontal region, one on either side of the middle line. These small patches correspond with two depressions on the frontal region of the brain, but I have been unable to discover their function.

The mouth parts consist of the upper lip, the hypostoma, and the mandibles. The first two of these have been described by Silvestri for P. polydesmoides (Classis Diplopoda, Portici, 1903), and my species offer no considerable variation. With the mandibles, however, it is different, as in both the species I have examined the mandible shows a considerable divergence from that of P. polydesmoides, as figured by

Silvestri. Here I must take the opportunity of correcting an error in my paper on the Skeat collection. The figure that I gave there as the mandible of P. kelantanicus is imperfect. The delicate chitin which forms a great part of the mandible has been torn away leaving only a part of the structure.

Fig. 14 shows the mandible of mexicanus. As will be seen in addition to the teeth and the small comb of bristle shown in Silvestri's figure the clump of bristles marked Z in his figure are, in my species, much enlarged, and the bristles are arranged in a concave space in a definite order (figs. 14, 15). It is possible that the curves may be exaggerated by the shrinkage of the chitin, but I have examined a great number under the microscope and think that the arrangement shown in my figure is correct.

Fig. 15 shows the two mandibles in situ on the hypostoma, and we can see that the effect of the configuration of the concave region in which the bristles are arranged is to form a sort of atrium set with bristles through which all food must pass before it reaches the gullet. In correspondence with this structure the muscles of the mandible are comparatively feeble, and only allow of a small amount of movement of the appendage.

There is another point in the composition of the exoskeleton which deserves attention. The suture passing along the back has been mentioned by Humbert and Saussure as only occuring in the Platydesmidæ and in Craspedosomidæ.

When one cuts a section through the dorsal region one sees the appearance shown in figs. 11, 12, 13. The longitudinal division between the two parts of the body-ring is complete, and not only that but there is a narrow longitudinal plate intercalated between the two parts. A comparison between the two species of Platydesmus and Craspedosoma shows that the conditions are nearly similar. In both cases the intercalated plate does not extend the whole length of the segment, but is only present in its anterior part and has the shape of a long narrow wedge.

It is not easy to account for these facts. Newport (3), in his monograph on the Chilopoda, 1844, held that the dorsal arch in the Chilopoda is composed of four pieces of which the two median ones are the last to be united, these two median pieces form the scutum and the two lateral ones the episcutum. Bode (1), in 1877, said that the body-ring of Polyxenus belongs to the pentazonal type, and describes the dorsal portion of the zonite as consisting of two large pleuræ and a relatively small tergum.

It would be natural to suppose that in the case of Platydesmus, the conditions of Polyxenus had been carried a little further, and that the tergum had been reduced to the thin median intercatated plate. It is possible that this may be the case. On the other hand it is possible that the intercalated plate may be a completely new structure, and that the two side plates may represent a single tergal plate that has been divided and jointed in the middle longitudinal line.

The difficulty in the way of accepting the first of these explanations, is that the keels which should be a prolongation of the sides of the terga, judging by the analogy of the keels of Polydesmidæ, would in this case be an extension of the pleuræ. I thought at first that the foramina repugnatoria might throw some light on the subject. Humbert and Saussure describe them as beginning in the fifth segment. Careful dissection and examination by means of sections failed to show me any trace of these glands, and I thought that as these glands are typically organs of the terga their absence was evidence that the two side divisions were not terga but pleura. This, however, is contradicted by Craspedosoma, which shows, as I have already said, the same formation of the zonite but has well-marked repugnatorial glands and foramina. Of course, it is not impossible that the keels and repugnatorial glands should have been formed in the pleuræ instead of in the terga, still their occurrence is a difficulty to my mind.

The second possibility that I have mentioned would seem the most probable to me if there were anything in the way of

evidence to support it, but, so far as I know, the condition of matters in Platydesmidæ and Craspedosoma is one that is only known in Polyxenus, if Bode is correct in his view (I think he is). So that in favour of the first we have the fact that Polyxenus shows a connecting-link between the almost vanishing tergum and the big pleuræ of Platydesmus on the one hand, and the common condition of a large tergum and small pleuræ on the other, while I can think of no evidence in favour of the latter.

It will be observed that to those who hold to the continuity of species evolution, the first view will appear most probable, while from the standpoint of discontinuous evolution the formation of a new structure like the narrow intercalated plate will not offer the same difficulty.

There is, however, another point of view which is worth considering. In a paper by Ray Lankester ("Structure and Classification of the Arthropoda," 'Quart. Journ. Micr. Sci.,' 1904), his thirteenth law or generalisation of metamerism asserts that homologous meromes of adjacent somites tend to fuse with one another, as in the case of the double somites of Diplopoda. In Heymon's investigation on the embryology of the Scolopendridæ ('Die Entwickelungsgeschichte der Scolopendrer,' Stuttgardt, 1901) he has made out that in the development of the exoskeleton of the dorsal region two parallel longitudinal furrows appear in the tergite, and divide it into three parts—a median and two lateral. Now if the original form of the tergum in the Diplopoda was originally, as in the Scolopendridæ, composed of three parts it would be easy to understand that in most forms the three parts of the tergum have fused in accordance with the law just mentioned, while in the forms known to have the pentazonal character, the original three pieces have persisted. This law or generalisation seems to apply to the fusion of meromes in adjacent somites only, but I think that it can be extended to the fusion of bilaterally symmetrical meromes in the same somite. The neuromeres in a single segment of Peripatus; the two neural cords are completely separate, and we have every degree of fusion in

Myriapoda up to the almost completely fused cord of Diplopoda. It does not necessarily follow that because one primitive character has persisted that therefore the Platydesmidæ and Craspedosomidæ are primitive types. It may be noted that the division of the zonite and the intercalated plate are present in all the somites except the telson.

The ventral plate of the two species of Platydesmidæ shows considerable differences. In kelantanicus between each pair of legs there is a sternal plate of peculiar form (fig. 17) with a sinuous anterior edge, and with the posterior edge curved so as to follow the curve of the outline of the coxæ, with a blunt point projecting between the coxæ of the pair behind it. On the median anterior region of this point there is a tubercle ending in a stout bristle. The bristle is perforated and in connection with the perforation there is a small mass of gland cells. The whole organ is shown in fig. 19. The ventral plate of mexicanus is simpler (fig. 18) and without the organ. The tracheal openings in both species are situated on the coxæ and begin in the third somite, that is, not counting the tracheæ in the head. The tracheal openings (fig. 20) dilate into a wide pouch which runs some way into the body before giving off the tracheæ.

The Nervous System.

Our knowledge of the nervous system of the Myriapoda is largely due to the excellent and accurate work of St. Remy (6). His work on the Myriapod brain was confined to two groups as far as the Diplopods are concerned; these are Glomeris and Julus. Both of these have eyes, and were selected with reference to the subject at which he was working, so that, for the purpose of comparing the brain of Platydesmu with that of other Myriapods, it was necessary to make a number of dissections of the brains of other species of Myriapods. The brain of Myriapods seem to fall naturally into three classes, types of which are shown in figs. 1—4.

In the simplest of these there is no optic lobe ; the frontal lobe is the most conspicuous part of the whole, the antennal lobe being small and inconspicuous, and lying below the frontal, while the circumœsophageal commissures start from the junction of the two lobes. There is a distinct transverse commissure beneath the œsophagus, though it is neither so thick nor so far separated from the subœsophageal ganglion as in the more complicated brains. In this class of brain are included those of the Platydesmidæ and the Polydesmidæ.

In the next class are included the brains of the Julidæ. The organ is complicated by the addition of optic lobes; the antennal lobe is more developed than in the first class, and the transverse commissure is larger. St. Remy's figure of the brain of Julus maritimus is typical of the class.

The last class is that typified by St. Remy's figure of Glomeris. The antennal lobe is much larger than in the foregoing, and more separated from the frontal. The mass of the brain is not continuous above the œsophagus, but the frontal lobes are connected by means of a commissure, as also are the antennal. The whole brain is less compact.

These three classes of brains are not distinctly marked off from one another, but the interval between one class and another is bridged over by a number of intermediate forms. The greatest interval, as far as my limited experience goes, is between Classes 2 and 3.

It is an interesting circumstance that the differences in the brain do not coincide with the great divisions of the Diplopoda as I expected that they would. The most striking instance of this is in two species of Diplopoda from the Malay peninsular, Sphaeropoeus evansi and S. modigliana (figs. 2 and 3). These two animals, closely related to one another by all the marks that are usually taken into account in the classification of the Myriapoda, have totally different brains. S. evansi has a brain resembling the figure in St. Remys' work of Glomeris, but shows a higher degree of differentiation. The antennal and frontal lobes

are much more separate, and lie almost on the same horizontal plane, the antennal lobe being anterior to the frontal. It is worth noting that, assuming St. Remy's view of the primitive situation of the ganglia that make up the brain of Myriapods to be correct, this arrangement would be almost exactly the primitive one. There is also a consideration of interest in the disposition of the parts of the brain. If it is true that the arthropod brain has been derived from the development of an eliptical ring of nervous tissue, and that this condition is represented by the widely-separated nerve cords of Peripatus joined at the ganglia by commissures, then the structure of the brain of S. evansi strongly recalls this condition, inasmuch as the ganglia on each side of the head are widely separate, and are joined by transverse commissures.[1]

The brain of S. modigliani (fig. 2), on the other hand, shows a concentration and compression that removes it from Class 3 altogether, and places it in the same class as that of the Julidæ. There is no separate commissure uniting the antennal lobe, but the two sides of the brain are united above the œsophagus by a broad tract of nervous tissue. This striking fact is not alone, for in the Craspedosomidæ we find a complicated brain of the same type as of that Glomeris. The Craspedosomidæ are classed by Silvestri in the third of the five suborders in which he divides the Helminthomorpha (I. Diplopodi), and yet its brain is of the complex type of the third class (fig. 1), with the two commissures uniting the frontal and antennal lobes of the two sides. The species which I dissected is Craspedosoma polydesmoides. Polydesmus has a simple brain of the first class. Blanjulus guttulatus has a simple brain of the type of the Polydesmoidea.

The brain of Platydesmus mexicanus occupies a large part of the head when we take into consideration the small size of the latter. It belongs to the first of my three classes,

[1] It is right to state that I had only a small amount of material of *S. evansi.*

being much compressed, and extending as far forwards and downwards as laterally. The frontal lobes are short and thick, and at the end of each a short thick nerve is given off which runs to the sense organ described at the beginning of this paper. This is evidently one of the class of organs which St. Remy calls the organs of Tomösvary. The frontal lobes are united by a thick mass of nervous tissue, the pont pneumogastrique of St. Remy, from which the pneumogastric nerve is given off. Below the frontal lobes are the antennal lobes, which are not so well developed as in most of the brains described before, and it is not until they are examined by means of sections that they can be made out clearly, except for the antennal nerve which is given off from each and passes to the antenna. Just at the junction of the antennal lobes with the frontal, the œsophageal commissures pass off embracing the œsophagus and passing downwards to the ventral ganglionic cord. Below the œsophagus they are united by a slender transverse commissure, which, as seen in fig. 7, is small in comparison with the mass of the brain, and the space between the commissure and the ventral ganglion is very narrow in comparison with that in other brains.

The nerve from the sense organ before mentioned passes off from the frontal lobe just before the plane where the œsophageal commissure is given off, and is shown in fig. 5, a mass of ganglion cells is situated at the point where the frontal lobe is continuous with the nerve, the nerve itself is short and fibrous, the fibres continuing into the hypodermis. The pigment which covers the hypodermic cells lining the transparent chitinous exoskeleton of the head and gives it its colour is darkened at the edges of the clear chitin of the organ, so as to form a sort of diaphragm, and below the clear thickening of the organ, the hypodermic cells are modified, and form a thick mass, with which the fibres of the nerve are continuous. When the hypodermis is depigmented with nitric acid the shape of these hypodermic cells can be seen. The cell is prolonged on the one hand to come into con-

nection with the nerve fibres, and on the other with delicate hairs which pass through the thickening of the chitin and project on the outside. The hypodermic thickening is richly supplied with fine tracheal tubes.

The two external light-coloured patches on the front of the head in P. mexicanus (fig. 16) are connected with two small thickened marks on the brain shown in fig. 4, but my material was not sufficient to allow me to make out their nature.

The ventral ganglionic cord has no striking peculiarity. Two pairs of nerves are given off in each segment, the anterior pair supplying the anterior part of the segment, and the posterior the posterior part. Another pair of nerves is given off from the middle of the ganglion in a rather more ventral position.

General Conclusions.

The position assigned to the Platydesmidæ among the Myriapoda by naturalists differs very much. Lucas (7) placed them near the Julidæ on account of their mouth parts. Newport classed them near the Craspedosomidæ, Gervais with Polydesmidæ, Saussure also with the Polydesmidæ. Humbert and Saussure in the family of Polyzonidæ silvestri places them among the Colobognatha. Let us consider the characters that have led to this classification. The characters of the Colobognatha according to Silvestri (I. Diplopodi) are these:—"Head small, eyes distinct or none. Mandibles and hypostoma degenerating or none, with the labrum forming a suctorial organ more or less perfect. Plural scutes free, coalesced or membranous. Lamina pedigera free. Foramina repugnatoria disposed in two lateral series beginning from the fifth somite. Segments 30 to 108, third and two last segments apodal, first and second with a single pair of feet, rest with two pairs. In the male, external copulatory organs simple, in the seventh segment."

Now the characters of the two species of Platydesmidæ

that I have examined do not seem to me to be such as to warrant their being included in a sub-order with these characters. The small head, the free lamina pedigera, and simple copulatory organs are the only characters which agree with the definition, and these are common to other groups. The mandibles, hypostoma, and upper lip do not form a suctorial apparatus. The peculiar arrangement of bristles on the mandible point rather to a carnivorous diet than to a liquid, and there is no apparatus for piercing in order to obtain liquid nourishment. Compare the hypostoma with that of the other groups of the Colobognatha, and with that of Polydesmus, and it must be admitted that it most resembles that of Polydesmus. The want of foramina repugnatoria, if I am correct in my belief that they are absent from the Platydesmidæ, separates them from the Colobognatha as does the peculiar arrangement of the body-ring on the pentazonal plan. In the internal organs I can see no signs of degeneration. Verhoeff, however ('Zool. Anz.,' xxiv, No. 654, 1901), denies that the Colobognatha are degenerate forms. The nervous system, to which I have devoted most attention, seems to me to be quite as well developed as that of the Polydesmidæ, and to resemble the latter to a great extent.

When we come to consider what group the Platydesmidæ should be placed in, it is a harder question, and one that I feel myself little qualified to solve. I shall therefore content myself with saying that until evidence to the contrary is forthcoming I shall regard the Platydesmidæ as an aberrant group allied, as Gervais supposed, to the Polydesmidæ.

With regard to more general considerations, the nervous system has interested me deeply, and has led me to dissect a great quantity of Diplopod brains. I hoped that these dissections might have thrown some light upon a question that most naturalists, I believe, consider already settled, the question of the unity of the group of Myriapoda. My investigations, though they do not in any way settle the

matter, yet strengthen my opinion that the question is still an open one.

We have in the Myriapod brain a great diversity of form in species that have always been considered closely allied. The variations in the structure of the nervous system are not as St. Remy pointed out allied intimately with the presence or absence of the ocular lobe, and therefore the difference is a more deep-seated one than can be due to the presence or absence of eyes. All these variations, however, keep within certain limits, and it is not possible to show as great a difference between the Chilopod and Diplopod brain as between the brain of Insecta, Crustacea, and Arachnida, and that of Myriapoda. I think that all the variations in their nervous system are such as might be expected in a large group which, though including a great number of different forms, yet deserves to be classified in a single class.

Books referred to.

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2. Heymons.—'Die Entwickelungsgeschichte der Scolopend,' Stuttgardt, 1901.
3. Newport.—"On the Class Myriapoda, Order Chilopoda," 'Trans. Linn. Soc.,' 1844.
4. Silvestri.—'I Diplopodi,' Geneva,' 1896.
5. ——— 'Classis Diplopoda Portici,' 1903.
6. St. Remy.—'Théses,' 1890.
7. Lucas.—"Sur un nouveau genre de la classe des Myriapodes appartenant à la famille des Julides," 'Annales de la Société Entomolique de France,' 1843.

EXPLANATION OF PLATE 29,

Illustrating Mr. F. G. Sinclair's paper "On some Points in the Anatomy of the Platydesmidæ.

Letters used in all the figures.

Ant. l. Antennal lobe. *Ant. n.* Antennal nerve. *Ch.* Chitin. *Fr. l.* Frontal lobe. *Gl.* Gland. *Hy.* Hypostoma. *N. t.* Nerve to organ of Tomösvary.

Oc. l. Ocular lobe. *O. n.* Ocular nerve. *Œs.* Œsophagus. *Œs. com.* Œsophageal commissure. *Tr. com.* Transverse commissure. *S.* Depression in brain corresponding to marks in external chitin. *Sp.* Perforated bristle.

Fig. 1.—Dissected brain of Craspedosoma polydesmoides.

Fig. 2.—Dissected brain of Sphæropœus modigliani.

Fig. 3.—Dissected brain of S. evansi.

Fig. 4.—Dissected brain of Platydesmus mexicanus.

Fig. 5.—Section through head of P. mexicanus, showing organ of Tomösvary.

Fig. 6.—Section through head of P. mexicanus, in region of antennal lobe.

Fig. 7.—Section through brain of P. mexicanus, showing œsophageal commissure and transverse commissure.

Fig. 8.—Organ of Tomösvary shown from the outside.

Fig. 9.—Section of hypodermis in organ of Tomösvary under high power.

Fig. 10.—Section through brain of Craspedosoma polydesmoides, showing transverse commissure.

Fig. 11.—Section through dorsal plate of P. mexicanus, showing intercalated plate.

Fig. 12.—Section through dorsal plate of Craspedosoma polydesmoides.

Fig. 13.—Section through dorsal plate of P. mexicanus.

Fig. 14.—Mandible of P. mexicanus.

Fig. 15.—Both mandibles of P. mexicanus in situ on the hypostoma.

Fig. 16.—Head of P. mexicanus.

Fig. 17.—Ventral plate of P. kelantanicus.

Fig. 18.—Ventral plate of P. mexicanus.

Fig. 19.—Ventral organ of P. kelantanicus.

Fig. 20.—Tracheal opening of P. kelantanicus.

Rhinosporidium kinealyi, n.g., n.sp., a new Sporozoön from the Mucous Membrane of the Septum Nasi of Man.

By

E. A. Minchin, M.A.,
Professor of Zoology at University College, London,

and

H. B. Fantham, B.Sc., A.R.C.S.,
University College, London.

With Plates 30 and 31.

1. Introductory.

The peculiar organism which forms the subject of this memoir was first brought to notice by Major F. O'Kinealy, I.M.S., occurring in a growth removed from a native patient at the Medical College Hospital, Calcutta, in May, 1894. The growth in question was described as "a small, vascular, pedunculated tumour, about the size and shape of a large pea, projecting into the vestibule of the left nasal fossa. It was a freely movable, painless growth, with all the appearances of a papilloma, and was attached by a short pedicle to the mucous membrane at the anterior and upper end of the cartilaginous septum, being entirely confined to that region." The tumour was examined microscopically by Major J. C. Vaughan, I.M.S. then officiating Professor of Pathology at the Medical College. A section of the tumour was exhibited by Major O'Kinealy before the Laryngological Society of London, and his descrip-

tion of the case, including the report of Major Vaughan, was published in the 'Proceedings' of the Society for April, 1903 (**1**). Later on a second report, with drawings of the clinical appearances of the growth, was sent by Major O'Kinealy and published in the Society's 'Proceedings' for December, 1903 (**2**). Since the original case seven or eight others had cropped up at the Medical College Hospital.

Our acquaintance with this interesting parasite is due, in the first instance, to Dr. Hemington Pegler, Curator of the Laryngological Society, who directed the attention of one of us to the slide sent by Major O'Kinealy, belonging now to the cabinet of the Laryngological Society. Dr. Pegler kindly entrusted the slide to us for examination. Major O'Kinealy also, when communicated with, very kindly sent a second slide, the counterpart of that in the possession of the Laryngological Society. Both the sections mounted on these slides were made from material fixed in absolute alcohol, and had been stained in picrocarmine and mounted in Farrant's solution. From the Laryngological Society's slide a photomicrograph was kindly made for us by Mr. E. T. Browne in the Research Laboratory of University College (Pl. 30). The second slide was unmounted, and the large section divided into smaller fragments, which were then restained in various ways and examined with the best Zeiss lenses. In this way we have been able to add to our knowledge of the parasite; but some points could not be made out clearly in our limited material, and it is to be hoped that a further examination of it will be made by observers in a position to obtain fresh or well-preserved material. From the published reports above referred to, these growths appear to be not uncommon in Calcutta among the natives.

For the clinical account of the growths produced by this parasite we refer to the two memoirs (**1**, **2**) by Major O'Kinealy. In this paper it is proposed only to describe the structure of the parasite so far as we have been able to make it out, and to discuss the affinities of the parasite with other Sporozoa and its position in the class. We propose

for this parasite the generic name Rhinosporidium, on account of its peculiar habitat, and we name the species after its discoverer, Major O'Kinealy.

2. Methods.

Since our material was limited, as has been said, to two sections, one of which, as the property of the Laryngological Society, was inviolable, our work was restricted to restaining in various ways the remaining section, which was unmounted and divided into smaller pieces, each of which was made a separate preparation. The section was of considerable size, and so full of the cysts of the parasite that even small fragments of the section contained some of them. Heidenhain's iron hæmatoxylin, Delafield's hæmatoxylin, gentian violet followed by orange, and safranin followed by licht-grün, were the combinations which gave the best results. A great obstacle to restaining, however, arose from the picrocarmine already present in the section, which could not be entirely dissolved out and caused the tissue to take up excess of the stains used, thus necessitating long differentiation and care in clarifying. Unfortunately also, the sections were cut too thick to show clearly the minute structural details of the parasite, and the fixation was not all that could be desired. In studying our preparations it was found that the 3-mm. homog. immersion of Zeiss gave clearer definition with the thick sections than the 2-mm., and was used in combination with the compensating eye-pieces 4, 8, 12, and 18, especially the first and the last of these.

3. The Structure of the Parasite.

In his second communication to the Laryngological Society (**2**), O'Kinealy states that "a section of the growth when fresh was seen to be studded with minute white dots, which under the microscope were found to be cysts filled with granular bodies." This description may be compared with

the photo-micrograph given us (Pl. 30), which shows the general appearance of a section under a low power. The growth, which has been compared by O'Kinealy to a strawberry, raspberry, or arbutus-berry, has the surface much folded and covered everywhere by stratified epithelium. In places the folds form deep "crypt-like involutions," as Vaughan has termed them (**1**), into which the epithelium extends. Below the epithelium is a stroma of connective tissue in which are lodged the parasitic cysts. Some cysts also occur deep down in the epithelium. The cysts vary very much in size, as Vaughan has already pointed out (**1**), and also in form. Usually nearly circular in outline, they may be oval or elongated, almost tubular in form (fig. 8). The cyst-wall is hyaline, of considerable thickness, and showing no trace of cell-structure. Its outer contour-line is very distinct, but its inner limit is not so sharply defined. The thickness of the cyst-wall in different cysts varies considerably. We have nothing to add to the description of the cyst-wall given by its discoverers, except as regards two points. In the first place, it is described by Vaughan as showing a concentric striation, but it appears to us to be perfectly homogeneous and structureless, and we regard the apparent concentric striation which may be observed occasionally when an oblique section of the cyst-wall is focussed up and down, as due to diffraction phenomena. In preparations stained with gentian violet and orange the cyst-wall takes up the gentian violet, and its extent and structureless character then become quite evident. With Delafield's hæmatoxylin also the cyst-wall stains pale blue, with slight concentration and darkening of colour on the inner side. Heidenhain's iron hæmatoxylin also shows up the cyst-wall very distinctly. Secondly, we are unable to confirm O'Kinealy's statement (**2**) as to the presence of "a pore in the cyst-wall through which the bodies escaped into the surrounding tissues." We have not found such a pore in any cyst that has come under our observation.

We come now to the most important feature of the parasite,

namely the contents of the cysts. They have been described by O'Kinealy (2) as granular bodies, containing refractile granules. Vaughan (1) has termed the granular bodies "sporules," but for descriptive purposes we will commence by employing the non-committal terms used by O'Kinealy. If one of the larger cysts be examined, it is at once obvious that the spherical or ovoid granular bodies vary greatly in size, showing every gradation from small bodies at the periphery to larger ones towards the centre, and, further, that the number of refractile granules in the bodies is also subject to great variation (figs. 6 and 7). For purposes of description it is convenient to consider the granular bodies as forming three zones or regions, namely peripheral, intermediate, and central, although in reality no hard and fast line can be drawn between these three categories. In preparations stained with safranin and licht-grün, however, the peripheral bodies stain green, the central ones red. The peripheral granular bodies measure about 1–1·5 μ in diameter, and contain a single refractile granule or nucleus (figs. 6 and 7). Those of the intermediate zone measure 2–2·5 μ in diameter, and contain two or three granules in addition to a distinct nucleus (figs. 6 and 7). This structure can be made out in a preparation stained by Heidenhain's method, in which the body appears to contain one nucleus and several granules in the form of more or less concentrated spherical masses of protoplasm (fig. 7), though in the elucidation of these structures in the intermediate zone thinner sections would have been most helpful, indeed desirable. The central granular bodies measure 5–6 μ in diameter and contain from 9 to 15 refractile granules. In the original picrocarmine preparation each refractile granule shows distinctly a central spot of different refringence from the surrounding part, and appearing darker or lighter according to the focus of the microscope. In preparations made by Heidenhain's method the central spot takes up the hæmatoxylin intensely and then appears in many cases deep black when carefully focussed. Delafield's hæmatoxylin also stains the central spot, though not so well. From

these reactions there can be no doubt that the central spot of the so-called refractile granules is a chromatic nucleus. Each granular body is thus seen to be a morula-like structure, containing on the average about a dozen nucleated corpuscles, for which we may now drop the term "refractile granules" and call them simply spores. Each spore-morula is bounded by a membrane, which in the original picrocarmine preparation appears highly refractile. Stained by Heidenhain's method, the membrane in question is still refractile but more distinct, while after Delafield's hæmatoxylin it is very clearly seen and darkly stained, though we have not been able to make out the "double fine contour line" described by Vaughan. The spore-morulæ appear more closely apposed and facetted in the iron-hæmatoxylin preparations, but slightly separated from one another in the slides stained with picrocarmine and Delafield's hæmatoxylin; either as the result of swelling in the former case, or shrinkage in the latter. Another curious point about the spore-morula, seen in the original picrocarmine preparations, is that some of them take up more of the carmine and have a red tinge, while the majority stain a light yellow colour; the former are apparently the fully developed, older bodies. Within the morula the spores have a more or less irregular arrangement (fig. 8).

The above description refers to what may be considered as a typical, fully developed cyst, and applies to the majority of the cysts in the sections we have studied. Besides these, however, we have found others measuring about 120 μ in diameter, less rounded in shape, in which no spore-morulæ are to be found, but the entire cyst is packed with spherical masses of protoplasm, each containing a single nucleus (fig. 5). In other words, the "granular bodies" in these cysts have not advanced beyond the condition of the peripheral zone in the cysts described above, and there can be no doubt that we are dealing with younger or immature cysts. Another very important feature of these smaller cysts is the presence of a peripheral zone of clear undifferentiated protoplasm immediately within the cyst-wall (fig. 5). We have searched care-

fully for a similar protoplasmic layer in the larger cysts, but have not succeeded in detecting it. Probably thinner sections of better preserved material would reveal at least traces of it. For showing up the smaller cysts Delafield's hæmatoxylin proved to be an excellent stain, as it coloured the cysts and their contents blue, while leaving the surrounding tissues unaffected. By means of this stain we were able to find still smaller cysts with thin walls and granular protoplasmic contents (fig. 2). The granules in them are of varying size, and some of the larger granules probably represent nuclei. Similar small cysts were also found in the slide stained with picrocarmine, which in many cases were of irregular outline, or even showed pseudopodia-like processes, giving the organism a resemblance to a small amœba with a clear envelope (fig. 4). These irregular forms were stained a yellow colour by the picrocarmine, and a slight concentration of the tissues round them was observable. A similar body was observed in a preparation stained with Delafield's hæmatoxylin, but this stain appeared to cause a certain amount of shrinkage and wrinkling in the cyst-wall (fig. 3), a result observed also in other instances—for example, in the membrane of the spore-morulæ (fig. 10). Finally, it should be mentioned that we have also found ruptured cysts, showing mechanical infiltration of the granular bodies into the surrounding tissues, as already noted by Vaughan (**1**). This appears to occur chiefly in places where the submucous tissue is in process of degeneration.

4. Systematic Position of the Parasite.

In the foregoing we have set forth the facts observed in a purely objective manner, so far as possible. It will now be useful to recapitulate and summarise our observations, and to construct what appears to us to be the developmental sequence of events, as a preliminary to the discussion of the affinities of the parasite.

(1) The youngest forms we have observed with certainty

are granular protoplasmic masses of irregular or even amœboid contours, enveloped in a hyaline membrane, and probably multinucleate (figs. 2, 3, 4). These bodies represent the early trophozoite stage, and occur in the submucous connective tissue, as do all other forms of the parasite, a few fully developed forms being also seen in the epithelium.

(2) The parasite increases in size, its hyaline envelope becomes thicker, forming a definite cyst-wall, and towards the centre of the body the protoplasm becomes segmented into spherical masses, each with a single nucleus and a delicate membranous envelope (fig. 5). As these bodies are destined each to give rise to numerous spores, they may be termed pansporoblasts, a word in use for the similar structures in the Neosporidia. Formation of the pansporoblasts goes on continually from the centre towards the periphery at the expense of the peripheral zone of protoplasm, which is at the same time growing and causing the cyst to increase in size as a whole.

(3) The pansporoblasts give rise to minute spores in their interior, first to one or two, then to a gradually increasing number, till about a dozen are found in each pansporoblast, forming a spore-morula (figs. 6, 7, and 10). The spores are minute rounded bodies, each with a single nucleus (figs. 7 and 10). The oldest pansporoblasts are towards the centre, the youngest at the periphery, thus forming the three zones described above. The peripheral zone of protoplasm becomes reduced to the vanishing point, and as the cysts do not exceed a certain size, it is perhaps used up entirely in the formation of pansporoblasts.

(4) In many cases it is evident that the cysts burst and scatter the spore-morulæ in the surrounding tissues. It is highly probable that this represents the usual method of endogenous reproduction of this parasite, both on account of the very great number of cysts present in the tumours, and from the tendency of the tumours to recur after extirpation. Thus in the original growth removed by O'Kinealy signs of recurrence were observed in less than a month after the

operation (**1**). With more experience, removal of the tumour with cauterisation of the base was found to be the best form of treatment (**2**), which shows clearly that unless the parasite be extirpated completely, it will multiply and produce fresh growths. We are unable, however, to bring forward any observations upon the manner in which the spore-morulæ give rise to new generations of parasites. And the method by which the parasite succeeds in infecting fresh hosts must remain for the present a complete mystery.

From the preceding summary it is evident that this parasite must be placed in, or close to, the sub-class Neosporidia of the Sporozoa. The multinucleate trophozoites of irregular form; the progressive and continuous spore-formation, commencing at an early period in the growth of the parasite; the formation of uninucleate pansporoblasts from which numerous spores arise; and the intercellular habitat of the parasite in the submucous tissue—these are points which are diagnostic, or at least characteristic, of the Neosporidia. On the other hand, its position in the sub-class is less clear. The amœboid trophozoite and the minute spores recall the polysporous Microsporidia, but a sharp difference is seen in the absence of any pole-capsule in the spores, a feature which distinguishes Rhinosporidium from each of the three orders at present included in the Neosporidia. Moreover, Microsporidia are not known to occur in any warm-blooded vertebrate, in which class of hosts the Sarcosporidia seem to take their place, but it cannot be said that Rhinosporidium has any special resemblance to the Sarcosporidia, though this is the only group of Neosporidia hitherto known to occur in man. Another feature which appears to differentiate Rhinosporidium from the typical Neosporidia is the manner in which spores are formed successively in the pansporoblasts. In this point it recalls the peculiar but still unnamed parasites described by Schewiakoff in Cyclopidæ (**4**; see also **3**, pp. 318–320, fig. 127). The simple nature of the spores, on the other hand, recalls the parasites for which Caullery and Mesnil have founded the order Haplosporidia, as for example

Bertramia (**3**, pp. 309–311, fig. 124.) The conclusion is, therefore, that Rhinosporidium is an annectant form, which shows marked affinities with the typical Neosporidia and also with the simpler Haplosporidia. It indicates that the order Neosporidia should be extended to include Rhinosporidium and the Haplosporidia. The Neosporidia thus constituted might then be further subdivided into: (1) Cnidosporidia (Doflein), forms with pole-capsules in the spore, to include the Myxosporidia sens. strict., Microsporidia, and (?) Sarcosporidia; (2) Haplosporidia, simpler forms without pole-capsules, including, besides the forms hitherto referred to this section, Rhinosporidium and Schewiakoff's parasites.

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EXPLANATION OF PLATES 30 AND 31.

All the figures of Plate 31 were outlined with the camera lucida (Zeiss), using compensating oculars 4 or 18 (Zeiss) and objective 3 mm. homog. immersion (Zeiss).

Significance of the Lettering.

Gran. pplm. Granular protoplasm. *Pplm.* Protoplasm. Other references are given in full.

PLATE 30.

Fig. 1.—Photo-micrograph of cysts and surrounding tissue. × 50 approx.

PLATE 31.

Figs. 2 to 6 indicate the life-history of the Sporozoön, so far as known at present.

Fig. 2.—Small cyst, with thin wall and granular protoplasmic contents. From a preparation stained with picrocarmine. × 480.

Fig. 3.—Small cyst, stained with Delafield's hæmatoxylin, showing shrinkage and wrinkling of the cyst-wall. × 480.

Fig. 4.—Small cyst, amœboid in shape, with granular protoplasmic contents. From a preparation stained with picrocarmine. × 480.

Fig. 5.—Cyst containing closely-packed spherical uninucleate masses of protoplasm (pansporoblasts), and undifferentiated peripheral protoplasmic layer. From a preparation stained with Heidenhain's iron-hæmatoxylin. × 480.

Fig. 6.—A fully developed cyst, with a portion only of its contents drawn, showing the three zones of granular bodies (pansporoblasts), which zones, however, show a gradual transition from the peripheral, through the intermediate to the fully-formed or central zone. As seen in preparations stained with iron-hæmatoxylin, Delafield's hæmatoxylin, or picrocarmine. In a picrocarmine preparation the fully-formed spore-morulæ take on a reddish tint. × 480.

The spore-morulæ indicated with dark contents in Figs. 6, 7, and 9 were stained red with picro-carmine, the others staining yellow.

Fig. 7.—Portion of fully-developed cyst, such as is indicated in Fig. 6, more highly magnified. This shows the formation of spores in the pansporoblasts, so far as can be ascertained in the relatively thick sections at our disposal. From preparations stained with iron-hæmatoxylin and picrocarmine. × 2000.

Fig. 8.—Fully-developed spore-morula, more highly magnified than in Fig. 7.

Fig. 9.—Outline of crescentic, almost tubular, form of cyst and its contained pansporoblasts. Some have fallen out of the preparation. The contents of the pansporoblasts are not shown. From a preparation stained with picrocarmine. × 480.

Fig. 10.—Spore-morula (fully-developed pansporoblast) and contained uninucleate spores. The enclosing membrane is wrinkled, after staining with Delafield's hæmatoxylin. × 2000.

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FEBRUARY, 1906.

THE QUARTERLY JOURNAL OF MICROSCOPICAL SCIENCE.

EDITED BY

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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, Imp.,] [London and Dorking.

CONTENTS OF No. 196.—New Series.

MEMOIRS:

With Title, Contents, and Index to Vol. 49.

Studies in Spicule Formation.

IV.—The Scleroblastic Development of the Spicules in Cucumariidæ; with a Note relating to the Plate-and-Anchor Spicules of Synapta Inhærens.

By
W. Woodland,
University College, London.

With Plates 32, 33 and 34.

Introductory.

The early stages in the form-development of the perforated-plate and stool spicules of Cucumariidæ and other holothurians have been described and figured by many previous observers, as e. g. by Théel (**10**) in 1882, by Hérouard (**4**) in 1890, by Mortenson (**7**) and Kishinouye (**5**) in 1894, by Gerould (**2**) in 1896, and others, but in no case has the morphogenesis of the spicule been systematically studied in relation to the disposition of the scleroblasts or cells concerned in the deposition of the spicule. It is true that Hérouard (**4**) has provided a very detailed account of this very subject—as to the manner in which the direction of growth of the holothurian plate spicule is due to the number and disposition of the formative cells,—but since he does not provide any figures in confirmation of his statements, and since my own observations tell a very different tale, I see no reason to qualify the assertion I have just made.

The reason for the fact that the scleroblastic development of the Cucumarian spicule has not hitherto been ascertained lies in the considerable difficulties attending the investigation, for, in the first place, most "säurefrei" plasma stains have but little effect on the cells contained in the Cucumarian body-wall, and hence, nuclei being alone visible, it is hard to say under these circumstances whether any scleroblasts exist at all; and secondly, supposing the first difficulty overcome, the spicules of most Cucumariidæ are so densely crowded in the thick body-wall as to render it in most cases impossible to determine which scleroblasts belong to which spicule, it being assumed that the observer has learnt to distinguish scleroblasts from the several other histological elements of the body-wall. My own partial success in solving these difficulties was attained, firstly, by persistently experimenting with various plasma stains (picro-carmine forming an excellent nuclear stain, as in the case of sponges, Alcyonaria, etc.) until I happened to discover that which alone gave satisfactory results; and secondly, by patiently searching through scores of preparations of portions of Cucumarian body-wall, drawing only those comparatively few spicules concerning the entirety and individuality of which no doubt could be entertained. In calcareous sponges, e. g. most spicules are easily observable and can be drawn as distinct individuals, but in the Cucumariidæ which I have studied only about 5 per cent. of the spicules are so situated as to permit of their being correctly figured. In no case have I drawn a spicule about which any uncertainty existed as to the number of the attached scleroblasts.

The method of preparation of the Cucumarian body-wall for high-power microscopic examination which I finally adopted was as follows:—The Cucumarias (not more than 8 mm. in length, and some considerably less) were fixed, as in the case of calcareous sponges, etc., in 1 per cent. osmic acid for ten minutes, washed with distilled water and stained for three hours in picro-carmine (Ranvier's or Weigart's), washed again, and graded up to absolute alcohol (all strengths

of alcohol being made up with absolute alcohol and distilled water to ensure complete neutrality—a precaution most essential to observe in the case of all reagents employed). Before staining in picro-carmine, each Cucumaria was cut in half longitudinally with a pair of scissors and the internal viscera and musculature on the inner side of the body-wall removed, the whole of the latter being carefully scraped away with a scalpel. From absolute alcohol the Cucumarias were then transferred to a saturated solution of lichtgrün[1] in absolute alcohol, in which they were left for a quarter of an hour, then washed in absolute alcohol, cleared in xylol, and finally mounted in Canada balsam, usually with the inner side of the body-wall placed upwards on the slide. The body-wall must be well flattened out on the slide before covering with the cover-slip (each Cucumaria should be cut into at least four pieces), and a lead weight placed on the cover-slip after covering until the balsam is fairly dry, which condition is seldom obtained under a week. In studying the small superficial spicules of the Cucumaria, the body-wall, of course, must be placed outer side uppermost on the slide.

The structure of the body-wall of the Cucumariidæ having been already well described in several of the larger text-books and papers dealing with this subject, such as those by Hérouard, there is no need for me to here re-describe it; it will suffice if I briefly mention the several kinds of spicules to be found in the two species of Cucumaria which I have studied—Cucumaria sp. and C. brunnea,[2]—and the

[1] In the plates a grey tint has been substituted for green.

[2] Of these two (possibly three) species of Cucumaria, I received from Plymouth at least one dozen specimens of C. sp., and six or seven of what I have called C. brunnea. Concerning the identity of this latter species I am tolerably certain that most of my specimens of it were brunnea, both on Mr. Pace's authority and on account of the external appearance (possibly, however, one or more C. saxicola were also examined with C. brunnea as possessing no nodulated spicules). But with regard to the identity of the species of which I received by far the larger number of specimens I am very uncertain. Though, as just stated, the greater number of the Cucumarias I received from Plymouth was of this species, yet, curiously enough, on

histological elements contained within the same body-wall. In both C. sp. and C. brunnea the spicules may be primarily classified into (1) the very small superficial spicules, situated in the outermost layer of the body-wall or epistroma, and (2) the much larger perforated-plate spicules situated in the deeper layers of the stroma. In both species the small superficial spicules are either triradiate (fig. 39 *h*) or X-shaped (fig. 40) in ground plan, and the plane of the three or four rays, as the case may be, is, as in the case of all other Cucumarian spicules, parallel with the plane of the body-wall. The principal features of these superficial or epistromal spicules consists in the fact that the extremities of the three or four rays curl outwards, i. e. towards the exterior of the animal, and either branch irregularly ending in knobs (figs. 40, 41) or join peripherally into a ring (after the style of the Auricularian wheel) which bears knobs on its external edge (fig. 42). With respect to the more deeply situated plate spicules, these differ somewhat in the two species. In C. sp.[1] (of which species I have by far the greater number of specimens) the plate spicules, which are densely crowded,

sending my slides to the Plymouth Marine Biological Laboratory for identification, Mr. Pace, who has recently been investigating the Cucumariidæ obtained at Plymouth, replied that he was unable to say what species it was, owing to the parts of the body-wall not being separate from each other, and that, so far as he could ascertain, it was a new species to him. Seeing, however, that many of the spicules are nodulated and are present in great numbers, C. sp. (originally forwarded to me as C. pentactes) is probably C. Normani ("Note on Two Species of Cucumaria from Plymouth, etc.," by S. Pace, 'Journ. Marine Biol. Assoc.,' n. s., vol. vii, part 2, 1904), not C. saxicola, which contains no nodulated spicules. However, the question of the identity of species is fortunately of little importance to me, since the process of spicule-formation is identical in all the species named; all I can definitely state on the subject is that I received specimens of Cucumaria presenting the two quite distinct sets of spicules I have enumerated in the text. I wish to thank Mr. Pace for kindly attempting to identify the specific nature of my specimens.

[1] It must be borne in mind that this enumeration of the several kinds of spicules only applies to young specimens of Cucumaria, not more than 8 mm. in length.

may be classified into three kinds: (A) regularly developed plates, somewhat thick, which bear prominent knobs on their internal face (figs. 17, 18, 19); (B) the more common, fairly regularly developed plates, which appear to be thinner than the knobbed variety, and bear no knobs (figs. 11, 12); and (C) very large (three or four times the size of varieties A and B), irregularly developed, generally elongated, thick plates with small perforations and no knobs (figs. 25—30). These last are usually few in number, and are developed on the innermost side of the wall. In C. brunnea the larger spicules are all of one kind, consisting of more or less irregular plates of larger size than usual, fairly thin, with the usual large circular perforations, and with no knobs (figs. 31—36). The plate spicules in this species are not so crowded as in C. sp., and therefore their development is easier to ascertain. Unfortunately for me, however, I possess very few specimens of this species, and in consequence I have not been able to trace the development of its spicules nearly as fully as I have in C. sp.

The scleroblasts or cells which possess the function of depositing the spicules are cells which, when free, are more or less spherical in contour, stain a light green with lichtgrün stain, possess a large spherical nucleus (stained red with picro-carmine) with a distinct nucleolus (often with several smaller ones as well), and usually also possess a number of dark, reddish-brown (so coloured by the lichtgrün) granules, which often collect at the cell periphery (figs. 1, 2). These granules only appear, according to my experience, when the cell is stained with lichtgrün; previously to so staining these granules appear as slightly refringent spots in the cell plasm, and are almost invisible. Occasionally, but very rarely, these granules are absent, and there is apparently no relation whatever between the number and size of these granules and the stage of growth of the spicule. Scleroblasts which are not free, but which are actively engaged in depositing a spicule, also tend to retain the spherical form and appear as subspherical masses on the surface of the spicule, the body of

which is otherwise uniformly invested by an extension of the scleroblast plasm, which is very difficult to render visible by staining, but which is quite evident on decalcification of the Cucumarian body-wall. The Cucumarian scleroblast in fact very much resembles that of the Echinoid pluteus, which also deposits the lime of the skeleton in a kind of ectoplasm and not in its internal substance, and differs, in consequence, from the scleroblasts of sponges and Alcyonaria, in which the deposit is internal. It is a remarkable fact that there should exist two such distinct types of scleroblasts as those just mentioned, the one in which the lime is secreted in the ectoplasm, and the other in which the lime is secreted in the endoplasm, and I know of no explanation of it; it is possible, however, granting this fact, that it explains some features of spicule morphology, and this I shall attempt to show later.

In addition to the scleroblasts adherent to the spicules and free scleroblasts, there exist two other classes of cells in the body-wall of C. sp. and C. brunnea, prepared as described above, which must be distinguished from the scleroblasts. The more numerous class, universally found, and which form a great impediment to the observation of the spicules and their scleroblasts, consists of cells running in strands which resemble scleroblasts in every particular save their form, which is elongated and drawn out after the manner of muscle-cells; I have no doubt but that they are the "fibres" already many times described (fig. 37). Similar cells—the strands of "endoderm"—are to be found in the common Alcyonium digitatum. The other class, comparatively rare, consists of cells about the same size and shape of the scleroblasts, but stained an intense opaque green, which colour is due to the presence of numerous spherules of a substance contained within the cell plasm which readily absorbs the lichtgrün. No dark granules are present in these green cells, and the nucleus resembles that of the scleroblast (fig. 38). I am not certain with regard to their identity; they are not pigment-cells, nor amœbocytes, but they are

possibly excretory in nature, and have doubtless been described under another form.

The Development of the Cucumarian Spicule.

As a type of the development of a Cucumarian spicule I shall describe that of the commonest form of spicule in C. sp. — the (B) of the above classification, — a description which will apply to all forms of the large plate spicules of Cucumariidæ with but slight, if any, modifications.

The spicule first makes its appearance as one or more small granules (fig. 3), which rapidly assume the form of a minute refringent needle (fig. 4). Despite the statements of Hérouard (3) and others, the spicule has not at any stage of its evolution the form of "un petit tetraèdre."[1] The remarkable feature about this initial spicular needle is that it can arise in connection with either two or four scleroblasts (fig. 4)— primary scleroblasts, as we shall call them. The origin of the spicule between two scleroblasts is by far the commoner mode, but, nevertheless, the needle does sometimes originate between four scleroblasts, two on either side of it, and hence Cucumarian spicules have two modes of origin. Scleroblasts recently divided are fairly frequently met with (fig. 2, *a*, *b*), and, since I have never yet seen a spicular needle enclosed by a single scleroblast, it is reasonable to assume that the bi-division of an ordinary scleroblast is the constant percursor to the production of a needle associated with two scleroblasts. On the other hand, the only explanation which occurs to me of the less usual, but equally certain, tetra-scleroblastic origin of the spicular needle is that it is the result of the apposition of two scleroblasts (fig. 2, *c*), which soon afterwards divide, thus forming four. The tiny needle thus originated elongates to form a rod (figs. 5 and 6), which is usually somewhat swollen towards the middle and at the extremities. Needles and rods possessing four scleroblasts (fairly commonly met with) exhibit no difference in size or

[1] See my paper, "On the Development of the Echinopluteus Skeleton."

form from those possessing only two (very common), and, indeed, throughout the growth of the spicule I have never been able to detect any distinction separating the spicules into two classes, according to their mode of origin. The swollen extremities of the rod later extend laterally (fig. 8), and, continuing to grow thus, the rod becomes definitely bifurcated at each end (fig. 9). About this stage it often happens that one of the scleroblasts of the bi-scleroblastic spicules divides, so that three cells are present (fig. 9, *b*, *c*). Similarly, one not unfrequently meets with a bifurcated rod with five cells—one of the primary four cells having divided (fig. 9, *f*). I say that these additional cells are produced by division of the two or four primary scleroblasts associated with the origin of the spicule, but it must be confessed that in Cucumarias there is no means of telling with certainty whether this additional number of cells may not have been produced by the arrival of a free scleroblast, which attaches itself to the already-formed spicule, and which, therefore, was not associated with its origin. Free scleroblasts are often to be found in the vicinity of growing spicules, and it is sometimes very difficult to distinguish between them and the primary scleroblasts. From which of these two sources additional scleroblasts are derived can only be definitely ascertained by studying spicule-formation in some species of Holothurian where the spicules are not so densely crowded as in the two species at which I have worked; I can at present simply record the number of scleroblasts associated with definite stages of growth of the spicule which I have observed, leaving the question as to their derivation unanswered. At the same time I may record my conviction that the additional scleroblasts mentioned originate by division of the primary scleroblasts and not by the arrival of new scleroblasts. One of my reasons for this conviction is that it is not uncommon to find scleroblasts attached to spicules with abnormally large nuclei—a sure sign of cell-division. Here, also, I may remark upon one striking fact, and that is that in C. sp. and C. brunnea, and pre-

sumably in other Cucumariidæ, the number and position of the scleroblasts adhering to a spicule bear no relation whatever to the stage of growth of that spicule [1]—which fact (also illustrated by Alcyonarian spicules) is in striking contrast to the conditions found, e. g. in calcareous sponges. The scleroblasts appear simply to function as localised stores of material needed for the enlargement of the spicule, and though, as explained below, it seems probable that the initial form of the spicule—the terminally-bifurcated rod—is determined by the position and structure of the primary scleroblasts, be they two or four in number, yet beyond this early stage of development, their position exerts no influence on the manner of growth of the spicule, which, as is well known, consists of continuous bifurcations of the extremities resulting in the familiar perforated plates. The body of the scleroblast undoubtedly becomes reduced as the mass of the spicule increases, though there is no diminution in the number or size of the contained black granules, which usually aggregate in the region of the nucleus.

To continue the account of spicule development. The bifurcations of the initial extremities of the young spicule rod elongate and themselves bifurcate (fig. 10), finally uniting both terminally and laterally (with respect to the length of the spicule rod) to form the first series of four apertures (fig. 11). As implied above, the number of scleroblasts present at this stage of growth is anything but constant, two, three, four, five or more being in different instances associated with the spicule, the form or size of which is quite independent of the particular number. Bifurcation of the spicule extremities continues (fig. 12), and finally results in a second series of apertures, and so on. But, in stages of spicule growth succeeding the formation of the first series of four apertures, it is quite hopeless, at least in C. sp. and C. brunnea, to attempt to ascertain the exact number of scleroblasts associated with individual spicules. The

[1] Differences of position in the body-wall of different spicules is probably largely accountable for this anomaly.

crowding of the spicules one upon the other, and the presence of free scleroblasts and multitudinous elongated cells ("fibres"), quite defeats any such endeavour; it is only possible to say that in young Cucumariidæ of the size above mentioned perforated plates of two, three, or more concentric series of perforations possess a very variable number of scleroblasts (I have rarely counted more than a score), quite irregularly arranged with respect to the form of the spicule, and differently arranged in different cases.

The criticism may be made that all the irregularity in disposition and number of the scleroblasts I have above insisted upon is due to disarrangement involved in preparation of the body-wall, and that in reality scleroblastic spicule-formation in Cucumariidæ proceeds after the beautifully symmetrical fashion described with so much assurance by M. Hérouard.[1] Such a criticism will have but little weight with those who observe the actual facts, since, for one thing, the body-wall and its contained spicules show no signs of disturbance, and this is very easy to detect when such has occurred (as at the extreme edges of portions of the wall which the observer of course avoids). Moreover, the body-wall, by my methods of preparation, is never subjected to rough treatment. The internal muscular layers separate quite easily from the hypostroma of the wall, and if each Cucumarian body-wall be cut into eight or more portions with a sharp scalpel, there is no need to subject these to any sort of tension in order to flatten them out on the surface of the slide.

In the foregoing I have given an account of the early scleroblastic development of the commonest type of spicule (B) to be found in C. sp. In the case of the knobbed (A), and the large irregular small-holed (C), varieties of spicule

[1] It is hardly necessary to point out that such a version of scleroblastic spicule-formation as that of Hérouard involves considerably more obstacles in the way of explanations than the truth. How does M. Hérouard account e. g. for the simultaneous arrival of the four cells forming a detachment, and, still more, their symmetrical disposition about the spicule when they have arrived?

of the same species, there is every reason to believe that their development is of the same type. The knobbed spicules very much resemble the common variety as regards approximate regularity of growth, but differ as regards their thickness, the small size of their perforations, and the development of knobs. Knobbed spicules containing four apertures are usually found with either two or four scleroblasts adhering to them, and indeed the stages depicted in figs. 17—19 are extremely common. They and older stages are usually found situated on the interior side of the wall with spicules of the (c) variety; as usual, the most massive spicules are situated internally. The reason for the development of knobs on these particular spicules I am quite unable to ascertain.

Of spicules of the (c) variety I have found several fairly young stages (figs. 20—24), from which it will be observed that the two types of development—the bi- and tetra-scleroblastic—also obtain here (cf. figs. 20—22). Another feature concerning this type of spicule is the small number of scleroblasts associated with the larger plates (figs. 28—30). Also most of the adult forms of these spicules are more or less elongated, and they lie with the long axis parallel with that of the animal.

In the other species, C. brunnea, the plate spicules are larger than any to be found in C. sp., but at the same time are fewer in number. Initially the development is as usual of two types; at least, judging from figs. 31 and 33, it is. Like the variety (c) of C. sp., the number of scleroblasts associated with the older spicules is small, and the plates (only the very young are shown in the figures) are elongated. The scleroblasts are very plentifully supplied with the dark granules.

With regard to the small superficial spicules common to both C. sp. and C. brunnea, I have been least successful in ascertaining their mode of origin and development owing to difficulties of observation.[1] Apparently they

[1] See further in section on Thyone.

arise in one cell (fig. 39, *c*, *e*), but of this I am not absolutely certain, since the other cell may have been detached from the young triradiate or rod, and young stages are so few in number, at least in my material, that I cannot come to a definite opinion. Older stages, where the original triradiate or terminally-bifurcated rod has branched, with one, two, three, four, and perhaps more nuclei, are quite common (figs. 40, 41). Some of these superficial spicules which happen to be more deeply situated in the epistroma have their main branches joined peripherally into a ring which then bears knobs (fig. 42). I hope, in the near future, to be able to more definitely ascertain the development of these superficial spicules,[1] and to settle what is rather an important question from a theoretical standpoint, viz. whether a triradiate spicule is capable of originating in a single scleroblast. I believe that in the Cucumarian epistroma it is capable of doing so, largely because so many of the adult superficial spicules only possess a single scleroblast, as shown in fig. 40.

Previous Literature.

Previous literature relating to the subject of the present paper is rare. The first observations known to me, made by an undoubtedly competent authority, are those of Théel, the results of which were published in the Challenger monograph on the Elasipoda in 1882. Théel attempted to study the development of the simple elongated spicules contained within the thin walls of the pedicels of young Oneirophanta, but owing to the rough methods adopted in the preservation of the material, no very definite results were obtainable. Théel's theory, which was evidently suggested by an inspection of the material, was that the sheath of the spicule—"a thin membrane, which, when treated with hæmatoxylin, became deeply coloured and very manifest"—was developed first, "the calcareous matters being subsequently produced by it." "The sheaths increase and gradually assume the

[1] See further in section on Thyone.

shape of a spicule. I have first discovered calcareous matter inside them when they have attained a length of about 0·20 mm." All who have investigated the development of calcareous spicules know the extreme susceptibility of these structures to traces of acid, and there can be no doubt but that Théel's empty sheaths simply represented the protoplasmic investment of dissolved spicules. It is true that the "sheath" produces the spicule, but it is no less true that the form which the deposited lime assumes determines the shape of the "sheath"—the two grow pari passu.

The same criticism probably also applies to Chun's wonderful account (1) of the development of the calcareous wheels of the Auricularia larva of Holothurians published ten years later. As Théel remarks concerning this account, "there seems to be scarcely anything similar observed in other Echinoderms." Chun states that when the scleroblast has attained a size of 0·03 mm., "there appears within the old cell-membrane a new one, which has an undulating outline towards the circular margin and speedily assumes a star-shaped form," and ultimately "the rays become united by a peripheral membranous ring. It is now impossible to mistake the mould of the subsequent calcareous wheel, prepared as it is by the complex folds of an internal membrane. . . . Moreover, the calx is actually secreted into this organic matrix formed by the skeletogenous cells as into a mould, and in such a way that (as the older accounts already teach us) calcification takes place first in the nave, then in the spokes, and finally in the felly of the wheel"—the nuclei present migrating from the centre to the periphery in correspondence with the extension of calcification. Before accepting this account of this "unique" mode of origin of the Auricularia wheel, I should have to be thoroughly convinced as before that the presence of empty sheaths or moulds was not due to traces of acid contained in the reagents employed in the preservation of the wheels. As I know from experience, the calcareous portion of the skeleton of the echinopluteus larva, despite the utmost precautions taken to ensure

the absence of acidity, generally vanishes within a month or so, leaving, at any rate in the case of sponge, Alcyonarian and Cucumarian spicules, a distinct "sheath" as its sole representative, with traces of contained calcareous matter. As I have above stated, the "mould" is probably as much the product of the contained sclerite as this is of the "sheath" or investing cœnocyte.

Hérouard (**4**), like Chun, has provided a very interesting account of the development of Holothurian spicules, and more particularly those which concern us in the present paper, viz. the perforated-plate spicules of Cucumariidæ; also, like Chun, he gives no figures in confirmation of his statements and, to again draw a parallel, I am sorry to say that these latter are equally as questionable, if not more so. Hérouard, whose excellent work on Holothurians should guarantee the trustworthiness of his statements, fully developed a preliminary note of his "Sur la formation des corpuscules calcaires chez les Holothuries," published in 1887, into an elaborate description of the process in his "Recherches sur les Holothuries des côtes de France," published in 1890. This description is, in brief, as follows:—The spicule commences as "un petit tétraèdre de carbonate de chaux," secreted in the interior of a cell resembling an amœbocyte. Apparently four of these cells associate, and "le calcaire se dépose le long des parois de contact de quatre de ces cellules accoleés l'une à l'autre et donne une production en forme d'*x* [see my fig. 9]. C'est lá le centre du developpment, la charpente en quelque sorte du corpuscule. Les choses n'en restent en général par lá; quatre autres cellules placées symétriquement dans les angles formés par la réunion des quatre premieres entrent en jeu; elles déposent à leur tour du calcaire sur leurs parois de contact avec les précédentes et on obtient ainsi un corpuscule de la forme représenteé fig. B ci-dessous [a diagrammatic equivalent of my fig. 11*f*.; Hérouard does not supply even diagrams of the cells]. D'autres cellules occupant les angles formés par ce groupe de huit cellules, entrant en jeu à leur tour, il se formera par le même processus de nouvelles branches, et nous aurons

ainsi la forme de la fig. E [see my fig. 12], et ainsi de suite." Hérouard, amongst other things, attributes the existence of the perforations in the plates to the presence of the cell-nuclei which, he says, prevents the deposit of lime in their immediate vicinity, and, to thoroughly round off his theory, he subsequently enters into half a dozen other "explanations"—referring the primitive form of the spicule to the contractility of the body-wall, etc.—which I have not space to recount in, much less examine. What is to be said of the foregoing, which professes to be an account of actual facts, written by a capable observer? M. Hérouard tells us that if we take the more or less macerated stroma of the body-wall of C. planci and place it in "carmin acétique," "nous voyons sous l'action du réactif, le corpuscule calcaire disparaître et être remplacé par un réseau hexagonal à peu près régulier, coloré en rouge, et au centre de chacune des mailles de ce réseau, un noyau : ces noyaux occupant précisément la place où se trouvaient primitivement les trous du corpuscule." I can only say that I have followed M. Hérouard's instructions, and cannot find a trace of "un réseau hexagonal." [1] In fact I must categorically deny the whole series of M. Hérouard's statements. The spicule does not arise as a minute tetrahedron; there are rarely four cells in connection with the terminally bifurcated rod (the more usual number being two), and then they are not hexagonal in form; four newly-arrived cells do not regularly attach themselves to the spicule for its further growth, and however many cells may ultimately be associated with the spicule they have, by no means, that symmetrical disposition claimed for them by M. Hérouard. I once showed my preparations of Cucumarian spicules to a friend who, merely glancing down the microscope tube, immediately exclaimed, "Ah, I see, one cell in every hole." I regret that M. Hérouard has chosen to adopt a like method of arriving

[1] All that is to be seen in a carefully decalcified body-wall of a Cucumarian are the globular scleroblasts in connection with the thin film of plasm (now rendered more visible) which covered the mass of the dissolved spicule.

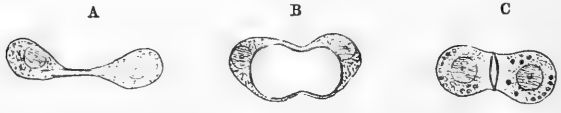
at a conclusion, and, worse still, publishing it as an ascertained fact.

Gerould, in 1896, supplied a figure showing a young terminally-bifurcated rod of Caudina arenata with five scleroblasts either near or attached to the spicule, but he evidently did not attempt a systematic research in this direction.

A few Theoretical Considerations.

Those who have read Prof. Minchin's admirable account (6) of spicule formation in the Clathrinid Ascons, or Study I (and II) of the present series, will have been struck by the

Text-fig. 1.



A. Young monaxon spicule of a calcareous sponge. B. Young Alcyonarian spicule. C. Young Cucumarian spicule.

curious fact that, whereas the young spicular needle or rod of sponges (and Alcyonaria) is situated in the line joining the two primary scleroblasts, that of Cucumariidæ is disposed transversely to this line (fig. 4; and text-fig. 1). Is this fact to be correlated with any other conspicuous difference in the structures associated with the spicule in the respective groups? The only difference, and that an important one, which suggests itself to me is the evident difference of constitution of the scleroblast substance in sponges and Cucumariidæ. As remarked upon above and also in Study II, whereas in sponges the young spicular needle originates in the deep interior of the substance of the scleroblast, and, in correspondence with this quality of the cell substance, the scleroblast of the grown spicule adheres to its mass as a sloping mound containing the nucleus, in the echinopluteus the young spicule originates in the ectoplasm

only of the scleroblast, and, similarly correlated with the quality of the cell substance, the scleroblast adheres to the grown spicule as a spherical mass with a very small basis of attachment. Now in regard to the character of the cell substance, the Cucumarian scleroblast agrees with that of the echinopluteus, and in consequence the young sclerite originates in the ectoplasm or extreme peripheral layer of the cell and the scleroblasts of grown spicules adhere as spherical masses, as shown by the figures. If we imagine say two indiarubber balls covered with a layer of some still softer material, which, we will also suppose, is secretory in function, to be situated between two boards (to represent the position of the scleroblasts between the external wall and internal flat layers of fibres), and whilst in this position to come into close contact, we can realise that this apposition, under such conditions, would produce the particular stresses in the soft layer lying between the two balls necessary to result in the formation of a transversely-disposed rod of matter secreted by the soft layer. The secreted matter cannot elongate in the direction of the line joining the two balls, since the indiarubber is not secretory. On the other hand, the soft layer, which is secretory, is already elongated at right angles to this line by the mutual apposition of the two balls, and the secretion will occur in the plane parallel to the boards owing to the compression exerted on either side by these. Substitute endoplasm for indiarubber, ectoplasm for the soft layer, and lime for the "secreted matter," and my meaning in reference to the possible reason for the deposition of the Cucumarian needle at right angles to the line joining the two primary scleroblasts will be evident. The same argument applies to the bi-division of a scleroblast since here also the plane of separation of the two cells is occupied by a layer of ectoplasm necessarily situated at right angles to the line joining the two cells. In sponges, on the other hand, the young sclerite naturally elongates in the direction of the line joining the two primary scleroblasts, since the elongated portion of endoplasm joining the two cells is the plasm that secretes the lime. Further, in

Cucumariidæ, the presence of two masses of endoplasm on either side at the middle of the young rod also possibly explains why this expands at places when the endoplasm is not situated, viz. at the extremities, ultimately leading to bifurcation—bifurcation, because the sources of material for spicule growth are situated at the sides of the rod and not at its extremities, and deposition would naturally occur in the direction from which the greatest supplies are received. However, no such supposition can account for the subsequent bifurcations of the four-holed spicule, since in many cases no additional cells make their appearance, and if additional cells do arrive, either by division of the two or four primary scleroblasts, or by the adherence of new scleroblasts, these bear no such relation to the spicule as would be required either by the supposition just made or by the theory of Hérouard.

It is possible that the perforations of the plates are in some manner related to the necessity for communication between the upper and lower strata of the stroma, for it is evident that if the spicule plates were all solid, they would, on account of their great number, form a serious impediment to the nutrition of the external and the respiration of the internal tissues of the animal. But a desideratum, and, as I have just implied, the perforation of the spicule plate is in all probability a desideratum, cannot constitute a cause to bring about the desired variation, and even if it could we are not as yet justified, owing to our ignorance of the inheritance of spicule characteristics, in assuming that the variation would be inherited and perpetuated by natural selection. The form of the spicule is a product of physical causes, but what these causes are I cannot yet say.

On the Mode of Deposition of the Spicules in Thyone fusus.

Since completing the work above described on the spicules of Cucumaria I have been fortunate enough to obtain from

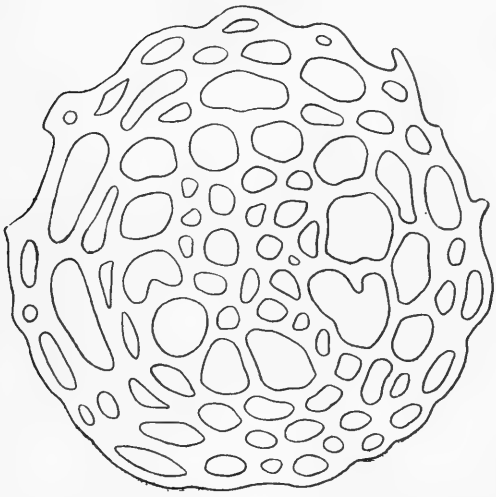
Plymouth two living fairly adult specimens of another common genus of the Cucumariidæ—Thyone. These I prepared for microscopic examination of the spicules by the method usually employed by me (vide supra), and I am thus enabled to describe the scleroblastic mode of development of some of the spicules characteristic of this genus. Needless to say, this mode of development is essentially identical with that described for the Cucumarian spicules. In preparing Thyone it is very difficult, if not impossible, to fix the animal with osmic acid in a fully-distended condition, and one has to be content (unless, perhaps, anæsthetics are employed) with those few portions of the body-wall which happen to be so fixed, since the remainder is of little use. In Cucumaria the multitude of spicules present in the body-wall prevents this undue contraction. The muscle-layers have, as before, to be carefully removed before mounting the portion of integument to be examined.

In Thyone fusus—the species obtained—there exist five distinct classes of spicular elements, which may be briefly enumerated as follows :—(*a*) the circular perforated-plate or "wheel" spicules situated at the extremities of the podia (text-fig. 2); (*b*) the "stool" spicules (figs. 50—56), chiefly, but not exclusively, found in the region of the integument situated just posterior to the ring of tentacles, and which is devoid of podia, the plates of which spicules are also circular in outline, but larger meshed centrally and thicker in limb as compared with the podia wheels, also they are smaller in total circumference (cf. text-figs. 2 and 3); (*c*) the much smaller irregular superficial spicules (figs. 57—65) found in the same region of the body as those last mentioned, more particularly at the bases of the tentacles; (*d*) the spicular "sponge-work" composing the oral plates, and (*e*) very large, massive, approximately-circular, perforated plates (three or four times the size of the podia wheels) situated in the main body-wall. These last I only discovered in isolating the spicules from the softer parts of the animal by means of eau-de-javelle, and I am ignorant as to their exact situation in the body-wall. My

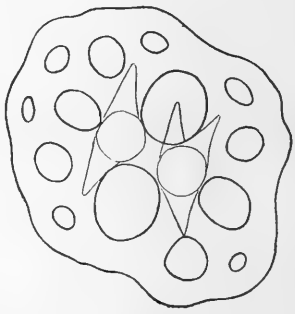
supply of properly-prepared material being strictly limited, I am only able at present to describe the scleroblastic development of spicules of the varieties (*b*) and (*c*) above enumerated. The development of the remaining varieties, however, doubtless proceeds on the same lines.

The youngest stages of the (*b*) variety of spicules I have been able to find are those represented by figs. 45 and 46, and these, it will be observed, are strictly of the more usual

Text-fig. 2.



Text-fig. 3.



Cucumarian type. Even at this early stage the "stool" process, which points towards the exterior of the animal, is originating by slight protuberances at the bases of the bifurcations of the rod extremities. Spicules in which the second series of bifurcations has taken place (figs. 47 and 48) are, as also in the case of Cucumaria, of two classes—those possessing two scleroblasts and those possessing four. I have observed, in one fair-sized specimen of T. fusus, at least four or five of these young spicules clearly with four scleroblasts attached. Since, as in Cucumaria, the majority of these spicules originate from only two cells (as is proved by the fact that in the majority of adult spicules only two

scleroblasts are present), it is probable that in Thyone, as in Cucumaria, these spicules have two modes of origin—the bi- and tetra-scleroblastic—the former being by far the more common. Owing, however, to my specimens of Thyone not being very young, I have not been able to find in this genus the youngest stages of these two modes.

The further development of these Thyone spicules is not precisely the same in every case, as will be seen by comparing figs. 50 and 51. Also, unlike the Cucumarian spicules, the perforations succeeding the first four formed are much smaller, attaining a minimum at the extreme periphery of the nearly circular plate. As before remarked, the vast majority (at least 70 per cent.) of the adult forms of these spicules possess only two scleroblasts (figs. 49—52 and 56), and so again differ from the spicules of Cucumaria. A few (an appreciable percentage) possess four scleroblasts (probably tetra-scleroblastic in origin), and also a few undoubtedly possess three scleroblasts, so that division of the scleroblasts evidently occurs. The reason why it is difficult to suppose that all spicules with four scleroblasts attached are derived from spicules possessing only two scleroblasts is that four scleroblasts are so often (relatively) found associated with young stages of these spicules (I believe I have seen a terminally-bifurcated rod possessing four scleroblasts, but it was not sufficiently certain[1] to figure). I must further mention that I have occasionally come across well-developed spicules with only one scleroblast adhering (once or twice with none at all), but, of course, in these few cases it may safely be assumed that one scleroblast has become detached during preparation of the slide, since, apart from this, there is no evidence whatever for supposing that these large spicules of the (*b*) variety can originate in one cell.

As regards the position of the nuclei on the spicule, in almost every case the two (or four) nuclei are situated at the

[1] As in Cucumaria the observer has to distinguish between scleroblast nuclei and the nuclei of adjacent fibres, but in Thyone this is not so difficult. "Green" cells are also present, but they are scarce.

sides (usually, but not always, one on each side) of the primary rod at the centre of the perforated plate—the "corpuscule calcaire fondamental"; occasionally, however, but very rarely they are situated more peripherally. Also in every instance the nuclei are placed rather more towards that side of the perforated plate on which the "stool" is borne than towards the inner aspect of the spicule. No nuclei ever adhere to the "stool" itself, and this develops entirely unaided in this respect into the form shown in fig. 55, where the spicule is viewed from a lateral aspect.

The whole spicule is in Thyone, as in Cucumaria, entirely enveloped by cytoplasm—is contained in a protoplasmic sac—and each of the nuclei is, as usual, situated in a more or less aggregated portion of it lying to one side of the spicule limb. Black granules are only rarely present in the scleroblasts of young spicules, and are apparently quite absent in those of adult spicules.

The small irregular spicules of T. fusus [variety (*c*) above] undoubtedly arise in one cell, as is also the case with the small superficial spicules of Cucumaria. When first observing these latter I was, as the reader is already aware, strongly disinclined to believe this, and I expressed myself very tentatively upon the subject. But later observations on additional specimens of Cucumaria and my still more recent examination of the similar spicules of Thyone banish all doubt upon the subject. I have not, in Thyone, been able to discover the primary rod stage of development of these small spicules (the youngest forms which I have observed being represented by figs. 57—61), but this undoubtedly occurs. As the spicule grows, it assumes an irregularly-branched form, the ramifications of which, however, do not anastomose (figs. 62—65), as in the larger types of spicule to form perforated plates, and at a certain stage of growth the initial nucleus divides into two (figs. 63 and 64). Still later the nucleus further divides forming three and four scleroblasts (fig. 65)—a "scleroblast" consisting of a nucleus with its associated mass of protoplasm—but I have never observed more than four. In this type of

spicule there is thus observable a rough relation between size of spicule and the number of attached scleroblasts, and such is the case with the similar type of spicule of Cucumaria. In the large spicules of Cucumaria, it will be remembered, I was unable to trace any such relation, but it is probable that, as I then observed in a footnote, this relation was obscured by the situation of the spicules in different planes of the body-wall—this factor in spicule development defeating comparison.

With regard to the spicular sponge-work composing the oral plates of Thyone [variety (*d*) above] many scleroblasts can be observed adhering to the various parts of the reticulum, but no relation can be traced between any particular local conformation of the skeleton and the presence of a scleroblast. This sponge-work doubtless arises in the same manner as the similar sponge work of Asteroids, Echinoids, and Ophiuroids arises, viz. by the fusion of primitively separate elements, each of which is equivalent to the individual plate-spicule of Cucumaria or Thyone, and therefore probably develops in the same manner, i. e. as regards the number of scleroblasts, etc. I have myself observed the perforated plates of Amphiura elegans and of the recently-metamorphosed Echinus esculentus prepared by the picro-carmine and lichtgrün method, and the number of scleroblasts attached to them amply confirms this supposition. On the other hand, the lateral spines of many Ophiuroids and the axial skeleton of Crinoid arms, judging merely from observation of the adult structures, appear to develop in a manner different from that just stated. Whether such is the case, however, I hope to ascertain at some future date.

In conclusion I wish to express my thanks to Dr. E. J. Allen, who kindly afforded me every facility during my visit to the Plymouth Marine Biological Laboratory in 1904, in obtaining material, and to Prof. Minchin, who originally suggested to me that I should turn my attention to holothurian spicules.

Note on a Stage in the Scleroblastic Development of the Plate-and-Anchor Spicules of Synapta inhaerens.

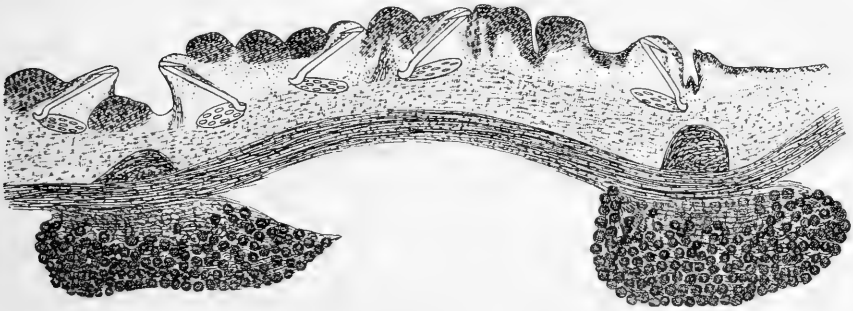
Whilst staying at Plymouth in the spring of 1904 I was able to obtain, with the kind assistance of Dr. E. J. Allen, about a score of young specimens of Synapta inhaerens. I hoped with these specimens to be able to ascertain the scleroblastic development of the characteristic spicules of these animals, but, young though the specimens were, all the spicules were more or less fully formed, and I only succeeded in procuring what is probably the final stage of growth. Since I am not aware that any scleroblasts have previously been figured in connection with the spicule, I think it worth while publishing the sole result that I have obtained.

The accompanying text-figure (text-fig. 4) shows well the position of the plate-and-anchor spicules in the body-wall of Synapta. As is already well known, the length of the anchor, curiously enough, is disposed transversely to the long axis of the animal, and the anchor encloses with the plate an angle which is fairly constant. It is possible that Östergren (**8**), following Quatrefages, is right in considering that the anchors aid in the locomotion and burrowing of the Synapta, but the suggestion is no explanation of the evolution of these curious structures.

Semon (**9**) has supplied excellent figures illustrating the morphogenesis of the plate-and-anchor spicules of S. inhaerens. As I have above remarked, all the spicules I observed were adult in form, and the Synaptas, having been treated in the same manner as the Cucumarias, showed well the cell-plasm and nuclei associated with them. As illustrated by figs. 43 and 44, the most conspicuous features of the spicule are the two elongated strands of protoplasm containing many nuclei which run on either side from the arms of the bow to the handle. This strand thickens at its bow end, forming a blob at the point of the arm, and the

small contained nuclei usually aggregate both towards the point of the arm and towards the handle. A certain number of nuclei are also to be observed in the perforations of the anchor-plate, along the shaft, and on the bow. There is also in every case a great concentration of protoplasm at the junction of the anchor with the plate, as shown. I have observed scores of spicules showing precisely the same features as those just described, and these are evidently constant for the adult spicule. Fig. 44 shows a similar spicule in side view. The angles which the arms of the bow make with the shaft and which the shaft makes with the

Text-fig. 4.



anchor-plate can be easily observed. It would be interesting to ascertain whether or no the recurvature of the arms of the bow is at all due to a tractive action exerted on these by the possibly muscular strands of protoplasm just described.

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EXLANATION OF PLATES 32, 33 & 34,

Illustrating Mr. W. Woodland's "Studies in Spicule-Formation."

With the exception of Figures 25—30 and 43, 44, all have been drawn with a magnification of 750 diameters.

PLATE 32.

Development of the (*b*) variety of spicule in Cucumaria sp.

Fig. 1.—Free scleroblasts.

Fig. 2.—Division of the scleroblast. *c* is apparently an association of two scleroblasts.

Fig. 3.—The initial calcareous granules.

Fig. 4.—The spicular needle. *a* and *b* bisclerobiastic, *c* tetrascleroblastic in origin.

Figs. 5, 6, 7.—Growth of the young needle. In Fig. 7 the rod is tetrascleroblastic in origin.

Fig. 8.—Initial broadening and bifurcation of the rod. In *c* one of the cells has divided.

Fig. 9.—Bifurcation of the rod. *a*, *b*, and *c* are bisclerobiastic, *d*, *e* and *f* tetrascleroblastic in origin.

Fig. 10.—Second series of bifurcations. *a* and *b* bisclerobiastic, *c* and *d* probably tetrascleroblastic in origin—probably because the two primary scleroblasts may have divided.

Fig. 11.—The formation of the young plate containing four perforations. The number of the cells ranges from two to five.

Fig. 12.—Further growth of the young plate; eight cells present.

Figs. 13—16.—Other forms of the (*b*) variety of spicule in C. sp., all biscleroblastic.

Fig. 17.—Knobbed spicule-plate (*a*) with two scleroblasts.

Figs. 18, 19.—Knobbed spicule-plates with four scleroblasts.

PLATE 33.

Figs. 20—30.—Stages in development of the (*c*) variety of spicule in C. sp. Mostly, if not all, biscleroblastic in origin. Fig. 22, however, is probably tetrascleroblastic. Figs. 25—30 × about 375.

Figs. 31—36.—Young stages in the development of the plate-spicules of C. brunnea. In fig. 35 the elongated cell to the left probably does not belong to the spicule.

Figs. 37, 38.—Elongated cells—"fibres"—and the green cells found in great numbers in the body-wall of both C. sp. and C. brunnea.

Fig. 39.—Stages in the development of the superficial spicules common to both C. sp. and C. brunnea. *c*, *e*, and *h* are stages found, which, if perfect, prove the one-celled origin of this form of Cucumarian spicule.

Figs. 40, 41 show the adult form of superficial spicule, with either one or two scleroblasts attached.

Fig. 42 shows another form of adult superficial spicule, with four scleroblasts.

Figs. 43, 44 show the final stage of scleroblastic development of the plate-and-anchor spicules of Synapta inhaerens. Fig. 44 shows the spicule in side view. Both figures × about 375.

PLATE 34.

Stages in development of the spicules of Thyone fusus.

Figs. 45—56 represent a developmental series of the stool spicules. Figs. 48 and 54 probably represent spicules of tetrascleroblastic origin. Fig. 55 represents a stool spicule in side view.

Figs. 57—65 represent a similar developmental series of the superficial spicules. In Fig. 60, one end of the spicule is apparently broken.

On the Maturation of the Unfertilised Egg, and the Fate of the Polar Bodies, in the Tenthredinidæ (Sawflies).

By
L. Doncaster, M.A.,
Mackinnon Student of the Royal Society.

With Plates 35 and 36.

1. Introduction.

The work described in this paper was undertaken to determine, if possible, what is the function of the polar bodies, and whether they are connected with the origin of the germ-cells, in a parthenogenetic insect. It has been maintained by Petrunkewitsch (**9**, **10**) that in the unfertilised egg of the honey bee, which gives rise to a drone, the second polar body unites with one half of the first, and that the "copulation-nucleus" so formed gives rise to the testes of the bee. Castle (**1**) has further taken the facts given in the papers of Petrunkewitsch and used them in formulating a suggestive hypothesis as to the heredity and determination of sex on Mendelian principles. It seemed, therefore, desirable that the matter should be investigated in other cases, and the sawflies were chosen as a likely group in which to work at the problem.

The sawflies are in many ways admirably suited to such work; many species are common and easy to rear; the eggs frequently develop when unfertilised, and in some species males, in others females, and in a third group both males and females are produced from parthenogenetic eggs. The eggs also are readily laid in captivity, and can be preserved at

intervals, so that their age is known with some exactness. They have, however, some disadvantages; the eggs are not readily penetrated by preserving agents, and the great amount of yolk, and the smallness of the mitotic figures, cause considerable technical difficulties.

According to Castle's hypothesis every germ-cell is either male-bearing or female-bearing, and when fertilisation takes place a male spermatozoon meets a female egg, or vice versâ, and the sex is determined by the dominance of one or the other sex. In the bee he supposes that all the eggs are male-bearing, and hence, when unfertilised, give rise to drones, but the queen bee was derived from a fertilised egg, and hence contained both sexes. The separation of the sexes is supposed to take place at the second maturation division of the egg or spermatozoon, and, since all the eggs of the bee are male, the second polar body must always be female-bearing, since it is at this division that the separation of the male from the female character takes place.[1] Petrunkewitsch finds that the second polar body, with one of the two nuclei formed by the first, gives origin to the genital cells of the drone, and Castle, therefore, supposes that the spermatozoa of the drone, descended from these polar bodies, are all female bearing, and that the female character is dominant, so that the fertilised egg always produces a female.

If the hypothesis here outlined were correct it would give a satisfactory solution of that most difficult of problems, the determination of sex, and it therefore seems of great importance to test it as thoroughly as possible. In animals which resemble the bee in having two polar bodies and producing males from parthenogenetic eggs we should expect to find the same course of events as described by Petrunkewitsch,

[1] The view that the separation of the sexes may take place at a nuclear division is supported by the case of Sagitta where the ovaries and testes are derived from the halves of one original cell; and by Trypanosoma, where the zygote nucleus divides into two, and when one of these develops the organism becomes female, when the other, male. See Schaudin's paper ('Arbeiten a. d. kais. Gesundheitsamt,' Berlin, vol. xx, 1904, p. 387). In neither of these cases, however, is the division a "reducing" one.

but, when females arise from unfertilised eggs, it seemed possible that the fate of the polar bodies would be different, as Petrunkewitsch found it in the fertilised eggs of the bee. The present paper gives the results of my investigations of this matter as far as they have yet gone.

Before proceeding to describe the maturation of the egg it is necessary to give some account of the evidence for the statement that virgin eggs of some species produce males, of others females.

In two of the species used there can be no doubt, viz. Croesus varus and Poecilosoma luteolum. In the first the male is not certainly known, one observer only having described it, and all agree that females alone arise from virgin eggs. In P. luteolum the male is extremely rare, and Miss Chawner, of Lyndhurst, tells me that she has bred thousands of this species for several years in succession without obtaining a male, and without finding any diminution in the fertility of the females. A third species used, Hemichroa rufa, is known to give chiefly females from virgin eggs, but occasionally males are produced.

Of the male-producing species I have assumed that Nematus pavidus yields only males on the authority of Cameron's 'Monograph of Phytophagous Hymenoptera' (Vol. ii, p. 173), and N. lacteus is so closely related to it that it probably belongs to this group, but of this I have no certain evidence, since my larvæ all died off.[1] The greater part of the work was done on N. ribesii (N. ventricosus of von Siebold), and in this case the evidence is not quite conclusive. It is agreed by all who have bred this species in quantity that all, or very nearly all, the flies reared from virgin eggs are males. Von Siebold (**11**, p. 121) obtained thirteen ♀ ♀ among about 1700 ♂ ♂ from virgin eggs, and supposes that these were introduced with the food. Other observers have obtained only males. It is always possible, however, that the females die off as larvæ; that they do not

[1] Miss Chawner writes to me that she has reared males only from virgin eggs of N. lacteus.

do so in the pupa is shown by von Siebold's experiments, and since often all the eggs hatch, it cannot be in the egg. I have reared a number of eggs in the hope of answering this question, but my results are hitherto uncertain, for I have never succeeded in rearing as much as 50 per cent. of the unfertilised eggs up to the imago. This is partly due to the exceptionally dry summer of 1904, which caused a greater mortality among the young larvæ than in 1903. It seems certain, however, that a much larger percentage of eggs of impregnated females grow up to imagines than from unfertilised. From eggs of impregnated females a large but varying percentage of female flies is produced; frequently considerably over 50 per cent. are females, and this suggests that the male larvæ are more delicate, so that the high mortality among larvæ from virgin eggs may be so accounted for, and since it is the common experience of those interested in sawflies that the females are more hardy than the males, it seems unlikely that all should die off when the eggs are not fertilised.[1] I must, however, leave the question open until next summer, when I hope to be able to settle it definitely.

2. Material and Methods.

The eggs are generally laid on the under side of the leaf of the food plant, and they are nearly always arranged so that the anterior end of the egg lies away from the base of the leaf. In N. ribesii the female, when laying, stands with the head directed towards the base of the leaf, and in attaching the egg to the groove made by the saw turns the abdomen so far over that the front end of the egg, which lay forward in the ovarian tube, lies backward when it is laid. The eggs are laid usually at intervals of about a minute, and of any row that nearest the edge of the leaf is the first laid, that nearest the base the youngest. I have not observed the

[1] It cannot be said that the unfertilized female lays the male eggs and keeps back the female, for a female will lay about 100 eggs and then die, and I find that only three or four eggs remain in the ovarian tubes.

egg-laying of the other species in detail, but in C. varus and H. rufa the eggs are also arranged with the anterior end towards the free end of the leaf. In N. pavidus and lacteus the eggs are laid in groups on the lower side of the leaf, and in P. luteolum are embedded between the laminæ.

In obtaining the eggs of different ages, the time was noted as nearly as possible when any row of eggs was laid, and it was then preserved at the desired time. In this way a large series of eggs of all ages up to about four hours was preserved, and a smaller number of the later stages. The rate of development varies considerably with the temperature.

In most cases a row of eggs was preserved and embedded entire, and the eggs cut one after another still attached to the leaf, since by this method it was possible to get more accurate orientation. Various methods of preserving were tried, including absolute alcohol and acetic acid, Flemming's solution, and other osmic mixtures; but much the best results were obtained by Petrunkewitsch's modification of Gilson's solution (water 300 c.c., absolute alcohol 200 c.c., glac. acetic acid 90 c.c., nitric acid pure 10 c.c., sublimate to saturation).

Some difficulty was experienced in getting paraffin to penetrate the eggs preserved in this mixture, and it was ultimately found that the best method was to put the eggs from absolute alcohol into cedar oil, where they were left for a considerable time, and thence transferred to xylol and through paraffin dissolved in xylol into pure paraffin. Even then the embedding was frequently defective, especially in C. varus, of which a large amount of material was destroyed in cutting the sections.

A number of stains were tried, but the only one that gave satisfactory results was Heidenhain's iron hæmatoxylin. This has the disadvantage that it stains the yolk granules more deeply than anything else, but when the position of the nuclei in the egg is known they can be found in most cases with no great difficulty.

3. Maturation and Early Development of the Egg in N. ribesii.

A number of eggs of N. ribesii were preserved very shortly after they were laid, but in most cases the nucleus had already begun to divide, so that the earliest stage commonly found was the first maturation spindle. In a few eggs preserved about five minutes after laying, the egg nucleus was found just beginning to divide, and at this stage it bears a remarkable resemblance to the figures given by Henking of the corresponding stage in Rhodites (Pl. 35, fig. 2, cf. Henking, **6**, Pl. 7, fig. 203). The mitotic spindle always lies in a little mass of protoplasm embedded in the yolk near the anterior end of the egg and on the dorsal side, i. e. the side of the egg away from the leaf. The "polar protoplasm," as I propose to call this mass, stains differently from the surrounding yolk, and when once its appearance is known it greatly facilitates finding the egg nucleus, or at a later stage the polar nuclei. There is also a somewhat similar mass of protoplasm just below the surface at the anterior end of the egg, from which in some cases a line of protoplasm and more finely granular yolk can be traced backward, and I find that this is the point where the spermatozoon enters when the egg is fertilised.

The first maturation mitosis begins almost immediately after the laying of the egg, and forms a large spindle lying perpendicular to the egg-wall. In the anaphase it is seen that the spindle fibres are thickened in the middle (Pl. 35, fig. 1); this is shown in the figures of Petrunkewitsch (**9**, Pl. 43, figs. 5, 6), and described in detail by Gross in Syromastes (**3**, p. 468), where he ascribes the thickening to chromatin left behind on the fibre. The mitosis is never quite completed, for as soon as the chromosomes arrive at the poles of the spindle, at each end a new spindle begins to be formed, and the first maturation division passes into the second (fig. 3). In neither of the maturation divisions have

I seen any trace of a centrosome; the chromosome number appears to be eight (Pl. 36, figs. 24, 25) (see below). The spindles of the second maturation division lie in the same line as the first, and when the chromosomes have reached the ends, each group gives rise to a large reticular nucleus with well-developed nuclear membrane. In this way arise four similar nuclei, lying in a line nearly perpendicular to the edge of the egg, the outer one being close to the edge, the two middle ones near together in the middle of the little mass of protoplasm, and the inner one on the inner edge of the protoplasm, almost embedded in yolk-granules (figs. 4, 5, 6). Of the four nuclei, the two outer are the two halves of the first polar nucleus, the third is the nucleus of the second polar body, and the innermost is the female pronucleus, which in the parthenogenetic egg will give rise to the embryo. After the nuclei have been formed the remains of the spindles are still seen between them, and at a rather later stage one or two round, rather faintly stained bodies are found near the nuclei (fig. 8); these bodies are probably derived from the spindles, and correspond to what Henking called the "thelyid."

At first all four nuclei are similar in size and shape, and lie nearly at even distances from one another, but very rapidly changes begin to take place. The outermost gets pressed against the wall of the egg, becomes flattened in shape, and in a short time becomes unrecognisable, and disappears. Meanwhile the innermost (the female pronucleus) begins to move deeper into the egg, and at the same time travels towards the anterior end, and in so doing becomes so closely embedded among the deeply staining yolk-granules that after a very short time it is very hard to find. While these processes are in progress, the two middle nuclei, viz. the second polar nucleus and the inner half of the first polar nucleus, move towards one another, and soon come so closely into contact that each becomes nearly hemispherical, the flat sides being pressed against each other. The chromatin of these two nuclei, which has hitherto been distributed in a fine

reticulum, next becomes aggregated in the centre of each as a closely-packed mass of chromosomes, and the nuclear membrane then disappears, and the two sets of chromosomes form a single group (Pl. 35, figs. 7, 8, 9). In the fusion of the two inner polar nuclei to form what Petrunkewitsch calls the "copulation-nucleus" there is no appearance of rays in the surrounding protoplasm, as sometimes occurs in the fusion of a male and female pronucleus in fertilisation; there is simply the disappearance of the nuclear membrane and the mingling of the two groups of chromosomes.

The stage just described is reached between two and three hours after the egg is laid, and the subsequent changes up to the seventh hour are comparatively small as far as the polar nuclei are concerned. The "copulation-nucleus" does not form itself into a definite nucleus with a nuclear membrane, but very shortly after the chromosomes of the two polar nuclei have mingled with one another to form one group, a separation takes place, and they come to lie in two groups near together in the little mass of protoplasm. Each of these groups is seen in several sections to consist of about eight separate chromosomes, lying at varying distances from one another, but never far apart. Neither group forms a nuclear membrane, and sometimes the two sets lie so near together that the space between them is hardly visible, but in most cases they are separated by a definite, though small space. In some sections of a stage about an hour after the fusion of the two nuclei (i. e. at about three and a half hours) in one at least of the two groups of chromosomes each appears to be double. And when the later stages are examined (fifth and sixth hours) it is sometimes, but not always, found that one of the groups of chromosomes contains about twice as many as the other. In a few sections of the later stages three groups of chromosomes appear lying side by side, but distinctly separated into three sets, and in this case each group appears to contain eight chromosomes (figs. 10, 11).

It appears, therefore, that not very long after the separation of the chromosomes of the "copulation-nucleus" into

two groups, those of one group may each divide into two, and either remain associated together or, in some cases, separate into two sets, so that altogether three groups are found lying side by side. There is no regularity, however, in these processes; the chromosomes in the polar protoplasm sometimes remain as a compact group, in others become scattered; their number varies in different eggs, but otherwise they remain unaltered until the nuclei derived from the egg-nucleus begins to come to the surface to form the blastoderm. Beyond this stage I have been unable to trace them.

Although nearly all the eggs examined follow the course here described, it should be mentioned that in two cases at least abnormalities have been found. In one of these, an egg preserved six hours after it was laid, in addition to a group of chromosomes in the "polar protoplasm," there is, nearer the edge of the egg, and slightly more anteriorly, an oval reticular nucleus with well-marked membrane. It is possible that this is the outer half of the first polar nucleus, which usually disappears several hours earlier, but in another egg, the exact age of which is, unfortunately, unknown, though probably between four and five hours, two such nuclei appear. One of these is close to the edge of the egg, and the other slightly deeper, i. e. in just the positions of the two halves of the first polar nucleus at an earlier stage; the chromosomes derived from the "copulation-nucleus" lie grouped in several irregular masses in the "polar protoplasm" near the two nuclei described, and the yolk contains a number of scattered nuclei, doubtless derived from the egg-nucleus in the ordinary way (Pl. 35, fig. 12).

It will be seen below that the arrangement here described as abnormal is exactly that commonly found in the species which yield females from virgin eggs, and it is known that something under 1 per cent. of females may be reared from virgin eggs of N. ribesii.

By the time the two inner polar nuclei have coalesced the outermost has usually quite disappeared, but in this respect different eggs vary somewhat, and in a few cases traces of it

remain for a short time longer flattened against the edge of the egg. Immediately after the second maturation mitosis the innermost nucleus begins to move deeper into the yolk, and, at the same time, towards the anterior end, so that at the time of the fusion of the polar nuclei it is found several sections further forward, closely embedded in yolk granules. The distance which it travels varies somewhat in different eggs, but about at the time when the chromosomes of the "copulation-nucleus" have separated into two groups it begins to divide, and in a short time numerous nuclei are found scattered in the yolk, especially in the front half of the egg. These "yolk-nuclei" are generally embedded in little masses of protoplasm, which makes them easy to find. I have not succeeded in finding the first division spindle of the egg-nucleus, but at a slightly later stage mitotic spindles are abundant, and differ considerably in form from the polar spindles. They are smaller and much narrower, so that the chromosomes are closely packed together, but the chief difference is that in the yolk-mitoses there are very conspicuous centrosomes, of which I have seen no trace in the polar mitoses (Pl. 36, fig. 32). Since the earliest stage of the division of the egg-nucleus that I have found consists of two nuclei lying near together just after division is completed, I cannot say at exactly what stage the centrosomes first appear. Another point of considerable interest is that in the segmentation mitoses the chromosome number remains the same as it is in the maturation divisions; this is seen clearly when a mitosis in the yolk is cut across transversely (fig. 26).

Several hours after the egg is laid, when the yolk nuclei have become very numerous, they begin to come to the surface and form a blastoderm, just as in other insect eggs. In the blastoderm nuclei a deeply-staining nucleolus is commonly found; it appears to consist of chromatin, and may be double. I have not seen it in stages younger than about fifteen or twenty hours.

4. Fertilised Eggs of N. ribesii.

I have not yet succeeded in working out thoroughly the development of the fertilised eggs. The maturation divisions occur as in virgin eggs, and conjugation of the two inner nuclei takes place in the same way, but the subsequent development of the polar nuclei shows some slight differences. In many eggs from impregnated females the outer nucleus is found much further from the edge of the egg than in normal unfertilised eggs, and instead of rapidly degenerating it becomes resolved into a group of chromosomes, which persist for some time. The two inner nuclei form a group of chromosomes, but each of these commonly splits into two or more chromatin granules at a rather early stage, so that a large mass of small chromatin bodies is often found in the polar protoplasm.

I have not been able to observe the conjugation of male and female pronuclei, and although spermatozoa have been found in some recently laid eggs, I have not been able to trace their subsequent development with certainty. There are indications that the spermatozoa develop into nuclei, which then disintegrate without ever conjugating with the egg nucleus, in which case the eggs of impregnated females would also be parthenogenetic. If this is the case, it would solve the difficulty which arises concerning the chromosome number, which is the same in somatic mitoses as in the maturation divisions. Since, however, the supply of material at present available is insufficient to determine the matter for the present, it must be left undecided.

5. Nematus pavidus and N. lacteus.

Of these species N. pavidus is said by Cameron and others to yield only males from unfertilised eggs. With regard to N. lacteus I have obtained no information,[1] and

[1] See p. 563, footnote.

my own experiments were unsuccessful, since although the eggs hatched the larvæ died off at an early stage. But the two species are so closely related in habit and structure that it seems very probable that both belong to the same group, and that virgin eggs of N. lacteus would produce males if the larvæ were successfully reared.

In both species the eggs are laid in large groups on the under side of the leaf of the willow; the group consists of a number of transverse rows of eggs, the eggs in each row lying side by side.

In N. lacteus the maturation mitoses take place as in N. ribesii, and four nuclei arise lying in a line perpendicular to the edge of the egg (Pl. 35, figs. 4, 13). The two middle ones, i. e. the second polar and inner half of the first polar nucleus lie from the first near together; they come into contact and apparently fuse as in N. ribesii. The fusion, however, does not seem to so complete as in this species, for at a stage not many minutes older two groups of chromosomes are found lying near together in the polar protoplasm. One of these groups clearly consists of about eight chromosomes, but the other group is more irregular, and not so many can be counted (Pl. 35, fig. 14). In some eggs only one group of about eight is seen, and it seems possible that in some cases the two nuclei come into contact, but do not fuse, and that one of them breaks up into chromosomes, while the other merely degenerates. This idea is supported by the fact that in some eggs of this age by the side of the small group of chromosomes a faint circular space is seen, resembling a degenerating nucleus. The chromosomes from the polar nucleus do not appear to persist for any length of time, for in eggs very little older they are already becoming scattered and difficult to count. The outer polar nucleus disappears as in N. ribesii, and the egg-nucleus sinks in and develops as in other cases.

Of N. pavidus very few eggs were obtained, and all of these unfortunately represent stages later than the maturation divisions. Their ages are between two and five hours,

and in the youngest egg a group of eight chromosomes is seen in the polar protoplasm, with what appears to be the degenerating remains of another group by its side. The later eggs mostly show a very compact group of eight rather large chromosomes, which may be so regularly packed together that they rather resemble a small nucleus, but have no nuclear membrane. Occasionally the number appears to be more than eight, and probably division has taken place as in N. ribesii.

It is likely from these observations that the course of events in N. pavidus does not differ from that described in N. lacteus; the two inner polar nuclei probably come into contact and break up into chromosomes, which form two groups lying close together. Those in one group rapidly disintegrate, while those of the other remain aggregated together and persist for several hours.

6. Poecilosoma luteolum.

In P. luteolum unfertilised eggs produce only females. The eggs are laid in little groups inside the tissue of the leaves of Lysimachia vulgaris, and the flies almost always choose for this purpose the small leaves near the apex of the plant.

The polar mitoses take place as in N. ribesii, and result in four nuclei lying in a line perpendicular to the edge of the egg. They differ from those of N. ribesii in being nearly always of different sizes; the outermost is smallest, the next larger, and the inner or second polar nucleus largest of all, but not differing much from the egg-nucleus (Pl. 36, fig. 15). The egg-nucleus sinks very rapidly into the yolk and moves forward for some distance, so that it is rarely found in the neighbourhood of the polar nuclei; it soon begins to divide, and gives rise to scattered nuclei in the yolk which develop, as in N. ribesii.

The second polar nucleus is generally separated from the others by a wider interval than in the species hitherto described, and the first polar nuclei are often found very close

together. The two outer nuclei move slowly to the edge of the egg and often become flattened together, and are soon followed by the inner nucleus, so that all three come to lie together at the outer edge of the polar protoplasm. From this stage onward there appears to be some variation in the course of events. Most commonly they all gradually degenerate, becoming crowded together and shrunken so as to be hardly distinguishable apart, and at a stage later they appear as an indefinite faintly-stained mass, which is not traceable further (figs. 16, 17). In other cases, before this happens, the chromatin of at least the two inner nuclei becomes aggregated in the centre of each, as if they were preparing to break up into chromosomes, and in a few eggs this actually takes place, and one finds one or two irregular groups of chromosomes in the polar protoplasm. In no case have I found any certain evidence of actual conjugation of nuclei such as I have described in N. ribesii, but some eggs suggest that all three polar nuclei may come together and break up to form a common mass of chromosomes, as has been described by Henking in Pieris brassicæ (**5**, p. 545). Such an irregular mass of chromosomes is found in some eggs, while others of the same batch show the nuclei degenerating as described above. In a few eggs two of the polar nuclei, instead of degenerating at once seem to remain at the edge of the egg as large rather indefinite bodies containing numerous chromatin granules; sometimes they have the appearance of irregular mitotic figures, and it seems probable that such nuclei might develop into the structure described below, which in a few cases has been observed at a rather later stage (fig. 18, *a* and *b*).

Usually, in eggs preserved more than three hours after being laid, all traces of the polar nuclei had disappeared, but one lot, all of which were laid on one day, and almost certainly by the same female, differed from others in this respect. These eggs are of ages between three and six hours, and in some of them, but not in all, there is a conspicuous compact mass of chromosomes rather deep in the polar protoplasm. In some it is so compact that it looks like a large, rather ab-

normal nucleus, and seems to be enclosed in a faint membrane; in others it is plainly a group of separate chromosomes (fig. 19). It seems to be quite irregular in its occurrence, since it may be present in one egg and absent from another lying by its side in the same leaf. All the eggs of this batch are well advanced, with numerous nuclei scattered through the yolk, so that I have no means of determining the origin of this nucleus with certainty, but it seems probable either that the mass of chromosomes which sometimes arises from the polar nuclei has persisted, or that exceptionally a conjugation of nuclei has taken place, which has given rise to the structure described.

The mitotic figures of P. luteolum are more clear and distinct than in the other species observed. The chromosome number is plainly seen to be eight both in the maturation mitoses (figs. 27, 29), and in those found later during segmentation and in the blastoderm (figs. 28, 30), so that, as in N. ribesii, no doubling of the number takes place in the somatic nuclei. Centrosomes are visible in the segmentation mitoses, but are smaller and less conspicuous than in N. ribesii, and only appear in rather deeply-stained figures (fig. 31). I have not been able to find them in the maturation spindles.

7. Cræsus varus.

C. varus also belongs to the class in which virgin eggs yield females only, and the male is not certainly known. The eggs are laid in rows on the veins on the under side of the leaf of the alder. I obtained only a small amount of material, and failed to get the flies to lay in confinement, so that it was necessary to put them in muslin bags on bushes. For this reason I could not determine the age of the eggs with complete accuracy, and my material was further reduced by the fact that the eggs of this species are very difficult to embed, and many were spoiled in cutting.

The earliest stage observed is shortly after the second

polar mitosis, and shows two polar nuclei, and the remains of the third nearer to the edge of the egg; the egg-nucleus has already sunk into the yolk, and is not seen in the same section (Pl. 36, fig. 22). The two inner nuclei lie very near together in the polar protoplasm, which is very small, often being visible in only one section. Rather later, a small compact group of chromosomes is found, which at this stage appear always to be seven or eight in number (fig. 23 *a*). Since I have not been able to find the maturation mitoses, I cannot say certainly that these chromosomes are derived from one nucleus, but analogy with the other species makes it highly probable that this is the case. The stage here described is reached probably about two hours after the egg is laid, and in eggs preserved an hour or two later a group of about fourteen to sixteen chromosomes is found in the polar protoplasm (fig. 23 *b*). It seems, therefore, that as in N. ribesii the chromosomes of the polar nucleus may split, but remain aggregated together in one group. Beyond this stage I have not been able to trace them.

C. varus seems to occupy a position intermediate between N. lacteus and Hemichroa rufa (described below); it resembles N. lacteus in apparently showing incipient conjugation of the two inner polar nuclei, but, as in H. rufa, only one of these seems to break up into chromosomes, while the other disappears completely.

8. Hemichroa rufa.

In this species, according to Cameron, unfertilised eggs yield both males and females, but females are much more abundant. The eggs are embedded in rows in the larger ribs of alder leaves, each egg lying in a separate incision and being completely covered by the leaf-tissue. In this case also it was necessary to let the flies lay on branches enclosed in muslin.

The earliest stage found is the second polar mitosis, in which, as in other species, two spindles lie in a line perpen-

dicular to the edge of the egg. They give rise to four vesicular nuclei, which resemble those of P. luteolum in their arrangement, the two outer, i. e. the halves of the first polar nucleus, are close together near the edge of the egg, and are separated from the second polar nucleus by a wider interval than in N. ribesii. The outer nucleus degenerates rapidly, and is soon followed by the second; the inner or egg-nucleus sinks in and begins to divide as in other species (fig. 20, *a* and *b*). Meanwhile the second polar nucleus breaks up into chromosomes, the number in this case as in others being eight or about eight (fig. 21). This group of chromosomes appears to persist without change for some time, but at a stage two hours later (probably about five hours after the egg is laid) they have disappeared, and nothing is found but nuclei in the yolk derived from the egg-nucleus. As in N. ribesii centrosomes are visible in the yolk-nuclei mitoses, but I have not been able to distinguish them in the polar spindles.

This species, therefore, seems to resemble C. varus in the course of its development; in each the two outer polar nuclei degenerate, and the inner one breaks up into chromosomes, which may each split into two, but which finally disappear. It resembles P. luteolum in the fact that no conjugation of polar nuclei takes place, but in the latter species all three nuclei usually disappear without resolution into chromosomes.

9. Chromosomes and Centrosome.

In all the species of which satisfactory sections across a mitotic spindle were obtained, the number of chromosomes going to each end of the spindle appeared to be eight. In some cases only seven, or even six, were observed, but more than eight were never found except when chromosomes already split were seen all together in the equatorial plate (i. e. fig. 25, *a* and *b*, which were superposed in the same section).

In both N. ribesii and P. luteolum the number in both the polar mitoses is undoubtedly seven or eight, and if reduction takes place in the ordinary way this must be the reduced number. But in both species spindles of somatic nuclei in the yolk or blastoderm show clearly that the number is not changed; eight (or possibly seven) chromosomes go to each end of the spindle. It is evident, therefore, either that a doubling of chromosomes takes place at a later stage, or that no reduction can take place in the next generation. The maturation spindles are so small that the question whether reduction occurs is not easily answered, but I have seen no evidence of it. If, however, the pairing (pseudoreduction, synapsis) takes place just before the maturation divisions, as has been described in so many insects, then the chromosomes at their first appearance in the laid egg would have the reduced number. Some of my sections of first polar spindles have a considerable resemblance to the figures given by Gross (**3**, Pl. 31, fig. 64) of the reduction divisions of Syromastes, particularly in the thickening in the middle of spindle fibres during the anaphase (fig. 1), but I have not been able to find any trace of tetrad formation or other evidence of normal reduction.

And in this connection a point of great theoretical interest arises, for in P. luteolum, in which the mitotic figures are most clearly shown, the species is normally parthenogenetic, only females being produced. It has been maintained by Häcker (**4**), Sutton (**12**), Montgomery (**8**), and others that of each couple of chromosomes which pair together at the stage of pseudoreduction one is of paternal, and the other of maternal, origin. But in a species which is regularly parthenogenetic there are no paternal chromosomes, and hence either pseudoreduction cannot occur at all, or it must take place in an abnormal manner. My comparison of the maturation mitoses with those seen in the blastoderm leads me to the view that no reduction takes place at any stage, and that all the divisions in this species are equation-divisions, and this view receives some support from the similarity of the

very early stages to those of Rhodites, in which Henking concludes that there is no reduction (**6**, p. 156).

My observations on centrosomes require a word of comment. In no case could I find centrosomes in the maturation spindles, but in all the species of which good preparations of the later stages were obtained they were easily seen in the segmentation divisions (Pl. 36, figs. 31, 32). Since the eggs were unfertilised they cannot be derived from the spermatozoon, and it is certain, therefore, that they are produced from the egg-nucleus itself. Jenkinson (**7**, pp. 429, 449) concludes that in the Axolotl the centrosome arises by precipitation of the egg-cytoplasm under the influence of the sperm-nucleus, and mentions other cases of the same kind. Wilson (**13**, p. 566) observed that centrosomes arose de novo in parthenogenetic eggs of Echinoderms, so that when all these observations are taken together it may be taken as proved that the statement so commonly made that the centrosome is derived from the middle piece of the spermatozoon is at least not universally true.

The centrosomes found in eggs of impregnated females of N. ribesii do not differ in any respect from those seen in virgin eggs.

10. Summary and Discussion of Results.

The chief facts described are as follows:

(1) In all the species investigated the first maturation spindle is found on the dorsal surface of the egg, in a little patch of protoplasm not far behind the anterior end. The first polar mitosis is never completed, for, at the end of the anaphase, a new spindle is formed at each end, and the second polar mitosis begins.

(2) The second maturation division gives rise to four nuclei lying in a line perpendicular to the edge of the egg; of these the innermost is the female pronucleus or egg-nucleus, the next is that of the second polar body, and the two outer are the daughter-nuclei of the first polar body.

(3) In all the species the egg-nucleus at once sinks into the yolk and travels for some distance toward the anterior end of the egg; it then begins to divide, and gives rise to the segmentation nuclei scattered in the yolk. At the same time the outermost polar nucleus usually becomes flattened against the edge of the egg, and in all cases degenerates more or less rapidly.

(4) The fate of the two inner polar nuclei varies in different cases. In the species which produce males from virgin eggs they lie from the first near together, and rapidly approach one another until they come into contact. The extent to which fusion takes place seems to vary in different species, but in all cases the nuclear membrane disappears, and they break up into chromosomes. The chromosomes may form a single group, which contains twice as many as are found in the polar spindles, or when the fusion has been less complete two groups may be found, lying very close together and each consisting of the same number as occurs at each end of the spindle.

(5) In the species in which virgin eggs yield females the inner polar nuclei usually lie farther apart, and do not approach one another. They may all move slowly outwards to the edge of the egg and gradually disintegrate (P. luteolum); or this may happen to those derived from the first polar nucleus, while the second polar nucleus loses its membrane and becomes resolved into chromosomes, which then show the normal number.

(6) The chromosomes derived from the polar nucleus or nuclei remain in the "polar protoplasm" for a varying length of time, but seem eventually to disappear. In N. ribesii, in which fusion of the polar nuclei takes place, they usually become separated into two groups, in one at least of which each chromosome may split into two, but finally they become scattered and are not traceable further. In N. pavidus and N. lacteus those of one group disappear early, while those of the other persist unchanged for a considerable time. In C. varus, in which only the inner polar nucleus gives rise to

chromosomes, they may each divide so that double the number is found, but they do not appear to develop further.

(7) In all the species the chromosome number is eight or nearly eight. This number is found at each end of both the first and second maturation spindles, and in the mitoses of the segmenting egg and in the blastoderm. No doubling of the chromosome number occurs during the early development of the egg, such as is described by Petrunkewitsch in the drone-egg, and it is concluded that at least in N. ribesii and P. luteolum no reduction division occurs, both the maturation divisions being equational.

(8) Centrosomes are not visible in the maturation spindles but are found in the segmentation mitoses of both eggs from both virgin and impregnated females.

The facts here described resemble, in many ways, those obtained by Petrunkewitsch in his work on the bee. The form of the maturation spindles, and the fusion of the second polar nucleus with the inner half of the first are closely similar in the two cases, the chief difference being that in the bee the whole process takes place on the ventral side of the egg, in the sawflies on the dorsal.

In the bee the "copulation-nucleus" divides into a group of nuclei which aggregate protoplasm around themselves, and according to Petrunkewitsch ultimately give rise to the testes of the drone; and during all the earlier part of their development these cells are distinguished by having each a "double nucleus" ('Doppelkern'). In the sawflies the conjugation of the polar nuclei takes place only in male-producing species, and the group of chromosomes which results ultimately disintegrates; but the fact that it commonly divides into two groups lying side by side may be compared with the production of "double nuclei" in the bee.

There are, however, several fundamental differences apart from the complete disintegration of the polar nuclei. In the bee a true reduction division is described as occurring, since sixteen chromosomes are figured in the equatorial plate of the first polar spindle, and only eight in the second polar

mitoses. In the sawflies the number in all the divisions is constant, and no trace was seen of tetrads or dyads to indicate the occurrence of reduction. In the parthenogenetic egg of the bee the somatic number of chromosomes is said to be restored by a process of doubling, which occurs before the first division of the egg-nucleus, and which does not take place if the egg is fertilised. In the sawflies no such doubling takes place, and the somatic number is the same, at least up to the formation of the blastoderm, as the number in the polar mitoses.

Among the most important papers on the maturation of the insect-egg are those of Henking (**5**, **6**), and many points of interest arise from a comparison of my work with his. The Hymenoptera investigated by Henking were Rhodites rosæ and Lasius niger, the first of which is parthenogenetic and produced chiefly females from virgin eggs. The early development of the polar nuclei in this species and in fertilised eggs of L. niger is closely similar to that found by Petrunkewitsch in the bee; the three polar nuclei consist of groups of chromosomes, and the two inner groups move together until they lie side by side in a clear space near the edge of the egg. Their development was not traced further. In virgin eggs of L. niger this course was sometimes followed, but in others no attraction between the chromosome groups took place, and in a third lot all three groups joined together. A fusion of all three polar nuclei also occurred in fertilised eggs of Pieris brassicæ and Musca vomitoria, in both of which species all the polar nuclei were vesicular.

Fusion of the two inner nuclei, or at least a convergence resulting in a group of chrososomes lying close together, took place also in some beetles, viz. in some eggs (but not all) of Agelastica alni, and in Adimonia tanaceti and Crioceris asparagi. In other beetles, e. g. Donacia sp., Lampyris splendidula, no fusion of nuclei took place, and in L. splendidula and Pyrrhocoris apterus both polar bodies are extruded from the egg and the first does not divide. Division of the first polar nucleus without subse-

quent fusion with the second was found in some unfertilised eggs of Lasius niger, and in both fertilised and virgin eggs of Bombyx mori.

It is evident, therefore, that the fate of the polar nuclei may differ very considerably in different insects. The case which one would expect to be most closely parallel with the sawflies is that of Rhodites rosæ, for this species is not only placed near them in classification, but is also parthenogenetic. In several points Henking's description is similar to that which I have given, the peculiar arrangement of the chromatin in the egg-nucleus at the beginning of the first polar mitosis is very like what I have observed in N. ribesii, and both forms seem to be peculiar in having no true reduction division, both the polar mitoses being probably equational. But Rhodites differs in two important points; according to Henking there is a doubling of the chromosome number at the first division of the egg-nucleus, which he supposes is brought about by the separation of chromosomes which have paired before the first polar mitosis; and there is at least a partial fusion between the inner polar nuclei, all of which in this case remain as groups of chromosomes, and do not develop a nuclear membrane. Among the sawflies investigated, with the possible exception of C. varus and a few abnormal eggs of P. luteolum, a tendency to fusion of these nuclei was observed only in male-producing species, and Rhodites produces females from virgin eggs. Since conjugation of the two inner polar nuclei takes place also in the fertilised eggs of the bee and of N. ribesii, which yield females, and apparently quite irregularly in other insects, it must be concluded that it alone can supply no evidence as to the causes which determine sex; but when all the facts which I have given are considered together, it seems to me that some indication is offered of the direction in which the solution of the problem may be sought.

Castle (1) has suggested that the second maturation division may consist in the separation of a male-bearing from a female-bearing nucleus. And Le Dantec has propounded

the view that maleness and femaleness are of the nature of molecular forces, which are mutually attractive, or which cause an attraction between bodies bearing them, just as opposite poles of a magnet or bodies charged with positive and negative electricity attract one another.[1] If these two hypothesis are combined it may be supposed that in the male-producing sawflies, such as N. ribesii, the four nuclei which result from the second polar mitoses are alternately male and female bearing, i. e. are arranged in order from within outwards, as in Diagram 1 :

Diagram 1.

there will then be an attraction between the two inner polar nuclei, which will cause them to move together and fuse. The nuclei of the female-producing species may be imagined to have two male-bearing nuclei in the middle, and a female at each end (Diagram 2),

Diagram 2.

in which case there would be no attraction between the inner polar nuclei. The question at once arises, "Why do not the daughter-nuclei derived from one mitosis move together and fuse again if they bear opposite sexes?" But it is evident that the force which brings a mitosis about, and drives the chromosomes to opposite ends, is a different force from that now imagined, since it occurs quite apart from sex, and it may be supposed that the force which causes the chromosomes to separate remains sufficient to keep the nuclei from coming together again. But that this may cease to operate

[1] F. Le Dantec, 'Traité de Biologie,' Paris, 1903, pp. 154—166.

is indicated by the fact that in P. luteolum the two outer nuclei (halves of the first polar body) do nearly always come together into such close contact that it is often hard to decide whether they are one or two.

It has been pointed out that in C. varus, which yields females from unfertilised eggs, the two inner polar nuclei lie very close together, although they do not fuse. It is possible that in this species the nuclei have a third arrangement with respect to the sex which they bear, as in Diagram 3—

Diagram 3.

i. e. the converse of Diagram 1, which would lead to the same type of behaviour as is found in the male-producing species.

It is of considerable interest that in rare cases eggs of N. ribesii are found which exhibit the female-producing type of development (Diagram 2 above, Pl. 1, fig. 12), and it is known that in this species occasional females are reared from virgin eggs. It may be supposed, therefore, that in a small fraction of the eggs of this species the maturation divisions occur in such a way as to cause the nuclei to be arranged as in P. luteolum or H. rufa, and that in such cases females are produced.[1]

The ideas here suggested are of course regarded as merely tentative, and cannot be tested until the course of events in the fertilised egg is accurately known. If it be found, as has been suggested above, that in fertilised eggs of N. ribesii no conjugation of pronuclei takes place, but that the egg is

[1] If in the bee the two polar nuclei which conjugate are respectively male- and female-bearing, and if, as Petrunkewitsch maintains, they give rise to the testis, then the spermatozoa of the bee should bear both sexes. But Meves ('Anat. Anzeiger,' xxiv, 1904, p. 29) has shown that in the spermatogenesis of the bee a sort of polar body-formation takes place, and it may be supposed that the male-bearing cells are thus eliminated, so that the mature spermatozoa would all be female-bearing, as Castle has suggested.

really parthenogenetic in this case also, it must be supposed that the entrance of the spermatozoon causes a reversal in the arrangement of the polar nuclei, and so females result. And if the two inner nuclei of the four produced at the second polar mitosis differ from one another only in that they bear different sexes, and it is to some extent a matter of chance which one becomes the egg-nucleus, it suggests an explanation of the fact that in all the species observed the second polar nucleus breaks up into chromosomes, each of which may split, but never form a true mitosis. The nucleus has in it the power of further development, but some part of the division mechanism seems to fail, and after what looks like a fruitless effort to divide, it gradually disintegrates. In this respect it differs from the outer polar nuclei, which usually disappear at an earlier stage, and show much less tendency to divide.

Zoo. Lab., Cambridge;
February, 1905.

Since writing the above, I find that in Pœcilosoma pulveratum, which yields only females from virgin eggs, the maturation is exactly as in normal eggs of P. luteolum. In Hylotoma rosae (males from virgin eggs) the inner polar nuclei approach one another as in other male-producing species, but apparently disappear without breaking up into chromosomes.

December, 1905.

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EXPLANATION OF PLATES 35 & 36,

Illustrating Mr. L. Doncaster's paper, "On the Maturation of the Unfertilised Egg, and the Fate of the Polar Bodies, in the Tenthredinidæ (Sawflies).

The figures were all drawn with a 2 mm. immersion lens, and in nearly all cases a camera lucida was used for the outline. The magnification is about × 1000, except in Fig. 5, where it is about × 600. In some of the figures two or three successive sections have been combined in one drawing; when this has been done it is mentioned in the description of the figure.

PLATE 35.

Fig. 1.—*N. ribesii*. First polar mitosis (combined from two successive sections).

Fig. 2.—N. ribesii. First polar mitosis [cf. Henking (**6**), pl. vii, fig. 203].

Fig. 3.—N. ribesii. Beginning of second polar mitosis.

Fig. 4.—N. lacteus. Second polar mitosis, anaphase.

Fig. 5.—N. ribesii. Close of second polar mitosis; three polar nuclei and egg-nucleus.

Fig. 6.—N. ribesii. Egg-nucleus and three polar nuclei; two inner polar nuclei in contact (combined from three successive sections).

Fig. 7.—N. ribesii. Two inner polar nuclei beginning to fuse.

Fig. 8.—N. ribesii. Fusion of two inner polar nuclei; outer polar nucleus degenerating.

Fig. 9.—N. ribesii. Resolution into chromosomes of "fusion-nucleus."

Fig. 10.—N. ribesii. Chromosomes in polar protoplasm. Two hours later than Fig. 9.

Fig. 11.—N. ribesii. Chromosomes in polar protoplasm; three groups.

Fig. 12.—N. ribesii. Abnormal egg developing according to the female-producing type. Two outer polar nuclei, and scattered chromosomes in polar protoplasm derived from the inner polar nucleus (combined from three successive sections).

Fig. 13.—N. lacteus. Telophase of second polar mitosis (combined from two successive sections).

Fig. 14.—N. lacteus. Two inner polar nuclei resolved into chromosomes.

PLATE 36.

Fig. 15.—P. luteolum. Three polar nuclei; the egg nucleus has sunk into the yolk (combined from two successive sections).

Fig. 16.—P. luteolum. Polar nuclei beginning to degenerate at edge of egg (combined from two successive sections).

Fig. 17.—P. luteolum. Degeneration of polar nuclei.

Fig. 18, *a* and *b*.—P. luteolum. Two successive sections of an abnormal egg. Polar nuclei forming a large mass at the outer edge of the polar protoplasm.

Fig. 19.—P. luteolum. Abnormal egg. Large nucleus deep in the polar protoplasm.

Fig. 20, *a* and *b*.—H. rufa, *a*. Two inner polar nuclei; the outermost has already degenerated. *b*. Egg nucleus in the yolk of the same egg.

Fig. 21.—H. rufa.—Inner polar nucleus; resolution into chromosomes.

Fig. 22.—C. varus. Two inner polar nuclei.

Fig. 23, *a* and *b*.—*a*. Resolution into chromosomes of inner polar nucleus. *b*. Later stage; splitting of the chromosomes.

Fig. 24.—N. ribesii.—Transverse section of first polar spindle, showing chromosomes.

Fig. 25, *a* and *b*.—N. ribesii. Transverse section of second polar spindle, showing eight chromosomes travelling to each end. The two groups were superposed in the same section, showing sixteen chromosomes at slightly different levels.

Fig. 26.—N. ribesii. Transverse section of somatic mitosis in yolk, showing chromosomes.

Fig. 27.—P. luteolum. Transverse section of second polar spindle.

Fig. 28.—P. luteolum. Transverse section of mitosis in blastoderm, showing chromosomes travelling to each end.

Fig. 29.—P. luteolum. First polar mitosis.

Fig. 30.—P. luteolum. Mitosis in blastoderm. The section is not stained deeply enough to show centrosomes.

Fig. 31.—P. luteolum. Mitosis in yolk, showing centrosomes.

Fig. 32.—N. ribesii. Mitosis in yolk, showing centrosomes.

The Rôle of Mucus in Corals.

By

J. E. Duerden, Ph.D., A.R.C.Sc.(Lond.),

Professor of Zoology at the Rhodes University College, Grahamstown, Cape Colony.

Within recent years much advance has been made in our knowledge of the physiology of actinian polyps by investigators such as Nagel, Loeb, Parker, Carlgren, and Torrey. Comparatively little, however, is yet known of the living activities of the closely-related madreporarian polyps. As regards actinians several contributions have appeared giving the results of observations and experiments upon ciliary activity and its significance in the feeding of the polyps, and it was while carrying out similar studies upon the madreporarians that it became evident that mucus likewise holds a place of much importance in the ingestion of food. The present account deals in the main with only this factor in the physiological processes of corals, namely the part played by mucus. The observations and experiments were carried out in the Hawaiian Islands, mostly upon the living mushroom coral Fungia, and the compound astraeid Favia.[1] The work

[1] Several species of Fungia have been described from the Hawaiian Islands. Mr. T. W. Vaughan, who is at present engaged upon a systematic study of the corals from this region, informs me that he recognises three species, viz. Fungia scutaria, var. verrilliana, Quelch; F. patella (Ell. and Sol.); and F. oahensis, Döderlein. Amongst many hundred living specimens collected I have as yet been unable to determine any important differences in the polyp itself. Similarly with the genus Favia; two species have been identified by Verill as doubtfully Hawaiian, viz. Favia Hombroni (Rous.) and F. rudis, Verrill, but the living polyps show no differences of any significance as regards the present work.

has been assisted by an appropriation from the Carnegie Institution.

Experiments with Fungia.

On account of its size and simple (not colonial) character the coral Fungia was found to be especially suitable for experimental studies on the feeding and other processes of madreporarian polyps. The disc is large and flat, Hawaiian specimens sometimes measuring as much as 15 cm. along the larger axis, thus readily admitting of observations on the transference of food and other substances across it. In their

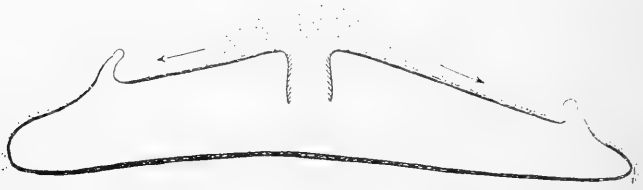

Fig. 1.—Diagram to show the distribution of particles dropped over the mouth of Fungia when an exhalent current is in progress. The particles are wafted away from the mouth, fall on the disc, become embedded in mucus, and are carried towards the edge of the polyp over which they fall.

living condition, resting on the sea-floor, the polyps are usually fully expanded, and the column is unable to close over the disc on retraction. The mouth is elongated and slit-like or oval according as it is closed or open. The tentacles are comparatively short (about 5 mm.), arranged at some distance from one another, and distributed over nearly the entire discal area; when fully extended they are sickle-shaped, broad below, and narrow above.

When very light non-nutritive particles, such as accumulate on the bottom of vessels containing cloudy sea-water, are gently dropped upon the disc of an expanded Fungia they produce no decided response on the part of the polyp. If the mouth be open many of the particles are wafted away

rather quickly over the sides of the polyp, the others settling upon the surface (fig. 1), while if the mouth be closed they all drop upon the disc and there come to rest. The particles which fall on the polyp are afterwards no longer free to move independently ; even a strong stream of water from a pipette is unable to dislodge them.

It is evident that on settling the foreign particles become embedded in a layer of mucus covering the polypal surface, for by means of a camel's hair brush or a stream of water from a pipette a layer of this character can be detached in shreds, and in it are contained the objects which have fallen upon the polyp. Moreover, if the débris be allowed to remain undisturbed for a time, and the polyp should open its mouth, a mucous layer is detached by the activities of the polyp itself, and is soon broken up into shreds and patches, which are then moved slowly towards the edge of the disc, over which they fall to the bottom of the vessel. After removal of the mucus the surface of the polyp is perfectly clean and free from foreign particles.

Likewise if carmine or sepia mixed in sea-water be allowed to fall upon the disc the coloured particles are somewhat uniformly distributed as they slowly settle in the mucus, and will remain thus for several minutes or even hours if the mouth remain closed. Ultimately, when the mouth is opened, the layer of mucus containing the particles becomes broken up and arranged in more or less radial strands and shreds, which are slowly transferred to the margin. Very rarely the coloured shreds are drawn in the reverse direction, that is, towards the mouth, and are then ingested.

When, instead of non-nutritive particles a filtered nutritive solution, such as meat or crab extract, is discharged over the disc of Fungia, a series of very decided responses are observed :—(1) The tentacles begin to move about freely, their inclination being mainly towards the mouth ; (2) the disc is partly retracted upon the skeleton ; (3) the mouth opens widely, and the solution can be seen passing from the disc down the stomodæum, an inhalent current having been

established; (4) after a few moments the disc and tentacles recover their normal condition of expansion; (5) the mouth remains open until all the nutritive solution is entirely indrawn, when it again closes, and both inhalent and exhalent currents cease for a time.

When carmine, sepia, or talcum powder is mixed with the nutritive solution similar responses to the above are brought about, and the presence of the colouring matter enables the course of the solution to be more readily followed. Some of the particles remain floating for a short time, and are drawn directly down the stomodæum as soon as the inhalent current is established, but many of them come to rest on the discal

Fig. 2.—Diagram to illustrate the flow down the stomodæum of particles resting upon the disc of Fungia when an inhalent current is set up.

surface. The grains of carmine or sepia which fall upon the disc are at first scattered somewhat uniformly over the surface, but, embedded in slime, they soon become disposed in more or less distinct radial streams along the interseptal grooves, and, as such, are gently drawn across the disc into the open mouth (figs. 2, 3). The nutritive solution evidently leads to a copious exudation of thin mucus, for sometimes as many as a dozen different mucous streams will be slowly moving at one time over the disc and down the stomodæum, distributed all round the mouth with some degree of regularity. When the indrawal of the mucus and its contained particles is completed the mouth closes, and the disc is altogether free from foreign objects.

The thin watery character of the mucus making up the streams containing nutritive substances is very different from its shred or membrane-like nature in the first experiments

with non-nutritive particles. With a pair of forceps or bent needle one can lift up or detach a stream, when it is found that the fluid is viscid, and presents all the usual characteristics of mucus.

The continuance of both the inhalent current and the streams of mucus is found to be altogether dependent upon the amount of the nutritive solution. When the pigmented meat extract is weak an inhalent current is nevertheless set up, but ingestion continues for only a short time, and then

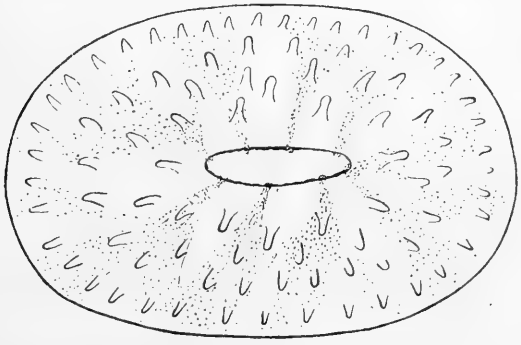

Fig. 3.—Distribution of nutritive particles over the disc of Fungia; streams of mucus are formed around the mouth and carry the particles down the stomodæum.

the flow stops; the reaction has ceased with the exhaustion of the supply of the nutritive solution. After a time the mouth opens and the stomodæal current is renewed, but in an opposite direction, and any non-ingested pigment particles remaining on the disc are then wafted outwards over the edge. Moreover, when shreds or patches of mucus are being floated away over the disc the direction of their movement can be readily reversed by the addition of nutritive solutions around the mouth. Upon the application of the stimulus the outward movement of the objects at once ceases, and they then begin to move slowly towards the mouth, where they are ingested. Should the indrawal not be completed before the action of the nutritive juice ceases, the bodies come to

rest, and are a second time wafted outwards upon the reversal of the current. On an active polyp it is thus possible, by the application of limited supplies of meat juices, to change a number of times the outward or inward movement of the mucus and embedded particles over the disc. Parker,[1] in experimenting with pieces of the lip of Metridium, also found that the ciliary reversals which bring about the change in direction of the current could be repeated many times without impairing the tissues.

As the mouth in Fungia is rather large, it is possible, by means of a fine pipette, to apply meat juice over any restricted region without its spreading to the neighbouring parts. It is then found that an inhalent current is set up over the area to which the solution is applied, while elsewhere an exhalent current is in progress. The phenomenon is thus presented of streams of mucus being indrawn over one portion of the polypal disc, while shreds of mucus are being wafted towards the margin over the remaining part of the disc.

If, instead of numerous small particles, a fragment of meat, a few shreds of crab's muscle, or a piece of an annelid is placed upon the disc of Fungia it causes a momentary depression of the disc and tentacles among which it falls, but recovery takes place almost as readily. The juices from the food soon diffuse to other parts of the discal area, and, on reaching the mouth, the latter responds by opening widely, and, at the same time, an inhalent stomodæal current is established, which leads to the formation of the mucus in streams. Should the fragment be small it is drawn somewhat quickly over the disc as part of the inflowing current without any assistance from the tentacles, and, on reaching the mouth, it can be seen to glide directly downwards without coming into contact with the lips or stomodæal walls. The latter take no direct part in the swallowing. After ingestion the mouth closes, and neither incurrent nor excurrent can be detected for some time unless further stimuli be applied.

[1] "The Reversal of Ciliary Movements in Metazoans," 'Amer. Journ. Physiol.,' vol. xiii, 1905, p. 3.

Should the fragment of meat be large or too heavy for the stomodæal current alone to set it in motion, its transference is assisted by negatively thigmotactic reactions on the part of the disc and tentacles. In a general way these may be understood by saying that the depressed parts of the disc and tentacles immediately under the fragment attempt to recover and elevate themselves, and, in so doing, displace the object resting upon them, causing it to move feebly one way or another. In the meantime the food has become entangled in mucus, and, as this is being drawn towards the mouth, it generally happens that the first movements of the meat are also mouthwards, and, once a definite transference towards the mouth is set up, it usually continues uninterruptedly, the force of the incurrent becoming greater the nearer the approach to the mouth. In like manner any object, nutritive or otherwise, resting upon the disc is ingested once it becomes entangled in the inflowing mucus altogether independent of its nutritive value. On account of their smallness the tentacles in Fungia are found to be of very little importance in transferring food to the mouth, though in other corals and actinians this is one of their principal functions.

In the feeding experiments it was frequently demonstrated that it is not the food itself which brings about the reversal of the stomodæal current and accompanying reactions, but the extractives from it, and further, that these must reach the mouth or its immediate neighbourhood. Often small fragments of meat or crab's muscle were placed on the marginal parts of the disc without calling forth any responses on the part of the mouth or changing the direction of the stomodæal current; the juices diffusing from them evidently failed to reach the lips, and no reaction followed. The particles of food in this case were ultimately wafted from the disc by the ordinary outward current in the same manner as non-nutritive substances. If, however, the meat were brought nearer the mouth the latter opened more widely, a positive current was established, and ingestion took place.

If shreds of meat from which all the juices had been extracted were placed near the lips they either brought about a feeble reaction or none at all, while pieces of paper soaked in meat juices served as stimuli just as effectively as meat itself or meat extractives. It was thus plainly demonstrated that the opening of the mouth, the institution of the inhalent current, and the copious exudation of mucus were altogether due to the nutritive extractives, irrespective of any mechanical stimuli.

Stomodæal Currents.

With regard to the wafting away of certain objects from the oral disc, and the indrawal of others, the above experiments prove conclusively that in Fungia there is at times a current of water flowing from the mouth, and that the direction of this current can be reversed by nutritive substances or their extractives. Such currents are produced by the lining of cilia always strongly developed along the whole stomodæal surface of polyps. Parker,[1] in the experiments with Metridium, invariably found the direction of the current over the lips to be outward in quiescent animals with the mouth open, while it is reversed when pieces of crab meat are applied to the lips; Carlgren's[2] experiments with other actinian species indicate similar changes in the stomodæal currents.

At first it seemed scarcely possible that any ciliary current from the mouth could influence such a large surface as that of the disc of the mushroom coral without any assistance from either discal or tentacular cilia, but the following considerations appear to leave no doubt that such is the case. When the mouth is completely closed no movements

[1] "The Reversal of Ciliary Movements in Metazoans," 'Amer. Journ. Physiol.,' vol. xiii, 1905, p. 2.

[2] Über die Bedeutung der Flimmerbewegung für den Nahrungstransport bei den Actiniarien und Madreporarien," 'Biol. Central.,' Bd. xxv, 1905, p. 308.

of even the lightest particles can be observed around it or anywhere else on the discal area; light objects may remain resting on the disc for hours, which would certainly not be the case if the disc itself, or even the tentacles, were ciliated. When, however, the mouth is opened and fine inert particles are dropped around it, they are seen to be at once wafted away, passing rapidly outwards in a continuous stream along with other light objects which may be lying upon the disc.

Objects resting upon the disc are moved in a very uniform manner once their translation commences; there are no subsidiary movements in the neighbourhood of the tentacles or any restricted part of the disc such as would be expected were cilia present. In some instances the mouth closed while the bodies were in the act of gliding towards the discal margin; the gliding motion then ceased, and the objects came to a state of rest upon the disc and remained there. Further, when the living tentacles or parts of the discal surface of Fungia are examined under the microscope there is no evidence that cilia are present.

From all these we may conclude that the outer surface of Fungia is not ciliated,[1] and that any motion of particles upon its disc or in its vicinity is entirely due to currents of water produced by the cilia lining the stomodæum.

The experiments also render it manifest that under ordinary circumstances the outward beat of the cilia is the more usual in Fungia, and that the inward beat is produced by the action of what we may consider to be nutritive substances. Further, the inhalent current is maintained only so long as the stimulating agent continues to act; after this there is a cessation, and then, following a greater or less interval, there is a reversal of the dominant beat, leading to an exhalent current.

[1] Carlgren has recently published a paper in which he shows a considerable variation in the extent of the ciliated surface both in actinians and corals ("Über die Bedeutung der Flimmerbewegung für den Nahrungstransport bei den Actiniarien und Madreporarien," 'Biol. Central.,' Bd. xxv, 1905, p. 308).

Parker[1] has determined the time interval between the moment of applying a stimulating agent (2·5 per cent. potassic chloride) to the lips of Metridium and the occurrence of reversal, and also the interval between the application of pure sea-water and the return of the normal stroke. In five experiments the former varied from 30 secs. to 2 min. 30 secs., and the latter from 9 min. to 13 min. 10 secs. The rapidity of reversal in Fungia when nutritive juices were employed was usually greater than in Metridium, taking place almost immediately upon application, but the return of the normal stroke varied much.

In corals there must necessarily at times be incurrents set up independently of any external stimuli. An exhalent current, if continued, would tend to exhaust the fluid within the polypal cavity were there no incoming supply, and the cœlenterate gullet has to serve as the passage for both the incoming and the outgoing streams. In this necessity for renewal we probably have an explanation of the fact that occasionally the non-nutritive particles of carmine, sepia, or even fine sand grains and fragments of shell were ingested, though usually they were rejected. Torrey[2] has likewise found that in the actinian Sagartia davisi chemically inert objects like cork, paraffin, glass, and paper are at times swallowed, but he considers that these substances may cause a ciliary reversal by mechanical stimulation; Parker, however, was unable to obtain any such reversals by mechanical stimulation from inert materials. The two opposite currents, inhalent and exhalent, proceed simultaneously in actinians like Metridium, which are provided with one or more siphonoglyphs, for Parker has demonstrated that the siphonoglyph current is always an inward one, whereas that over the lips may be either inwards or outwards[3]. As siphonoglyphs

[1] "The Reversal of Ciliary Movements in Metazoans," 'Amer. Journ. Physiol.,' vol. xiii, 1905, p. 6.

[2] "On the Habits and Reactions of Sagartia davisi," 'Biol. Bull.,' vol. vi, 1904, p. 212.

[3] Hickson ('Phil. Trans.,' 1883, p. 694) long ago found that in the

are absent in all coral polyps the conditions governing the inhalent and exhalent currents are simpler in corals than in actinians. In the latter the water expelled over the general surface of the stomodæum can be replaced synchronously by that flowing down the siphonoglyph, whereas in corals the two streams must be intermittent. Hence the greater probability that in corals, at any rate, inhalent streams may be established without the influence of external stimuli, and carry with them whatever foreign particles may be upon the disc.

The actual conditions determining the reversal of the cilia have been studied by Parker[1]. He shows both by experimental means and by actual observation that the labial cilia do not reverse when in contact with carmine, Indian ink, sand, pellets of filter-paper moistened with sea-water, or with solutions of sugar, quinine, or picric acid in sea-water, but that they reverse to dilute crab-meat juice. Further, the reversal occurs only over the area where the stimulus is applied, as was found to be the case in Fungia. Investigating the subject chemically he finds that the ciliary reversal due to crab-meat is probably dependent upon some organic combination containing potassium, for the cilia reverse in a $2\frac{1}{2}$ per cent. solution of potassic chloride in sea-water, though not in a large number of other chemical substances tested; reversal is found to be due to potassium ions, since it occurs in $\frac{5}{8}$ *m* Na Cl + $\frac{1}{3}$ *m* KNO_3, but not in $\frac{5}{8}$ *m* Na Cl + $\frac{1}{3}$ *m* Na NO_3.

Parker has fully demonstrated that the tentacles of Metridium are ciliated, but here the direction of the stroke is not reversed by the application of meat juices; likewise the inward beat of the cilia lining the siphonoglyph is non-reversible. Any part or all of the rest of the stomodæal cilia, however, reacts to the influence of nutritive juices.

alcyonarian, Alcyonium digitatum, the cilia of the single siphonoglyph produce a current of water from without inwards, whereas the cilia lining the rest of the stomodæum produce currents in the opposite direction.

[1] 'Amer. Journ. Physiol.,' vol. xiii, 1905.

It is manifest that ciliary activity is an important factor in the physiological activities of actinian and coral polyps, particularly in combination with mucus, as will be more fully discussed later. The exhalent currents produced thereby serve to waft or float away whatever inert particles accumulate upon the disc, while the inhalent currents are of much significance in ingestion. The cilia are shown to react locally, and only upon direct contact with the stimulating substance, that is, their activity is not dependent upon nervous impulses, either near or distant.

Experiments with Favia.

For a study of the reactions and feeding activities of colonial corals a species of Favia was employed. The individual polyps are here about 8 mm. across, and the tentacles are closely arranged at the margin of the disc, so that those of adjacent polyps intermingle on full expansion (fig. 6). Compared with those of Fungia the tentacles of Favia take a greater part in the activities of the polyp.

When very light débris is dropped upon a colony of Favia with the polyps fully expanded, the lighter particles are caught in the exhalent currents from the various stomodæa, while the heavier fall on the disc, and become embedded in the superficial layer of mucus. The free floating particles over any one polyp are wafted rather quickly from the mouth towards the tentacular region, beyond which they seem about to pass, when they are immediately checked, in such a manner as to demonstrate that between the individual polyps there is either an approximate neutral zone or one in which the current moves upwards (fig. 4).

The marginal regions, where the tentacles from adjacent polyps intermingle, thus become occupied by particles driven to and fro within very narrow limits; sometimes the objects pass more over the disc of one polyp, and then further over an adjacent polyp. Ultimately, however, the particles come to

rest among the tentacles in the somewhat neutral region, and settle in the interpolypal spaces below. Any new particles coming into the area of motion of an individual polyp are at once wafted from the mouth towards the periphery, and are there battered to and fro until in the end they settle among the tentacles. Sometimes the particles are whirled round and round over the oral aperture for some time before being driven away.

The superficial layer of mucus containing the heavier particles is ultimately freed, the mucus being formed into shreds or patches. These in their turn are wafted peripherally and accumulate among the tentacles, their further

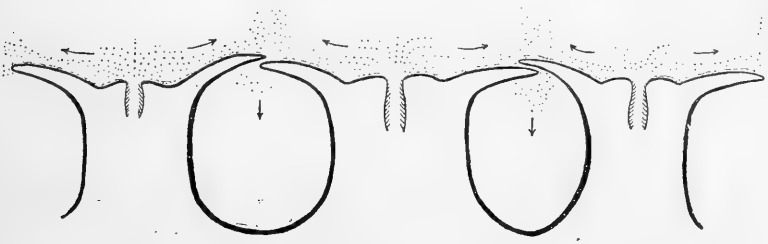

FIG. 4.—Distribution of particles dropped upon a colony of Favia when exhalent currents are in progress. Three polyps are represented in section.

progress outwards being arrested by the currents from adjacent polyps. The boli usually revolve round and round for some time, and then sink between the tentacles into the spaces between the surrounding polyps.

Somewhat similar results were obtained from experiments with carmine, sepia, and talcum powder in sea-water. The fine particles from the diffusion cloud were at first distributed uniformly over the surface of the disc and tentacles, having become embedded in the mucus. Usually after a few minutes the mucus began to break into patches or shreds, and was then floated towards the intermingled tentacles, through which it passed to the columnar areas below.

Very rarely the carmine or sepia, embedded in mucus,

would be slowly drawn down the stomodæum, instead of being driven away. This movement, however, was never carried out with the same readiness as described below for nutritive substances, and in some instances the indrawal would cease altogether, and after a short interval the shreds be rejected and wafted away over the disc.

When a clear solution of meat extract is poured over a colony of Favia, with the polyps fully expanded, the following reactions take place:—(1) The tentacles twist and turn mouthwards, and the individual polyps partly retract; (2) the polyps recover and the tentacles are fully extended outwards; (3) while 1 and 2 are in progress the mouth of each polyp opens very widely, the peristome protrudes somewhat,

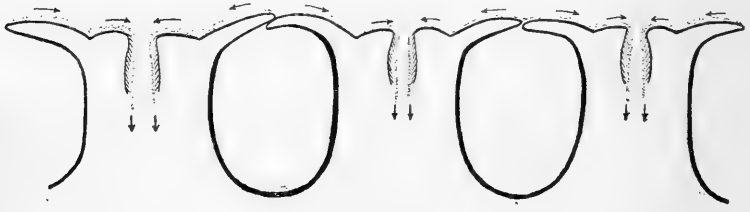

Fig. 5.—Nutritive particles dropped upon a colony of Favia and flowing in a mucous stream down the stomodæum.

and several streams of mucus are seen passing down the stomodæum (figs. 5, 6); (4) after the ingestion of the mucous streams the mouth closes and the polyps recover their normal condition.

If the nutritive solution be rendered conspicuous by mixture with finely powdered carmine or sepia, the polypal reactions are the same as with the extract alone. The particles of carmine or sepia fall in a uniform manner over the surface of the disc and tentacles, and later are arranged in mucous streams. The streams are seen to form first upon the peristome, and then to extend over the entire discal and tentacular areas. Moreover, towards the close of the indrawal, the streams entering the mouth of any one polyp are found to be continuous in a radiating manner with those entering the

mouths of the surrounding individuals, as shown in fig. 6. Manifestly under such circumstances any single mucous strand is pulled at from both extremities, and as the indrawal continues it reaches in time a state of tension. The strands are then seen to break or divide about the middle, and each moiety passes to its own polyp and is ingested. Evidently upon stimulation by nutritive juices the mucus in Favia is exuded in such quantity as to form a continuous superficial layer over the colony, and to bridge the intervals between adjacent polyps.

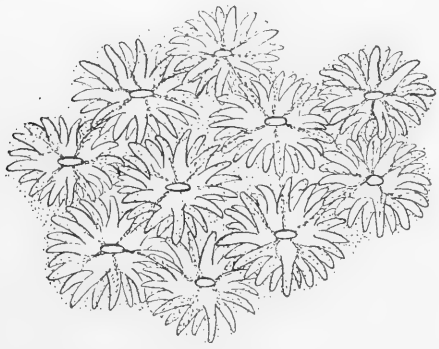

FIG. 6.—Several polyps of Favia showing the distribution of nutrient particles after falling upon the colony. The particles are becoming arranged in mucous streams and drawn down the stomodæa. Frequently the streams passing down the mouths of adjacent polyps are continuous.

The polyps of Favia very readily respond to the influence of nutritive juices, and many experiments can be arranged to illustrate the quickness with which the reversal of the dominant movement of the stomodæal cilia takes place. If when non-nutritive particles of carmine, sepia, or talcum powder are being wafted outwards towards the polypal margin a solution of meat extract is dropped upon a colony the outward movement at once ceases, a momentary rest occurs, and then a reversal in the direction of motion takes place. The mouth is widely opened and streams of mucus are indrawn, carrying

with them whatever foreign particles are resting upon the disc or tentacles.

The nature of the foreign objects ingested is of no significance. When the reactions set up by a nutritive solution are in progress, any substance whatever, coming within the influence of the incurrent stream of mucus, is swallowed. Neutral bodies, such as small grains of sand, fragments of shells, or pieces of paper, which have been resting upon the discal surface for some time become covered with mucus and are indrawn into the widely-opened mouth. Even particles situated as far outwards as the margin of the tentacular zone come within the influence of the stomodæal currents, and are slowly carried centrally and ingested. The act of ingestion is thus purely mechanical; the polyps exercise no such faculty as choice as regards the substances which enter the gastro-vascular cavity. It is simply a question of the influence of different solutions upon the stomodæal cilia. When the latter are stimulated they react directly, without any other part of the polyp being concerned, so as to produce an inhalent current, and any object upon the disc or tentacles is drawn downwards whatever its nature, whereas when the cilia are producing an outward current all objects upon the disc are rejected.

The reactions of the polyps of Favia towards solid nutritive substances, such as fragments of meat or crab's muscle, are of much the same nature as those towards nutritive solutions. The close contiguity of the polyps often renders it possible for several mouths to reach the same fragment and all attempt to ingest it. Under these circumstances the food is pulled at from many different directions, but in the end one polyp generally succeeds in securing the whole.

Distant polyps in the colony, that is those not directly touched by the food substances, are stimulated to activity by diffusion of the juices, and respond by opening their mouths and instituting an inhalent current. The polyps also possess to a remarkable degree the power of protruding the peristome and directing it towards the source of the stimulus (directive

reaction), a response which will be discussed in another contribution.

The experiments described suffice to show that the feeding reactions of the colonial coral Favia closely resemble those of the single polyp of Fungia. The differences, such as the more complicated character of the superficial currents and the continuity of the mucous streams, are such as are dependent upon the colonial condition. As in Fungia, there was no evidence that the external surface of Favia is ciliated.

Throughout the experiments there was no indication that the food during ingestion was actually grasped by the lips or that its indrawal was directly assisted by peristaltic movements of the stomodæal walls. When the particles were small they were simply carried along in the inhalent stream, whether of water or mucus, without coming into direct contact with the lips or stomodæal walls, or without any movements of these. When the fragments were large the oral aperture became wider and wider to admit them, and then closed after their admission. Even here their motion downwards was of a gliding character, though there can be no doubt that when, on account of their size, the fragments come into direct contact with the stomodæal walls they will bring about reactions of the organ. Torrey [1] considered that the peristaltic movements of the œsophagus in Sagartia davisi may assist the cilia, but found no evidence that they take more than a very subordinate part in the swallowing or disgorgement. In the case of Hydra, Wagner [2] found that as soon as food touches the hypostome the mouth begins to open and its edges fasten to the meat. The actual process of swallowing here depends entirely on the activity of the tissue of the hypostome and body. Wagner regards it as difficult to see how cilia could have enough strength to play

[1] "On the Habits and Reactions of Sagartia davisi," 'Biol. Bull.,' vol. vi, 1904, p. 215.

[2] "On some Movements and Reactions of Hydra," 'Quart. Journ. Micr. Sci.,' vol. 48, 1905, p. 609.

any part in the swallowing of entire entomostraca, annelids, insect larvæ, and the like which form the ordinary food of Hydra; the cilia, however, are certainly capable of such a performance in coral polyps and actinians.

Significance of Mucus.

The foregoing experiments indicate that mucus is an important factor in the feeding and other activities of corals. As is well known, the outer layer or ectoderm in all coral polyps is largely made up of unicellular mucous glands, separated by long narrow supporting cells, with nematoblasts here and there. When detached by maceration and also in sections the outer half of the mucous cell is generally found filled with a clear transparent fluid, the cytoplasm and nucleus having been pressed toward the inner mesoglœal aspect of the cell; sometimes the contents of the gland cells are finely granular, the latter being perhaps a stage in the formation of the clear mucus. The mucus usually stains intensely in Delafield's hæmatoxylin.

Under ordinary circumstances the exuded mucus forms merely a thin layer over the external surface of the polypal wall. But when the polyps are irritated, as when roughly handled, they send out enormous quantities of the clear, viscid secretion, though, on account of its transparency and absence of colour, it is not usually perceived; also on mechanically irritating certain actinians (Lebrunia) I have found the fluid to be exuded to such a degree as to give an almost gelatinous consistency to the water in which the polyps were contained.

The experiments with light débris and fine powders have shown that when such substances fall upon the polypal surface they become embedded or entangled in the superficial layer of slime, so that the particles are no longer free to move independently, but only as a part of the mucous layer. Further, the superficial mucus bearing the foreign particles

is from time to time separated in irregular patches from the general surface, and wafted away over the edge of the disc by the excurrent stream from the polypal cavity. In these movements over the discal surface the patches and strands of mucus may become more or less rolled into boli. Parker also found in Metridium that powdered carmine in sea-water when dropped upon the tentacles was matted together in threads as though by mucus, and was slowly carried from the bases of the tentacles towards their tips, but he nowhere attaches much significance to the secretion.

After removal of the impregnated mucus the polypal surface is fresh and clean, and there can be no question that one of its functions is thus to protect and keep clean the walls of the polyp, there being no other means by which this can be accomplished. Light bodies falling upon the polyp, whether nutritive or otherwise, do not come into direct contact with the ectodermal cells but are received in a thin mucous layer, which is shed from time to time and got rid of by means of currents from the stomodæum, aided perhaps by the movements of the polypal walls and tentacles.

On the column of many actinians, e. g. Phellia, Adamsia, the mucus is not regularly removed, as it is in corals, but accumulates and hardens somewhat so as to form a rather thick membrane, having sand grains, foraminifera, diatom frustrules, and other foreign bodies embedded in it. In these cases the mucus appears as a rough protective membranous coating close to the polypal wall, and is shed only at wide intervals, after which the column appears thinner walled and more delicate. The upper part of the column (capitulum) and discs of the polyps remains permanently smooth, the mucus and any adhering foreign particles being regularly removed from this region. Likewise the tube in Cerianthus is largely formed of exuded slime, in which nematocysts and foreign particles have become embedded. Loeb[1] has shown that the secretion of the tube of Cerianthus is

[1] Loeb, J., "Studies in General Physiology," 1905, p. 165.

produced only on mechanical irritation of the surface of the polyp against solid particles.

It is uncertain how far the exudation of mucus in corals is a continuous physiological process under ordinary circumstances or to what extent it is dependent upon stimuli. It can scarcely be imagined that the falling of light débris upon the disc is sufficient to influence the exudation, yet once the particles reach the disc they become embedded in such a secretion, and are not removable except along with the mucus. It is probable, as in the case of so many aquatic animals, that the polypal surface is always provided with a thin mucous coating, and that from time to time the outer stratum sloughs off and is then renewed. In addition, there can be no question that mechanical stimuli increase the amount of mucus exuded; this is certainly the case on handling or irritating specimens, and may also take place when any but the lightest objects are dropped upon the disc.

Nutritive solutions cause an increase in the amount of mucus exuded in corals to such a degree that it may envelop the entire disc and tentacles of the polyps concerned; after a supply of meat juices as many as five or six mucous streams may be seen passing down the gullet together. The amount exuded for the time being is, however, limited in quantity, and can be wholly ingested, as may be inferred from the fact that the streams cease after a time, and a strand undergoing ingestion at both ends ultimately breaks in the middle. The exudation can be renewed by the application of a further nutritive solution, but experiments are still needed to determine how long successive discharges will continue, or how voluminous they will remain. Parker[1] found that potassic chloride, which is capable of reversing the ciliary activity in Metridium, is also a stimulant for the production of mucus and the discharge of nettle capsules, apparently more so than meat-juice itself.

The mucus undergoes some physical or chemical change

[1] "The Reversal of Ciliary Movements in Metazoans," 'Amer. Journ. Physiol.,' vol. xiii, 1905. p. 4.

shortly after exudation. When freshly secreted, as from stimuli of nutritive juices, it is a clear, colorless, watery, viscous fluid, readily forming into separate streams; but after an exposure on the disc or tentacles for some time the superficial layer becomes more consistent, more membrane-like, and is then capable of being separated only in patches or shreds. In actinians where the mucus is rarely shed, as in Phellia and Adamsia, it forms a more or less permanent membranous coating. Such physical changes are well known in the mucus exuded by other organisms, such as molluscs and planarians. In these the secretion is also very thin or watery when first secreted, but rapidly changes so as to become more consistent.

The separation of the exuded mucus from the discal and tentacular surfaces in the form of patches or shreds is dependent upon the stomodæal currents, probably influenced by the movements of the disc and tentacles. If the mouth be closed and no incurrent or excurrent is produced the matted mucus simply remains upon the disc, may be for hours or days. In the forms studied there was no evidence of ciliation over any external part of the polypal surface such as could effect or assist in the removal. When, however, a stream of water from the stomodæum, or even from a pipette, was forced over the disc, either outwardly or inwardly, the mucus was detached from its adherence to the polypal surface and broken up into irregular patches, and then either drawn inwards or wafted outwards according to the direction of the current. By the action of the outward currents the mucous patches are often rolled into boli and then dropped over the sides, whereas when drawn into the stomodæum the mucus is in the form of distinct continuous streams. Whenever indrawal takes place as a result of external stimuli there is an accompanying increase in the secretion of mucus. The peripheral movement is a wafting or floating away of detached strands or patches, the centripetal is an indrawal of several watery streams.

Not only does the mucus serve as a protection to the polyp

under adverse circumstances, and assist in getting rid of foreign substances which may fall upon it, but it is of much importance in the process of nutrition, by serving as a vehicle or means of conveyance of nutritive substances to the mouth and down the gullet. In actinians the transference to the mouth is largely carried out by the tentacles, but these organs are seen to be of much less importance in Fungia and Favia. The experiments have shown that nutritive juices reverse the ordinary outward beat of the cilia and lead to the production of an inhalent current; at the same time the nutritive substances stimulate the exudation of mucus, and this is then drawn down the gullet in the form of streams, carrying with it whatever substances are embedded or entangled in it. Even the transference mouthwards of moderately-sized food fragments, which do not become altogether embedded in the slime, is assisted by the same means. As the fragment is moved across the disc it becomes more and more enveloped in mucus, and is thus brought more within the power of the streams already passing down the gullet. Further, an inhalent current being established, objects are ingested along with the mucus irrespective of their nutritive value.

The viscid mucus also serves to entrap small living organisms falling or resting upon the disc, organisms perhaps too small to stimulate the tentacles to activity. In this way protozoa, small crustaceans, and larvæ of different kinds may be secured as food, much in the same way as the mucus secreted by the endostyle in ascidians entangles food particles. In addition the secretion assists the tentacles in their attempts to hold and secure prey of a more active kind. When a living annelid or crustacean is placed upon the disc of a coral it at once becomes entangled among the tentacles; though at first wriggling violently it is soon rendered motionless, and on attempting to draw the victim away with the forceps adhering shreds of mucus are dragged along with it. Paralysation and loss of motion is here mainly due to the poisonous action of the nematocysts, but the loss of motion is

without doubt influenced by the envelope of mucus gathered in the struggles of the organism, the secretion of the mucus being much increased by the mechanical and chemical stimuli. It is probable that all food before ingestion becomes enveloped in mucus.

An interesting case illustrating the protective value of mucus under adverse conditions occurred in the course of the investigations. Colonies of Porites living in the tanks in which were kept other corals used in the experiments apparently found the conditions unfavourable, and the polyps became wholly retracted and inactive. To all appearances the polyps were dead, and fine particles of mud and débris from the somewhat cloudy water settled upon the colonies. However, having transferred the colonies to other and apparently more favourable conditions the polyps, after a time, began to expand, when it was found that they had to break through a rather dense felt of mucus and foreign particles, which had formed over the entire surface during the several days of inactivity. For some time the colony presented a ragged appearance, that is until all the mucous shreds and patches had by one means or another been removed. It is manifest that by forming such a coat of mucus, coral polyps have a certain power of protecting themselves during periods of inactivity, whether brought about by slight changes in the composition of the water, muddy conditions, or variations in temperature

Summary.

1. Under ordinary conditions the outer surface of coral polyps is covered with a thin, continuous layer of mucus, in which objects falling upon the polyp become embedded or entangled.

2. When first exuded the mucus is thin and watery, but later becomes more consistent. From time to time it is broken up into shreds and patches which are driven or wafted from the surface of the disc by the ordinary exhalent currents from

the stomodæum, along with any embedded foreign particles in it.

3. Nutritive substances and extractives placed upon the polyp increase the amount of mucus exuded, and also result in an opening of the mouth and the institution of an inhalent stomodæal current by reversing the dominant outward beat of the stomodæal cilia.

4. The mucus exuded as a result of nutritive stimuli is drawn down the stomodæum by inhalent currents in the form of distinct streams, and carries with it whatever substances, nutritive or non-nutritive, are embedded or entangled in it.

5. Ingestion in coral polyps is thus purely mechanical, depending upon whatever substances are capable of producing an inward beat of the cilia, the opening of the mouth, and the exudation of mucus. An inhalent current being established, objects are carried into the polypal cavity without regard to their nutritive value, and independently of any peristaltic motions on the part of the stomodæum.

6. In the ordinary activities of coral polyps inhalent currents may be occasionally instituted independently of any external stimuli, and these carry with them any inert objects resting upon the disc.

7. Mucus is of much importance in the protection of the polypal surface from foreign objects and in keeping it clean, and also in the entanglement and ingestion of prey and food substances.

Observations on the Structure and Life-history of Pleistophora periplanetæ, Lutz and Splendore.

By

W. S. Perrin, B.A.,

Shuttleworth Research Student of Gonville and Caius College, Cambridge.

With Plates 37 and 38.

Contents.

The subject of this research, which is still in progress, is a Myxosporidian parasite of the ordinary black cockroach Periplaneta orientalis, found in bakehouses, college kitchens, and similar places. The presence of this parasite in the cockroach was first recorded by Schaudinn, who states (6) that the spores "eines Nosema" occur in the fæces of cockroaches examined by him, and emphasises their similarity to the spores of Bacillus bütschlii, parasitic in the alimentary tract of Periplaneta orientalis. He describes the presence of a polar capsule in these spores, and

further states that, under the highest magnifications the spirally wound filament of the polar capsule is often quite plainly discernible.[1]

Lutz and Splendore (8) describe the presence of the spores of a Myxosporidian in the Malpighian tubules and neighbouring gut of the Brazilian cockroach Periplaneta americana, and refer them to the genus Nosema and add the species name "periplanetæ." The slight descriptions given by Schaudinn and Lutz and Splendore of the spores they find in Periplaneta orientalis and Periplaneta americana respectively, fit, as far as they go, the spores of the Myxosporidian which is the subject of the observations recorded in this paper. These observations show, however, that the parasite investigated belongs to the genus Pleistophora, a conclusion impossible to arrive at from a knowledge of the unstained spores alone.

In spite of the fact that all attempts to infect Periplaneta americana with the parasite by feeding them upon the infected fæces of Periplaneta orientalis have been unsuccessful, it is possible that the parasite is identical with that described by Lutz and Splendore. I therefore propose to retain the specific name periplanetæ, while referring the parasite to its proper genus Pleistophora. The reasons for adopting this course will be entered into later, after the account of the life-history has been given.

Material and Methods.

Pleistophora periplanetæ lives in the lumen of the Malpighian tubules of Periplaneta orientalis, of which it is the sole inhabitant. A few trophozoites are generally to be found also in the ileum together with a larger number of pansporoblasts containing ripe spores, and the ripe isolated

[1] The polar capsule is a structure occurring in the spores of the Myxosporidia, containing a filament which is extruded under the stimulus of the digestive juices, when the spore reaches the alimentary canal of its host. The filament serves to attach the spore to the wall of the alimentary canal.

spores themselves. The parasite lives freely in the lumen of the alimentary canal and the Malpighian tubules. Examination of the fresh tubule usually shows the trophozoites crowding up against the sides of the passage, although they are frequently present in sufficient numbers to block the whole lumen completely. When the trophozoites are present in any number the cells of the tubule are comparatively clear and hyaline, the very numerous granules ordinarily present in the cells of uninfected tubules being conspicuous by their absence.

Examination of the fresh tubules and sections, both transverse and longitudinal to them, has failed to reveal the presence of intracellular stages. The parasite appears to be wholly extracellular, and, in this feature, differs from the rest of the Cryptocystes, which are by definition cell parasites. The parasite is not apparently fatal to its host. Every adult Periplaneta examined contained the parasite, and frequently great numbers of it, without any apparent inconvenience. The reason of this is probably to be sought in the fact that although the parasite may be present in very large numbers its presence is confined to comparatively few of the Malpighian tubules, nor do the tubules themselves, however strongly infected, appear to be destroyed.

Confusion with tissue elements or with any other members of the singularly rich and varied fauna harboured by the cockroach is impossible on account of the very characteristic appearance of the trophozoites, more particularly when they are stained with Giemsa's eosin-azur solution. As before mentioned, every adult cockroach examined contained the parasite in larger or smaller numbers. The very youngest individuals still possessing the light brown tint characteristic of the infant cockroach were alone found to be uninfected. Spores occur regularly in the fæces.

For examination of the living plasmodia the Malpighian tubules were freed from adhering fat-body, cut in small pieces and examined in normal salt solution. Much of the structure revealed in the stained preparation can be made out in the

living cell. The addition of small quantities of vital staining reagents, neutral-red, brillant-cresyl-blau and methylene blue failed to disclose any further details of structure. For making permanent preparations of the parasite, films fixed and stained in various ways were found to be alone admissible. Sections of 2 μ thickness cut in paraffin were found to be of but little value.

Films were prepared in two ways:

1. The Malpighian tubules were cut up into small pieces, which were spread over a coverslip. The coverslip was then allowed to dry, immersed in absolute alcohol for ten minutes, dried, and stained with one of Giemsa's modifications of the Romonowsky-Nocht stain. The best results were given by leaving the films over night in a mixture of an aqueous solution of eosin and azur II freshly prepared according to Giemsa's well-known recipe.[1] The coverslips were washed with tap-water after staining, dipped for a moment in absolute alcohol to prevent overstaining, washed again with tap-water, dried, and mounted in cedar wood oil.

When a preparation was required immediately, Giemsa's ready prepared eosin-azur solution was employed. The specially powerful mixture of azur I, azur II, and eosin used by Schaudinn for the Spirochæte of syphilis was found to stain too deeply and was discarded.

The above method of fixation and staining, though apparently drastic, gives excellent results. Careful comparison with the living material and preparations fixed with osmic vapour shows that the trophozoites themselves are but very little deformed in general outline, frequently not at all, while no other method of staining differentiates nucleus and protoplasm so sharply (see figs. on left hand side of plate).

The method has an additional advantage in that the nuclei stain differently. This point will be referred to later. Nuclei of the cells of the Malpighian tubules upon the other hand are completely spoilt by this method (see fig. 15). The

[1] Ten parts of a solution of 1 gr. eosin B.A. in 1000 c.c. water is added to 1 part of a solution of ·8 gr. Azur II (Grübler) in 1000 c.c. of water.

reason for this is to be found in the different shape of the plasmodia and the nuclei. The plasmodia are thin and generally leaf-like, while the nuclei are spherical, and hence undergo great deformation in drying on to the glass.

2. The Malpighian tubules were cut up in a very small drop of filtered egg-white, and fixed either by exposure to osmic vapour or by immersion in boiling corrosive sublimate and absolute alcohol in the proportions 2 : 1. After osmic the coverglass was washed in water, after corrosive sublimate in water plus iodine, to precipitate the mercury, the film in each case being either first hardened by passing through the alcohols in grades of 10 per cent., or stained straightway in Delafield's hæmatoxylin. The excess of stain was then washed out in acid alcohol, the film being finally mounted in xylol, or, preferably, alcohol, Canada balsam. Films fixed by the above method were also stained with Heidenhain's iron-hæmatoxylin. Delafield's hæmatoxylin gave, however, better results. Fixation with osmic vapour and corrosive sublimate gave practically identical results. The network of the nuclei of the cells of the Malpighian tubule is somewhat finer with osmic than with sublimate, but the difference is very slight.

Structure of the Vegetative Stages of Pleistophora periplanetæ.

The trophozoites are amœboid nucleated masses of protoplasm varying from uninucleated specks of protoplasm, fig. 1, from 2—3 μ in diameter to masses measuring in some cases 30 μ by 55 μ, and containing 60 or more nuclei (fig. 13). The protoplasm may be homogeneous and hyaline (figs. 1—6), or exhibit a marked foam-like structure, produced by the presence of a large number of small vacuoles (figs. 10, 35). Vacuoles of every size are of frequent occurrence. The protoplasm of the smallest trophozoites possessing only a few nuclei is, as a rule, hyaline, that of the larger multinucleate

ones sometimes hyaline (fig. 20) and sometimes foamy (fig. 35). Granules appear to be absent, but large masses of a mucoid-looking substance are occasionally enclosed by the trophozoites.

In many cases differentiation of the trophozoite into a denser, more deeply staining, external region or ectoplasm, and an internal, more fluid, and vacuolated region or endoplasm is well marked (figs. 25, 34, 35). Pseudopodia are frequent, and generally lobose and rounded (fig. 34), though elongated and pointed pseudopodia also occur. The pseudopodia are, as a rule, formed from the ectoplasm alone, and probably serve to attach the trophozoites to the surface of the cells of the Malpighian tubules.

The nuclei are of two kinds, one which stains a bright red with Giemsa and feebly with hæmatoxylin, and one which stains a deep purplish red with Giemsa and very deeply with hæmatoxylin (figs. 9, 10, 31, 35). The deeply-staining nuclei are frequently contiguous to a small area of protoplasm staining bright blue (fig. 10). They appear to be composed of degenerating substance, since the residual nuclei of the pansporoblasts, which are sometimes at first bright red (fig. 14), stain a deep purple as the spores mature and their own degeneration becomes more advanced. The nuclei, which stain a bright red with Giemsa, are generally reticular, though not infrequently compact. In fig. 11 the nuclei are compact, in fig. 13 reticular. Division of the nuclei appears to be effected by simple fission (fig. 4).

Auto-infective Methods of Reproduction.

Multiplication of the trophozoites within the host takes place in four ways:

(1) The young uninucleate trophozoite may divide amitotically into two daughter cells (figs. 50 and 51).

(2) The nuclei of a young trophozoite may multiply amitotically, and proceed to the periphery of the cell (figs.

16 to 18, 36), while the protoplasm aggregates itself around each nucleus, the trophozoite dividing finally into a number of spores (fig. 19). A process similar to this has been observed by Doflein in the case of Glugea lophii. It is a method of reproduction which, in Pleistophora periplanetæ, strongly recalls the schizogony of the Telosporidia, e. g. the breaking up of the malarial parasite into a number of small sporozoites within the red blood-corpuscle.

(3) A multinucleate trophozoite may divide into two by a process of simple fission (fig. 11).

(4) Portions of a large multinucleate trophozoite may separate themselves from the parent cell and commence an independent existence (figs. 13, 20, 35). The piece budded off may have one or several nuclei, and it seems that the buds are all either uninucleate or multinucleate exclusively (figs. 13 and 20), a trophozoite never apparently producing buds with one and many nuclei at the same time. A similar process to the above, of a multinucleate cell breaking up into multinucleate fragments, has been styled plasmotomy by Doflein. Division of the trophozoite into two fragments he calls simple; division into more than two fragments, multiple plasmotomy. The production of uninucleated swarm spores from a trophozoite by budding does not appear to have been hitherto recorded among the Myxosporidia. It is a noteworthy fact that no dark purple nuclei are to be found in trophozoites undergoing the above processes of reproduction, and it seems likely, more particularly in view of the absence of pseudopodia, that the trophozoites containing these nuclei are about to form resting spores. This point will be referred to later (page 622).

Spore Formation.

All the above methods of reproduction subserve auto-infection, but resting spores are also produced which bring about the infection of fresh hosts. These resting spores

possess a spore-coat or shell which is highly resistent to the action of reagents. The spores gain the exterior in the fæces of the cockroach. Doflein calls the production of naked swarm spores for auto-infection, and resting coated spores for infection of fresh hosts "multiplicative" and "propagative" methods of reproduction respectively. Among the Myxosporidia it is usual as a preliminary to sporulation for the trophozoite to produce a number of internal buds, which give rise to a varying number of spores, the production of spores continuing within the trophozoite without any cessation of its activity or growth. These internal buds have been styled by Gurley pansporoblasts. In Pleistophora periplanetæ, however, no such formation of pansporoblasts occurs, the whole of the trophozoite simply withdrawing its pseudopodia, rounding itself off and producing spores. As, however, the classification of the Myxosporidia is largely based on the features exhibited by the pansporoblast, the number of spores it produces, etc., it seems advisable to regard the trophozoite of Pleistophora periplanetæ as producing one pansporoblast, although the point at which trophozoite ceases to be trophozoite and becomes pansporoblast may not be easy to define.

As a matter of fact I am inclined to regard the production of the deeply staining purple nuclei (figs. 9, 10, 12) as indicative of incipient sporulation for the following reasons:

(1) As mentioned above these nuclei never occur in trophozoites, which may be inferred to be actively metabolic, e. g. trophozoites in any of the stages of multiplicative reproduction.

(2) The presence of these nuclei is associated with an absence of pseudopodia and a general appearance, as though the trophozoite were rounding itself off to form a pansporoblast (figs. 9, 10).

(3) Apart from the spores it produces, the pansporoblast contains a considerable amount of protoplasm and a number of nuclei, which are not used in the production of spores. These nuclei are left behind, and, during the later stages of sporu-

lation nearly always, and during the earlier stages usually, stain exactly the same tint as the above-mentioned purplish nuclei. Occasionally, it is true, these residual nuclei of the pansporoblast remain of a bright red tint even when sporulation is comparatively far advanced. This is, however, exceptional.

The first stage, then, of sporulation consists in the rounding off of the trophozoite to form a pansporoblast, and the appearance of nuclei of two different kinds, the nuclei of the sporoblasts, and the residual nuclei, which disappear with the remains of the protoplasm of the pansporoblast. The pansporoblast (figs. 14, 30—33) is usually a more or less oval body, occasionally oblong and rounded at the ends. It is almost completely filled with developing spores, all roughly in the same stage of development. The interstices between the spores are occupied by protoplasm and nuclei, which ultimately disappear. The number of spores produced varies from three or four (fig. 33) to forty or more. From twenty to twenty-five is about the usual number. Each spore is formed from an oval mass of protoplasm—the sporoblast,—which rather exceeds in size the spore it produces. In stained preparations the sporoblast appears to lie in a vacuole. This vacuole is not, however, evident in the living cell, and is probably an artifact produced by shrinkage of the protoplasm during preparation. It is most marked in preparations fixed with osmic vapour and sublimate alcohol, although it also occurs in preparations fixed by drying on to the slide and immersing in absolute alcohol (figs. 14, 30—32). The number of sporoblasts and residual nuclei is roughly the same. The two kinds of nuclei present, however, considerable differences in appearance and staining power. The residual nuclei are compact and have a sharply-defined outline from the first. With Delafield's hæmatoxylin they stain a very deep blue; with Giemsa, as mentioned above, usually a deep purple. The nucleus of the sporoblast stains differently according to its state of development. In the earliest stages (figs. 14, 30, 38, 39) it is in the form of a sparse reticulum or

a number of discrete particles. With Giemsa it stains bright red, with hæmatoxylin, however, but faintly, in marked contrast to the residual nuclei (figs. 30—33). Later, the nucleus condenses to form a sphere containing a central particle (fig. 31). This sphere stains faintly at first, but as it contracts the staining becomes much deeper (figs. 32, 40—42). The now compact and deeply staining nucleus shifts its position from the centre to the end of the spore, in correspondence with the development of a vacuole with highly refractive contents.

Figs. 41—43 illustrate the gradual development of this vacuole and the shifting of the nucleus, which now divides into two, the daughter nuclei moving to the centre of the spore (figs. 44—47). Fig. 47 indicates that the two nuclei are once more dividing. Later stages than the above have not been satisfactorily stained owing to the very resistent nature of the spore coat. While these nuclear changes have been taking place the spore has been surrounding itself with a spore coat or shell. This, as shown in fig. 49, consists of two halves meeting in a median suture. It is presumably through this suture that the sporoplasm when infecting a new host makes its exit. The spore in its final form is a flat oblong structure rounded at the ends (figs. 48, 49), varying from 5 μ—6 μ in length, and 2·5 μ—3 μ in breadth. In the living state the sporoplasm is finely granular, and at one end of the spore a small highly-refractive globule is present. The relation of the polar capsule, which is almost certainly present, to the vacuole described above and the refractive globule I have, as yet, been unable to determine. Application of the usual reagents—ether, concentrated sulphuric, nitric, and hydrochloric acids, glycerin, iodine, and boiling water, etc.—has hitherto failed to procure extrusion of the filament of the capsule. Experiments now in progress will, I hope, throw further light upon this point. The spore coat is unaffected by the above-mentioned reagents, and also by 'eau de Javelle' (sodium hypochlorite). Attempts to produce the emergence of the sporoplasm from the spore by treating the fæces with the

digestive juices and contents of the alimentary canal of other cockroaches have been as yet unsuccessful.

Classification of Parasite and Discussion of Results.

With the above facts of the life history of Pleistophora periplanetæ to hand it is possible to classify the parasite accurately. From the minuteness of the spores, the fact that the pansporoblast produces more than two spores, and the invisibility of the polar capsule in the fresh state, the parasite belongs to the sub-order Cryptocystes of the Myxosporidia and the family Glugeidæ, which possesses the characters of the sub-order. In the fact that the spores are not pear-shaped, but oblong with rounded ends, and that the parasite is extracellular it differs, however, from the rest of the Cryptocystes. It belongs to the section Oligosporogenea (Doflein), because the trophozoite produces a single pansporoblast, and the production of numerous spores by the pansporoblast relegates it to the genus Pleistophora. It is this formation by the trophozoite of a single pansporoblast instead of a number of them, which removes the parasite from the genus Nosema, section Polysporogenea, to the genus Pleistophora, section Oligosporogenea. To sum up, the classification of the parasite is as follows:

Class.—**Sporozoa.**
Sub-class.—Neosporidia.[1]
Order.—Myxosporidia.
Sub-order.—Cryptocystes.
Family.—Glugeidæ.
Section.—Oligosporogenea.
Genus.—Pleistophora, Gurley, 1893.
Species.—Periplanetæ, Lutz and Splendore, 1903.

[1] Reasons, based on the life-history of Pleistophora periplanetæ, for objecting to this method of subdivision of the Sporozoa by Schaudinn are given on page 626.

There are several points of interest offered by the above scattered details of the life history of Pleistophora periplanetæ, both with respect to the Sporozoa in general, and the Myxosporidia in particular. In the first place it is seen that there are practically two very definite phases in the life history, a schizogonous phase, characterised by almost excessive multiplication with a view to auto-infection, and a sporogonous phase characterised by the cessation of growth and trophic activity and the formation of resting spores. The two phases are sharply marked off from one another and certain morphological differences are presented by the trophozoites of the two phases. In this feature, i. e. the sharp separation of schizogonous and sporogonous phases from one another, Pleistophora periplanetæ resembles Thelohania mülleri, a Myxosporidian belonging to the closely allied genus Thelohania, described by Stempell. Stempell distinguishes two kinds of trophozoites of Thelohania mülleri—"meronts," which multiply by a simple form of division, and "sporonts," larger elements, which produce resting spores. No such difference of size marks out the trophozoites characteristic of the multiplicative and propagative phases of Pleistophora periplanetæ, but the marked difference in behaviour of the two forms of trophozoites, and, perhaps, the appearance of the purple nuclei in the trophozoites about to sporulate, constitute an equally distinctive difference.

The occurrence of sporulation at the end of the schizogonous phase by no means agrees with Schaudinn's division of the Sporozoa into Telosporidia, i. e. Sporozoa in which spore formation occurs at the end of the trophic phase, and Neosporidia comprising the Sarcosporidia and Myxosporidia, in which spore formation continues during the trophic phase. As far as the point in the life history, at which spore formation occurs, is concerned, Pleistophora periplanetæ is in exactly the same position as a Hæmosporidian or a Coccidian. In each case a trophic phase characterised by vigorous multiplication is succeeded by a resting phase characterised by the

production of resting spores. As Stempell remarks, Schaudinn's grouping of the Myxosporidia and Sarcosporidia together as a sub-class separate from the rest of the Sporozoa, is probably well-grounded, but the difference between the two sub-classes must be expressed in other terms than the period in the life history at which sporulation occurs. The evidence afforded by the disporous Phænocystes, in which the trophozoite produces only one pansporoblast, which gives rise to two spores, while the residual protoplasm ultimately dies off, and the conversion of the trophozoites of Thelohania and Gurleya into a single pansporoblast, support this view.

Another point of interest in the life-history of Pleistophora periplanetæ is afforded by the existence of the residuary nuclei,[1] which, together with the protoplasm of the pansporoblast, die off, while sporulation is being effected. In the remaining members of the Cryptocystes and all the Phænocystes, with the exception of the Disporea—also "free" parasites—no such separation of residuary nuclei occur. In the Disporea two residuary nuclei are left behind to die off, one for each spore of the pansporoblast. The meaning of these residuary nuclei is obscure. At first sight it would appear that the residuary nuclei and protoplasm are together homologous with that part of the trophozoite of the Disporea which remains after the separation off of the pansporoblast, but if this is the case, what represents this residuary mass of nuclear material and protoplasm in the remainder of the Oligosporogenea, including Thelohania mülleri, Gurleya, etc., in which the trophozoite is converted bodily into the pansporoblast with no residuum ?

It is significant that in Thelohania mülleri, while the spore in its final form possesses two nuclei just like Pleistophora periplanetæ, these two nuclei increase to four in number while lying in the gut of a new host (Gammarus) before germination. Stempell regards this process as one of nuclear reduction, preliminary to conjugation with the amœboid contents of another spore. In view of the above fact it

[1] See spore formation, p. 621.

seems possible that the residuary nuclei of the pansporoblast of Pleistophora periplanetæ represent reduction bodies, and that in the case of Thelohania mülleri (and possibly other Oligosporogenea) the extrusion of these reduction bodies takes place later on from the spore itself. It is perhaps somewhat premature to attempt any homologisations or explanation of the meaning of these residuary nuclei in the present undeveloped condition of this branch of Protozoan research, more particularly in view of the fact that in no single case has conjugation been recorded in the case of a Myxosporidian, and in no single case has the development of an adult trophozoite from the sporoplasm of a resting spore been traced. In view, however, of the rapidly increasing number of cases in which conjugation has been shown to take place among the Protozoa, e.g. Actinosphærium, it seems very improbable that the Myxosporidia will prove to be an exception to what is being shown to be apparently the rule among even the most unspecialised forms of life. The presence of these residuary nuclei and Stempell's observations on the spore of Thelohania mülleri are certainly suggestive of a preparation for conjugation.

Among the bacteria themselves, a class of organism which has been regarded till lately as in no case exhibiting a conjugatory process among its members, conjugation in a modified form has been shown to take place.[1] The existence of the above-mentioned gap in our knowledge of the Myxosporidian life-history, and the silence of all observers on the subject of conjugation among the Myxosporidia are phenomena probably not unconnected with each other.

Note on a Hitherto undescribed Parasite (?) of Periplaneta orientalis.

In the course of these present researches certain peculiar structures, which occurred in many of the stained prepara-

[1] Schaudinn has recently (1902) shown that a kind of conjugation occurs in Bacillus bütschlii parasitic in the ileum of Periplaneta americana.

tions of the Malpighian tubules, attracted attention. Several of these structures are represented in figs. 21—24, and 37. They present an appearance similar to the trophozoites and spores of Pleistophora periplanetæ upon a smaller scale, and stain in similar manner with the same reagents. The trophozoites (figs. 21—23) are, however, of a more delicate structure than the trophozoites of Pleistophora periplanetæ, the nuclei being much smaller and more compact, while the protoplasm is also more delicate. The spores (figs. 22 and 37) are very much smaller, measuring not more than 1 μ—2 μ in length and about ·5 μ in breadth, instead of 5·5 $\mu \times 3\mu$ as in Pleistophora periplanetæ. No intermediate stages between these forms and Pleistophora periplanetæ have as yet been observed, and I am inclined to think that these structures are the trophozoites and spores of a new Myxosporidian parasite. The structures are, however, in need of further investigation, and pending further research I have decided only to mention their occurrence without giving them a name. They may turn out to belong to the life-cycle of Pleistophora periplanetæ, and indeed little beyond the statement that they are almost undoubtedly organised bodies is permissible, under the circumstances.

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EXPLANATION OF PLATES 37 & 38,

Illustrating Mr. W. S. Perrin's paper on "Observations on the Structure and Life-history of Pleistophora periplanetæ, Lutz and Splendore.

All the figures refer to Pleistophora periplanetæ from the Malpighian tubules and gut of Periplaneta orientalis with the exception of Figs. 21, 22, 23, 24, and 37, which refer to another Myxosporidian parasite from the same locality.

The figures are all drawn with the help of Zeiss's drawing camera from stained preparations. Those upon the left half of the plate are drawn and coloured from preparations stained with Giemsa's eosin-azur. Nuclear structures are in all cases tinged a bright red or purple and protoplasm a bright blue. The remaining figures are drawn from preparations stained with Delafield's hæmatoxylin, with the exception of Fig. 28, which was stained by Heidenhain's iron hæmatoxylin method.

A Zeiss microscope with an apochromatic 3 mm. homogeneous oil immersion objective (mm. ap. 1·40) and compensating eye-pieces 12 and 18 was used in all cases.

Critical illumination with strong artificial light, Welsbach incandescent, etc., was employed.

Magnifications.—Figs. 1—32, 34—37, 50, 51: 1750/1.
Figs. 33, 38—49: 2700/1.

PLATE 37.

Figs. 1 to 8.—Series of trophozoites of increasing size and with nuclei ranging from one to eight in number. Fig. 4 shows a trophozoite with three nuclei, one of which is in process of division. The series illustrates the development of a multinucleated plasmodium from a uninucleated sporozoite.

Fig. 9.—Multinucleated trophozoite with reticular protoplasm and numerous vacuoles. The nuclei are of two kinds, the more numerous variety being

reticulated and coloured a bright red, the less numerous compact and of a purplish tint. In the middle of the trophozoite an aggregation of these purple and probably degenerated nuclei is to be seen. The presence of these nuclei probably indicates approaching sporulation.

Fig. 10.—Another trophozoite of similar character.

Fig. 11.—An example of simple plasmotomy. A trophozoite is in the last stage of binary fission.

Fig. 12.—Small trophozoite showing the early occurrence of the purple nuclei.

Fig. 13.—Large trophozoites undergoing multiple plasmotomy, or, in other words, budding off small multinucleated masses of protoplasm, which will become independent trophozoites. One bud is already detached.

Fig. 14.—Pansporoblast at an early stage of development. Each of the oval sporoblasts appears to be lying in a vacuole. This appearance is produced by the shrinkage which the protoplasm undergoes in preparation. The nuclei of the sporoblasts are seen to be diffuse and reticular. The interstitial or residual nuclei retain in many cases the bright red tint of the normal nucleus. Two can be seen to be purplish. Fig. 30 represents a pansporoblast at a very slightly earlier stage.

Fig. 15.—Nucleus of cell of Malpighian tubule fixed by drying and showing the red colour assumed by nuclei, when stained by Giemsa. The figure should be compared with Fig. 27, which represents a similar nucleus fixed, however, with corrosive sublimate and alcohol, and stained with Delafield's hæmatoxylin.

Fig. 16.—Multiple amitosis; trophozoite about to break up into four sporozoites.

Fig. 17.—Similar figure, but nuclei more numerous.

Fig. 18.—Later stages of same process.

Fig. 19.—Group of sporozoites just after division.

Fig. 20.—Large trophozoite budding off unenucleated spores.

Fig. 21.—Trophozoite of hitherto undescribed Myxosporidian with the same habitat as Pleistophora periplanetæ. The nuclei are compact and much smaller than the nuclei of the Pleistophora.

Fig. 22.—Trophozoite and group of spores of the same Myxosporidian. Several of the nuclei undergoing binary fission.

Fig. 23.—Trophozoite of same.

Fig. 24.—Trophozoite of same, the nuclei being reticulated and surrounded by a vacuole.

Figs. 50 and 51.—Small trophozoites dividing into two.

PLATE 38.

Fig. 25.—Trophozoite of Pleistophora periplanetæ showing elongated non-nucleated pseudopodium of ectoplasm. Sublimate alcohol and Delafield.

Fig. 26.—Trophozoite giving off bud.

Fig. 27.—Nucleus of cell of Malpighian tubule, fixed with hot sublimate alcohol, stained Delafield. This figure, with Fig. 15, is inserted for the purpose of indicating the size of Pleistophora periplanetæ—the nucleus measures 16 μ in diameter—and showing the drastic effects of fixation by drying on such a spherical body as a tissue nucleus (see Fig. 15 as compared with Fig. 27). The leaf-like trophozoites are, upon the contrary, well preserved by this method of fixation.

Fig. 28.—Trophozoite fixed with osmic acid vapour and deeply stained with iron hæmatoxylin.

Fig. 29.—Trophozoite showing the mucoid masses which occasionally occur in the trophozoites.

Fig. 30.—Pansporoblast with sporoblasts containing a nucleus in the form of scattered dots or a reticulum. Stage is slightly antecedent to Fig. 14. The residual nuclei are seen as deeply stained masses between the sporoblasts.

Fig. 31.—Pansporoblast at a later stage. The nuclei of the sporoblasts are now in the form of a sphere containiug a central particle.

Fig. 32.—Still later stage. The nuclei are still more condensed and the spore coats are beginning to be formed.

Fig. 33.—Small pansporoblast with four spores. Stage much later than in Fig. 32. A thick spore coat is present and two nuclei, one each side of the central vacuole, are to be seen. Magnification 2700/1.

Fig. 34.—Trophozoite in an early stage of multiple plasmotomy.

Fig. 35.—Later stage in same process. One bud is just free.

Fig. 36.—Multiple amitosis; see also Figs. 16—19.

Fig. 37.—Spores of hitherto undescribed Myxosporidian fixed with sublimate alcohol and stained Delafield's hæmatoxylin.

Figs. 38 to 47.—Series of figures showing the changes in the nucleus in the development of the spore from the sporoblast.

Fig. 38.—Nucleus of the sporoblast as a number of discrete particles.

Fig. 39.—Nucleus somewhat more condensed than in Fig. 38. Particles fewer and larger.

Fig. 40.—Nucleus of spore in the form of a ring of four chromosomes.

Fig. 41.—Similar stage. A small vacuole is to be seen in the sporoplasm.

Fig. 42.—Later stage. Vacuole increased in size.

Fig. 43.—Vacuole occupying nearly the whole of the length of the spore. Nucleus situated at one end.

Fig. 44.—Nucleus divided into two.

Figs. 45, 46, and 47.—Showing change in position of two daughter nuclei from end of spore to its middle. The vacuole has increased in its refractive power. Fig. 47 represents the latest stage of the spore to stain satisfactorily. The two nuclei show indications of division.

Fig. 48.—Spore in about the same condition as in Fig. 47, seen from the side.

Fig. 49.—Ripe spore showing the suture between the two halves of the shell or spore coat.

For Figs. 50 and 51 see Plate 37.

A Study of the Life-history of Bucephalus Haimeanus; a Parasite of the Oyster.

By

David Hilt Tennent.

With Plates 39—42.

Introduction.

Among a number of oysters which I procured in February, 1902, for the purpose of a demonstration, were several which were badly infected with the cercaria Bucephalus. At the suggestion of Professor Brooks I undertook a study of the origin of the germ-cells, from which the cercariæ arise, the material at my command promising to be favourable for such an investigation.

Note.—Since I began my studies on the origin of the germ-cells in Bucephalus haimeanus two papers on the process in related forms have appeared. I refer to those of Reuss (**41**, 1903) on Distomum duplicatum, and of Haswell (**23**, 1903) on Echinostomum sp. Further mention of these papers will be made at another place in this paper.

Economic considerations have also made a study of the life history of the parasite of importance. Exact data as to the extent of infection, its means of spreading, the effect of infection upon the oyster, etc., have been desired. In my investigations I have endeavoured to determine not only facts of a purely scientific interest, but also those which will be of use to the oyster planter.

From this rather limited field my research gradually broadened until it included not only the study of the germ-cells, but also that of the older cercaria stages, as well as investigations along various lines for the purpose of determining the complete life history of the form under consideration.

This work, of which the present paper is an account, has been carried on in the Zoological Laboratory of the Johns Hopkins University, and in the Fisheries Laboratory at Beaufort, North Carolina.

It is with pleasure that I acknowledge my indebtedness to the Honourable George M. Bowers, United States Commissioner of Fish and Fisheries, for the opportunity of enjoying the advantages of the Beaufort Laboratory, and to Dr. Caswell Grave, Director of the Laboratory, by whom many courtesies and privileges have been extended. To Mr. R. E. Coker I am indebted for material from Pamlico Sound, and to Mr. O. C. Glaser for infected oysters from Cameron, Louisiana.

To Professor Brooks I express my most hearty appreciation of the unfailing interest, and of the many kindnesses which have characterised his direction of my work.

Material and Methods.

The abundance of infected oysters at Beaufort provided the opportunity of making extended observations on living material. That this has been done will be apparent from the account of my work.

For the continuation of my work in Baltimore, recourse was had to preserved material. In my work on 'Bucephalus' I made use of many fixing agents. Corrosive sublimate solution, either with or without the addition of acetic acid, picric acid liquids, and Perenyi's fluid gave extremely poor results. Tissues placed in Gilson's mixture for two hours were excellently fixed and gave very useful preparations. By far the best fixation, however, was obtained by the use of

Flemming's chromo-aceto-osmic mixture (weaker formula). Tissues were allowed to remain in this reagent for twenty-four hours and were then removed and washed in running water for twenty-four hours.

In my study of Gasterostomum I have tried but three fixing agents: acetic sublimate solution, Gilson's mixture, and cold saturated aqueous solution of corrosive sublimate warmed to 35° C. The material preserved by the use of the last-named reagent has been satisfactory.

For staining I have used borax carmine and acid borax carmine, both of which gave fairly good results; Delafield's hæmatoxylin and eosin, which were useful; gentian violet, which was unsatisfactory; Flemming's triple stain (safranin, gentian violet and orange G.), which was excellent in the study of the germ-cells, and Heidenhain's iron hæmatoxylin, with eosin as a counter stain. By far the best results were obtained by the use of this method.

The material was imbedded in paraffin and cut in one, two, and three micron sections.

In studying the nervous system I used methylene blue as an intra-vitam stain, by means of which I was able to make out the topography of the nervous system as well as the scattered ganglion cells.

For the study of the nervous system in preserved material I found Heidenhain's iron hæmatoxylin with a counter stain of eosin in 95 per cent. alcohol most useful. Several chloride of gold methods were tried, none of which proved satisfactory.

Historical Account of Gasterostomum.

Von Siebold (**1**, 1848) discovered and named Gasterostomum fimbriatum, which he found in the intestine of Perca and Lucioperca.

Wagener (**2**, 1852) described this species, giving also descriptions and figures of two new species, G. minimum, from Trigla microlepidota, and G. gracilescens =

Distomum gracilescens, Rud. (Synopsis, p. 409), from Lophius piscatorius. (Gasterostomum rather than Distomum because the mouth is situated in the ventral sucking disc.)

In 1857 Wagener (**3**) gave figures of an old and two young specimens of G. fimbriatum.

Wedl (**4**, 1857) redescribed and figured Distoma campanula, Dujardin ('Histoire naturelle des Helminthes,' p. 435), from intestinal slime of Esox.

Wagener (**5**, 1858) recognised the identity of this form with G. fimbriatum, v. Siebold.

In this paper (1858) he again discusses the three species then known.

1. G. minimum, Wagener, from intestine Triglæ microlipidotæ.

2. G. gracilescens, Wagener, from intestine Lophii piscatorii = Distoma gracilescens, Rud.

3. G. fimbriatum, v. Siebold = Distoma campanula, Dujardin, from intestine Esocis lucii, Percæ fluviatilis, Luciopercæ Sandræ; encysted forms on gills of Cyprinus, showing the points in which they agree, and calling attention to the fact that the first two are from the Mediterranean where B. haimeanus is found, while the last is a fresh-water form, as is B. polymorphus.

As a means of distinguishing the species he proposes making use of the difference in the direction of the gut, and the relative size of the sucking disc, and of the eggs.

Diesing (**6**, 1858) in a systematic paper separates the three forms above mentioned, retaining the genus Gasterostomum for the species fimbriatum, and creating a new genus, Rhipidocotyle, for the species gracilescens and minimum.

1. Rhipidocotyle gracilescens = Distoma gracilescens, Rud. = G. gracilescens, Wag.

2. R. minimum = G. minimum. Wag.

The complex and peculiar structure of the anterior end of G. fimbriatum, which was described by Wagener (1857),

seemed to him sufficient reason for the formation of a new genus.

Ziegler (**7**, 1883, p. 549) shows the error in Wagener's interpretation.

Diesing also confuses the posterior with the anterior end in the three species named.

Molin (**8**, 1858) describes a new species, G. armatum from Conger conger.

Gervais and Beneden, 1858 ('Zool. Med. II,' p. 207), describe G. crucibulum = Monostomum crucibulum, Rud.

Cobbold (**9**, 1858) gives a careful description and figures of G. gracilescens, which add little to the knowledge of that form.

Diesing (**10**, 1859), in a revision of his classification, accepts three species of Gasterostomum:

1. G. fimbriatum, v. Siebold.
2. G. armatum, Molin.
3. G. crucibulum, Gerv. and Ben. = M. crucibulum, Rud.

Molin (**11**, 1861) gives a complete description and figures of his G. armatum, describing a cornucopiæform acetabulum and distinct genital apertures.

Olsson (**12**, 1867) redescribes G. armatum, Mol., and G. gracilescens, Rud.

Maddox (**46**, 1867) found what seems to have been young G. gracilescens encysted in the nerves of the haddock.

He describes the vitellaria (sixteen on each side) and flame-cells, which he calls pulsating bodies, connected with the water vascular system.

Van Beneden (**13**, 1871) gives as new species:

G. viperæ—intestine of Trachinus vipera, Cuv.
„ triglæ— „ Trigla gurnardis, L.
„ clupeæ— „ Clupea sprattus.
„ vivæ— „ Trachinus draco, L.
„ nova, sp. „ Cyclopterus lumpus, L.

Of these new species he gives figures of two, G. triglæ

and G. viperæ, as well as giving new figures of G. gracilescens and G. crucibulum.

Olsson (**14**, 1875) redescribes:

1. G. gracilescens, Rud.; embryonic forms from brain of Gadus minutus, adult from Lophius piscatorius.

2. G. armatum, Molin, correcting Molin's error with regard to the genital aperture.

3. G. crucibulum, Rud.

= Monostomum crucibulum, Rud.
= Distoma crucibulum, Dujardin.
= Gasterostoma crucibulum, Diesing.

and gives a figure of the last-named form.

Rud. v. Willemoes Suhm (**15**, 1873) expresses the opinion that G. crucibulum = G. armatum. He gives figures of the embryos of these forms, and thinks that Bucephalus haimeanus is a stage in the life history.

Levinsen (**16**, 1881) redescribes G. armatum, confirming Olsson (**13**) in his observation as to the single genital aperture.

Linton (**17**, 1899) describes and figures:

1. G. ovatum, Lt. ("Monostomum orbiculare," Rud. Lt. in 'Proc. U.S. Nat. Mus.,' Vol. xx, p. 541) from Lobotes surinamensis.

2. G. arcuatum, Lt., from Sarda sarda and Carcharinus obscurus.

3. Gasterostomum sp. from Scomberomorus maculatus.

4. Gasterostomum sp. from Tylosurus marinus.

The form which is to be considered in the present paper is that designated by Linton (**17**) as Gasterostomum sp. from Tylosurus marinus.

For reasons which can best be discussed in a later section of this paper, I shall tentatively consider the form as G. gracilescens, Wagener.

Historical Account of Bucephalus.

Several varieties of Bucephalus, the larval form of species of Gasterostoma, are known.

B. polymorphus, von Baer (**18**, 1827), from Anodonta mutabilis, var. Anatina and Cellensis, and from Unio pictorum.

B. intermedius, Ulicny (**19**, 1878), from Anodonta cellensis.

B. haimeanus, Lacaze-Duthiers (**20**, 1854), from Ostrea edulis and Cardium rusticum.

B. crux, Levinsen (**16**, 1881) from Modiolaria discors.

B. cuculus, McCrady (**21**, 1874), from oysters at Charleston, South Carolina.

Cercaria bucephalus, Ercolani (**22**, 1880), from Unio and Anodonta.

Bucephalus von Baer, Haswell (**23**, 1903), from Mytilus latus.

Baer (**18**) describes and figures the thread-like germ-tubes and the cercariæ as they appeared under low magnification. He thinks it probable that the germ-tubes were propagated by sprouting ("Sprossen") and also through the development of young tubes from the tails of the cercariæ.

Siebold (**1**) recognises that the gut is simple, and sees that the mouth opening is upon the middle of the ventral side of the body. He is of the opinion that Bucephalus is related to Gasterostomum.

Wagener (**24**, 1858), from the anatomical resemblance, thinks it probable that B. polymorphus becomes G. fimbriatum. He also describes (**3**, 1857) a bladder-like structure ·01 mm. long from which are given off two slender tubes, which, both together, are one fifth of a mm. long. The bladder contained cells; the tubes were empty. This structure, I take it, may have been a rather young sporocyst. Pagenstecher (**25**, 1857, and **26**, 1863) found Bucephalus in Anodonta anatina. He describes and figures the animal

as having a paired digestion tract, an error which leads Diesing (**27**, 1858) to doubt Siebold's correct interpretation and to form a new genus (Bucephalopsis), to which he assigns Lacaze-Duthiers' B. haimeanus.

Lacaze-Duthiers (**20**) describes B. haimeanus and figures the sporocysts and cercaria. According to his description the mouth is at the anterior end of the body, and a narrow œsophageal tube passes backward to the gut. He describes the unhealthy appearance of infected oysters, the gonads in such oysters having shrunken in size, and the tissues having become transparent and watery in appearance.

Claparède (**28**, 1863) obtained B. haimeanus while towing in the open sea at Saint Vaast la Hougue. He also found it attached to the under side of Sarsia and Oceania.

Huet (**29**, 1889) found B. haimeanus in Cardium edule. He confirms Lacaze-Duthiers' observations as to the unhealthy appearance of infected specimens, but cannot find the mouth and œsophagus described by Duthiers. He says that B. haimeanus seems to cause the death of the host, and then escapes into the surrounding water.

McCrady (**21**), at Charleston, in July, 1868, found several oysters in which the gonads were filled with a mass of white fibres, the germ tubes of a Bucephalus. Concerning these fibres McCrady says: "The impression left upon me by the distinct resistance to the knife was that they would prove chitinous." Consequently, on account of this rigidity of the tubes, he describes the cercaria which he found as a new species, B. cuculus. He mentions the tail as bristling with cells like lasso cells. He has "no recollection" of a ventral aperture or sucker, and saw none of the regularity of construction of the body such as was observed by Lacaze-Duthiers.

In infected oysters the gonad was covered by a transparent skin, through which the branching tubes might be seen. Parasites were found only in the gonads, and in infected oysters there was no trace of spermatozoa or eggs.

He hazards the opinion that "In all probability the oyster is freed from its intruding guest before the winter sets in."

Giard (**30**, 1874) found in the viscera of Belone vulgaris, especially in the liver, genital glands, and peritoneum, many little cysts which contained untransformed B. haimeanus. He thinks it probable that B. haimeanus becomes transformed into some species of Gasterostomum in the intestine of some large fish which eats Belone, possibly the shark.

Badcock (**31**, 1875) describes the swimming of B. polymorphus as "flying like eagles through the water with a general upwards tendency."

Stewart (**32**, 1875) describes the pavement-like appearance of the surface of B. polymorphus. He describes the musculature of the body, and thinks that the three-lipped invagination at the point of the body is the mouth and the complex of glands is the pharynx.

Ulicny (**19**) describes B. intermedius from Anodonta cellensis, which differs from B. polymorphus chiefly in the fact that the median portion of the tail is more deeply constricted and lengthened at the sides, "der bisquitformige Anschwellung des B. polymorphus" being differentiated into two independent bodies.

Ercolani (**22**) describes Cercaria bucephalus, which differs from B. polymorphus in having a divided intestine and the mouth opening in the anterior end. He believes that the tails form sporocysts. It is quite possible that he has fallen into Pagenstecher's errors in both instances.

Levinsen (**16**) figures the transformation of the germ-balls into B. crux, which, in its older stages, is very different in appearance from the other forms of Bucephalus which have been described.

Ziegler (**7**) gives a most accurate account of the structure of B. polymorphus and of G. fimbriatum, together with an account of the biology of the animal. From the anatomical resemblance he concludes B. polymorphus to be a larval form of G. fimbriatum.

Haswell (**23**) describes and figures a Bucephalus from sporocysts in which the germinal epithelium contained a red colouring matter. In other respects there seems to be little difference between this and B. haimeanus.

Observations on B. haimeanus, Lacaze Duthiers = B. cuculus, McCrady.

The form upon which my observations have been made is presumably that described by McCrady (**21**) as B. cuculus. McCrady's account is not fully satisfactory as it seems to have been written some time after the observations were made and then from insufficient notes.

In the examination of a large number of oysters one finds them in many stages of infection. For convenience of description we may consider them under four stages.

I. Individuals in which infection is recent. Examination with a lens magnifying about ten diameters or more, or examinations with the naked eye reveals little irregular patches, somewhat whiter in colour than the surrounding tissue, lying just beneath the outer covering of the reproductive glands. Upon tearing the oyster tissue with needles similar patches may be found within the substance of the gonad. These patches are comparatively young sporocysts. They consist of a central bladder-like body from which short branches are given off (figs. 2 and 3).

They may readily be separated from the surrounding tissue, and examination with a higher magnification will show that there are opaque masses of cells, the germ-balls, within the cavity of the central body.

II. In more advanced stages of infection the branches of the sporocyst have grown in length perceptibly and have become "germ-tubes" (fig. 4), which occupy intercellular spaces within the host (fig. 14). The number of germ-balls has increased greatly and various stages of development are seen, some of the germ-balls having given rise to well-developed cercariæ.

III. In still more advanced stages nearly the whole substance of the oyster has been replaced by a mass of white fibres—the germ-tubes. At this stage the oyster may still appear plump and healthy, a rather chalky whiteness being the only difference in appearance between an infected and a "fat" uninfected oyster. So tightly is the visceral mass packed with the branching germ-tubes that a slight cut in its surface results in the bursting out of a considerable mass of tubes. Upon dissecting the oyster with needles it is seen that the germ-tubes not only penetrate the gonads but fairly riddle the liver and stretch out into the gills, the palps, and even into the walls of the pericardium.

The germ-tubes at this stage contain embryos in stages of development from a single cell to cercariæ which are about to be set free.

IV. In the last stages the tissues of the oysters seem almost completely wasted away. No trace of the gonads is to be seen, while the liver persists as a rounded mass about the size of a pea. Upon tearing the outer covering of the body germ-tubes may be drawn out; they have lost their whitish appearance, have become transparent, seeming, as one draws them out, much like viscid strands. Within their lumens are found only a few partially-developed cercariæ or germ-balls.

I have examined oysters during every month of the year, and on every occasion have found infected specimens. The growth of the parasite seems, however, to be most rapid in June. My observations lead me to believe that infection takes place during the summer months, from June to October.

At the time that McCrady made his observations he was unaware of those of von Baer and Lacaze-Duthiers. Upon gaining access to these later he was unable to correlate the form which he found with either of the then known varieties. Though he did not see anything which might have been taken for a mouth, he admits that there may have been one.

Because of the rigidity of the tubes which, as has been already noted, he described, the lack of the striation which Lacaze-Duthiers observed and some minor differences, he

believed B. cuculus to be more in accord with the observations of von Baer and Siebold on B. polymorphus than with those of Lacaze-Duthiers on B. haimeanus. In this opinion he was, it seems to me, to a great extent, justified. But with the aid of the additional light which Huet (**29**) has shed upon B. haimeanus, and from my own observations I am convinced that, as Braun (**33**) has already suggested, B. cuculus and B. haimeanus are identical.

The rigidity of the tubes which McCrady described, I have never been able to observe; on the contrary, I have found the tubes to be soft and yielding.

Lacaze-Duthiers called attention to the differences between B. haimeanus and B. polymorphus. After a consideration of these differences with Huet's modification of Lacaze-Duthiers' description, it is readily seen that, aside from the fact that the one is a marine and the other a fresh-water form, the chief difference is that one has long and the other short tails. Since the date of Lacaze-Duthiers' observations (1854), when von Baer's figures and descriptions (1828) were his chief source of information concerning B. polymorphus, Ziegler's most excellent account of this form has appeared (1883).

With the aid of the further data at my command it at once becomes apparent to me that the only difference in structure in the two forms is that of the shape of the tails, and this difference is most evident only when the tails are in an extended condition.

The shape and structure of the body with the tails removed is identical.

Observations on the Free Swimming of B. haimeanus.

The most abundant source of supply for cercariæ of all stages has been the sporocysts which occur within the oyster. Free swimming stages may also be obtained, though never in abundance, by towing with a fine-meshed net in the vicinity of the oyster beds. I have also obtained them by placing oysters, which I suspected were infected, in aquaria, when

small numbers of mature cercariæ were forced out in the stream of water from the exhalent siphon. These cercariæ swam actively for about two hours and then sank to the bottom.

By the addition of diatoms the water was kept fresh, and the cercariæ thus kept under observation for several days.

During this time the tails were broken off, but the cercariæ were still capable of making slow progress on the bottom of the dish by means of contractions of the body.

At the end of four days all were dead. None encysted themselves, and in none were any changes of structure to be seen.

The Sporocyst.

The youngest sporocysts that I have been able to identify positively as such, consist of a central, irregular, sac-like body from which one or more cylindrical branches are given off (figs. 2 and 3), the structure being not unlike the sporocysts of Leucochloridum which Heckert (**39**, 1889) has described. The cylindrical branches are the germ-tubes, and it is from these and from similar new branches of the central body that the great mass of fibres found in the badly-infected oyster is derived. The tubes grow to a length of several centimetres, repeatedly branching throughout their course. In branching many strange effects are produced, to some of which may be due Pagenstecher's (**25**) idea that the tails of the cercariæ give rise to new germ-tubes. Fig. 5 shows an appearance which at first sight might give such an impression. A further discussion of this point will be given later.

The germ-tubes are usually of a rather uniform diameter (0·1 mm.; fig. 4). In some instances portions of the tube become enlarged and form conspicuous sac-like swellings in which a considerable number of cercariæ may be found (fig. 3).

The growing end of the tube is rounded and of a somewhat greater diameter than the older portion (fig. 4).

Throughout its extent the lumen of the tube contains cercariæ in various stages of development.

Sections (figs. 7, 8, 17,) show that the tubes have a thin homogeneous cuticle beneath which lies a layer of circular and a layer of longitudinal muscle-fibres. In many cases sharp constrictions appear in the tubes; these, I believe, are caused by contraction of the circular muscles. I have never been able to see any indication of movement, other than this, in the sporocyst.

The wall of the tube in its younger portions (figs. 7 and 17) is about 0·002 mm. thick, the thickness varying greatly with the age of the tube. In this younger portion the limits of the cells which compose the wall are fairly well defined. These cells have a uniform appearance, the finely granular cytoplasm staining much more lightly than the vesicular nuclei with their scattered nuclear material (fig. 7).

In slightly older tubes the original definite cell boundaries have disappeared and the differentiation of germ-cells has commenced (figs. 6 and 17). Within the lumen of the tubes undivided germ-cells and germ-balls may be seen.

In the oldest tubes (fig. 8) the walls have decreased in thickness, cell boundaries have disappeared completely, the muscles may no longer be recognised, the differentiation of germ-cells and their subsequent endogenous proliferation has ceased, and only a few scattered nuclei remain.

The germ-tubes in their earlier growth push their way in the intercellular spaces in the tissues of the host (fig. 14). Later, an actual destruction of these tissues seems to take place, a definite zone of apparently disintegrated cells (Paletot?) lying between the wall of the tube and the unaffected cells (fig. 6). In whatever manner it is accomplished the cells composing the tissues of the host ultimately disappear in great part, only a few shrunken strands remaining. In the reproductive organs the formation of the sexual products is practically completely inhibited. While a few eggs or sperms may be present their appearance is abnormal.

At the beginning of this section I spoke of the youngest

sporocysts that I have been able to positively identify as such. In many oysters I found in the palps and gonads and entangled in the gills little cysts, which, when examined alive, seemed to consist of a simple sac enclosing a few cells. Until I had sectioned and stained some of these bodies I felt inclined to consider them as very young sporocysts, only shortly removed from a miracidium-like larva. Their appearance in sectioned material causes me to doubt this interpretation (fig. 12).

The Origin of the Germ-cells.

The origin of the germ-cells, from which the larval forms Trematodes arise, has been discussed at considerable length by various investigators. Leuckart (**34**, 1882) and Schwartz (**35**, 1886) trace them directly to the egg. Wagener (**36**, 1866) and Biehringer (**37**, 1884) describe their origin from the body wall. Thomas (**38**, 1883) thinks that the germ-cells may originate in either or both of these ways.

In B. haimeanus there is little doubt that the germ-cells arise in the wall of the sporocyst.

Figs. 6, 7, and 17 illustrate conditions frequently seen in the younger portion of the germ-tubes. In the growing end of the tube (fig. 7) the outlines of the cells which compose the walls are easily seen. These cells have lightly staining rather granular cytoplasm and a vesicular nucleus with scattered deeply staining nucleoli.

In the older portions of the tube the original cell boundaries have disappeared. The cytoplasm of these cells has apparently collected itself in dense, granular, rounded masses around the nuclei, the resulting structures, as their subsequent fate shows, being the germ-cells (fig. 7, *g. c.*) Scattered about within the wall are a few nuclei, which have retained their original appearance.

The germ-cells now pass from their place of formation into the lumen of the tube. In the great majority of cases, so far as I have been able to determine, there is no division

whatever of the germ-cell until it has passed from the wall into the lumen. In a few instances I have noticed that a small cell had been cut off from the germ-cell as it was entering the cavity of the tube (fig. 15, *a*, and 16).

Within the wall at this time there are also many small, rather transparent, yellowish bodies. These, after fixation with Flemming's solution, become extremely black. I believe them to be fat droplets.

The "Keimlager."

Besides the method of forming the germ-cells which is above described, another often occurs. This takes place towards the close of the first method. In this second method, instead of the scattered production of germ-cells, there is a localisation of production; a definite portion of the wall of the sporocyst, usually at one end of the tube, grows into the lumen as a blunt process (fig. 22), which functions as a special organ for the production of germs. In these processes the germ-cells, which are there differentiated, undergo their stages of segmentation and reach the condition of a germ-ball of considerable size, when they burst the tissue enclosing them and come to lie in the lumen of the tube where their further development takes place.

This process has been described by Heckert (**39**, 1889) for Leucochloridium, by Looss (**40**, 1885) for Amphistomum subclavatum, and also by more recent investigators.

The germ-cell, as it lies in the lumen of the tube, is a spherical body about 0·005 mm. in diameter, with a nucleus 0·003 mm. in diameter. The cytoplasm is dense, finely granular, and takes the stain easily. The nucleus takes an even, darker stain, while its round nucleolus stains black.

The first appearance of activity in the germ-cell itself is the cutting off of a small cell (fig. 15, *b*). (As has been already noted, this may take place within the wall.) This cell differs in appearance from the much larger germ-cell in that its

nuclear contents are more coarsely granular. These contents in a later stage are seen gathered together in two masses, the large nucleus of the germ-cell having, in the meantime, moved to the opposite periphery of the cell. In stages which I believe to be still older, a second small cell has made its appearance, and still later there are three, two of which seem to be the result of division in the first cell (fig. 15, *c*).

Reuss (**41**, 1903) has seen a similar phenomenon in the sporocysts of Distomum duplicatum, and has interpreted these small cells as being of the nature of polar bodies. Haswell (**23**, 1903), describing germ-cells of Echinostomum sp., has noticed in the vicinity of the ovary (Keimlager) a number of cells which have deeply staining homogeneous nuclei, although he has never seen a stage similar to that described by Reuss or by me. In this connection Haswell says: "If these are not of the nature of polar bodies, it seems difficult to account for them."

The Segmentation of the Germ-Cell.

The subsequent behaviour of the germ cell is similar to that of a segmenting egg.

It has been impossible to observe the actual processes of segmentation in living material, the opacity of the wall of the sporocyst rendering observations unsatisfactory. In entire mounts of preserved material and in sections the selection of succeeding stages depends only upon patient search.

The division of the cells is mitotic. Although it has been impossible to make out the process of spindle formation in living material, and although sections but rarely indicate such a process, the evidence furnished by these exceptional instances seems clear (fig. 21).

The germ-cell in its first division is cut into two cells of nearly equal size, one being somewhat larger than the other (fig. 15, *c*). In the second division the smaller of these two

cells divides, giving a three-cell stage in which the two smaller cells lie across the larger (fig. 15, *d*). In the five-cell stage (third division) the smaller cells have again divided, and lie somewhat irregularly about the large cell (fig. 15, *f*).

In the seven-cell stage (fig. 15, *g*) two of the smaller cells (found in the third division) have again divided, and two of these cells have taken a position on the surface of the irregular clump of cells. These two cells flatten, the flattened edges growing out and forming a membrane which encloses the young germ ball. These flattened cells apparently divide again at least once, since in a series of sections it is possible to find four nuclei lying at various points in the membrane. These nuclei ultimately disappear, leaving the thin membrane which persists during the entire development of the cercaria. I have never observed any indication of the throwing off of this membrane. It seems to form the basement membrane lying below the cuticle in the mature cercaria.

Up to this time (seven-cell stage) cell outlines in the developing germ-ball are fairly distinct. The amount of cytoplasm as compared to the size of the nucleus is small, but still a thin layer may be seen about each nucleus. In further stages of the developing germ-ball I have been unable to observe cell outlines, this structure appearing in the nature of a syncytium with an ever increasing number of nuclei.

This spherical mass of slightly separated nuclei, the germ-ball, reaches a diameter of about 0·02 mm., when it begins to elongate. Meantime a group of nuclei has appeared toward one side of the germ-ball from which has developed the pharynx, which does not, however, reach its permanent form until considerably later. A group of nuclei in the region just above the pharynx gives rise to the gut. This group at first forms a rather compact mass, which subsequently obtains a lumen (fig. 13, *gt.*).

After reaching this stage the germ-ball, as already noted, begins to elongate. From one end, which we are now able to recognise as the posterior end, two processes, the rudi-

ments of the tails, are pushed out (fig. 9, *tr.*). Their further development consists chiefly in an elongation, during the process of which the middle piece, which is formed from the basal portion of each branch, assumes definite form (fig. 13, *tr.*).

The remaining organs of the mature cercaria, which, with the exception of the nervous system, of the gut, and of the water vascular system are extremely rudimentary, are meantime being gradually differentiated from the general syncytium which composes the body of the larva (fig. 13).

This appearance can best be described under the section immediately following which deals with the structure of the older cercaria.

Before proceeding to this section I wish to speak of the the recent investigations of Reuss (**41**, 1903) on the development of the cercaria and sporocysts of Distomum duplicatum, and of those of Haswell (**23**, 1903) on the sporocysts of an Echinostomum.

Both of these papers reached me after the completion of my investigations on the sporocysts and cercaria in Bucephalus. It gives me pleasure to note that my observations confirm those of Reuss in every respect. With the observations of Haswell they are also in accord in nearly every particular.

The Anatomy of B. haimeanus.

The body of Bucephalus (fig. 1) is somewhat lance-shaped, varying in length in various individuals from 0·15 mm. to 0·20 mm., and in breadth from 0·04 mm. to 0·08 mm.; the thickness is but slightly less than the breadth.

The anterior end of the body is slightly pointed, terminating in a three-lipped invagination which is described as the mouth by the older investigators. The posterior end of the body terminates bluntly and passes abruptly into the tail, which consists of a median portion and two lateral appendages.

The body continually changes in shape, stretching out so that it becomes long and narrow, or suddenly contracting so that it is but slightly longer than broad. The tails, when fully extended, may reach a length of from five to ten mm.; when contracted they are about the length of the body.

As seen in transmitted light, the body is nearly colorless, and, with the exception of opaque masses of cells which are the rudiments of various organs, is perfectly transparent.

Through the clear integument may be seen the contractile sac of the water-vascular system, the pharynx and gut, the rudiments of the penis sheath and the testes, the cystogenous organ, and many unicellular glands (giant cells, sunken epithelial cells) (fig. 1).

In swimming the posterior end of the body is directed forwards, the tails rapidly lashing from above downwards.

When the creature is examined under a cover-glass the tails are moved slowly back and forth or twisted into intricate knots, ultimately being thrown off. With the loss of the tails, however, locomotion does not cease. By means of peristaltic contractions, passing from behind forwards, the creature is able to creep slowly forward. During these motions the anterior end is pushed out and broadened so that it assumes a disc-like appearance, while the cystogenous organ is pushed forward and constricted.

The Cuticle.

The entire animal is covered by a transparent cuticle ("Hautschicht," of the older German investigators) from 0·002 mm. to 0·0025 mm. in thickness, which in surface views appears to be divided into rectangular areas of uniform size (fig. 31). This appearance has been described in B. polymorphus by Stewart (**32**) and by Ziegler (**7**). It is to this peculiar pavement-like structure of the cuticle that the striated appearance of the body, described by several observers, is due. The mosaic effect is caused, I believe, by the growing spines which are arranged in rows parallel to

the long axis of the body, the crease or depression between each row causing the lines which give the impression of longitudinal striation, while the cross-lines, which are not continuous (fig. 31), and which are heavier, i. e. of greater breadth, are caused by the spines themselves.

The arrangement of the muscle-fibres immediately below the cuticle may add to the effect.

I am led to the view above expressed by the appearance in the most mature cercariæ that I have obtained. In these the longitudinal striation had disappeared, while the cross striation, which was still noticeable, was plainly seen to be due to the spines, whose ends projected above the general level of the cuticle.

I have never seen nuclei in the cuticle. Ziegler (**7**) has often been quoted by investigators desirous of obtaining support for one of the views of the nature of the outer covering of Trematodes, as describing nuclei in the Hautschicht of B. polymorphus. On p. 543 (loc. cit.) he says that he has seen nuclei in only one section, a section which passed tangentially through the integument. Later in his paper (p. 547) he speaks of the possibility of error in the observations of investigators who have described nuclei in the cuticle.

A comparison of Ziegler's figure with my own tangential sections convinces me that his section really passed so deeply as to include some of the nuclei of the parenchymatous tissue, lying beneath the cuticle.

It is not my purpose to review the literature bearing upon the much disputed question of the nature of the Hautschicht or investing membrane or cuticle of Trematodes. Such reviews may be found in nearly all the German publications on the subject. A most excellent and recent synopsis may be found in Maclaren's (**42**) paper.

Suffice it to say that there have been three major views as to the nature of this outer layer. They may be classified as follows:

1. It is a metamorphosed cellular epidermis.

2. It represents a basement membrane, the true epidermis and cuticle having been thrown off.

3. It is a true cuticle, i. e. the product of an epidermis.

There have been some minor views, which may readily be referred to one or the other of these classes.

According to the originators and supporters of the third explanation (Blochmann, **43**, 1897) (Kowalevski, **44**, 1895), and most of the recent workers, the epidermis is represented by deeply lying gland-cells, whose ducts traverse the muscle layers and the basement membrane and pour their secretion upon the surface of the latter.

While the form which I have studied is small and perhaps would not be chosen for an investigation of the cuticle, it has served most admirably for the purpose.

The large cells (giant cells) visible through the integument in living specimens claimed my attention early, and from their appearance gave the impression of being gland-cells. This was especially true of two lying in the anterior end, one on each side of the cystogenous organ, whose ducts were visible.

These cells are from 0·02 mm. to 0·025 mm. long and from ·01 mm. to ·015 mm. broad. In sectioned material they are seen to have finely granular cytoplasm, a prominent nucleus, and deeply-staining nucleoli. In some instances a duct passing outward to the sub-cuticular layer may be seen; in other examples the cells lie closely applied to the muscle-layers.

For a time I was at a loss as to whether these were to be considered as gland-cells or whether they were myoblasts, such as are described by Bettendorf (**45**, 1897).

A study of the embryonic stages convinced me that they were gland-cells. In the young stages of the cercaria they make their appearance after the appearance of the muscle-fibrils and before the formation of the cuticle.

An interesting staining reaction served to convince me still further that these are glands which give rise to the cuticle. In a series of sections which were overstained with

hæmatoxylin, these cells, in cercariæ in which the cuticle had not yet formed, were stained a very deep blue. In other cercariæ (on the same slide) in which the cuticle was present, the cells were stained only slightly, while the cuticle had taken the stain deeply; the reaction of the substance within the cell toward the stain in the one case, and of the cuticle in the other, being identical.

By reason, therefore, of all the evidence which I have been able to gather from my observations on B. haimeanus I have been led to believe that the outer body layer, in this case, is a secretion of the more or less deeply lying gland cells.

Although working upon a form very similar to that upon which Ziegler based his belief that the Hautschicht represented a metamorphosed cellular epidermis, I have reached a different conclusion. The more precise modern technical methods probably account for this.

The only cellular body covering present at any time is that which I have already described as being formed at the seven-cell stage. This enveloping membrane increases in size as the embryo grows, always being closely applied to the surface of the syncitium which forms the body of the larva. At no time in the development or in the later history is a definite, complete epidermis to be seen. The nuclei of the parenchymatous tissue may form a more or less regular row, between which cytoplasm may be demonstrated, but the cell walls of a continuous epidermis are never visible.

As has been already indicated, it is possible, in a series of sections through a well-filled germ tube, to obtain embryos in every stage of development. This being the case, if any portion of the body covering were thrown off during these larval stages, some evidence of the process would be seen. In no case whatever have I seen anything which would lead me to suppose that such a process took place in the embryonic stages of Bucephalus.

My evidence, therefore, seems to confirm the observations of Blochmann, Kowalevski, and others rather than those of Ziegler, Biehringer, Schneider, Kerbert, etc.

Sub-Cuticula.

Immediately below the cuticle lies a homogeneous layer of more deeply staining material in which the muscles are imbedded. This may be regarded as the sub-cuticular layer.

Musculature.

The body musculature consists of both ring and longitudinal muscle fibres, the former lying closely applied to the lower surface of the cuticle, the latter lying just beneath the ring muscles. In transverse sections the longitudinal muscles appear as a row of fine dots. In longitudinal sections the ring muscles have a similar appearance.

The Body Parenchyma.

The organs of the body lie embedded in parenchymatous tissue. The nuclei are prominent and stain readily, while cell boundaries and cytoplasm are difficult to demonstrate. The gland cells, of which mention has already been made, lie closely surrounded by the parenchymatous cells.

The Cystogenous Organ.

In the anterior end of the body, immediately behind the three-lipped invagination, lies a pear-shaped complex of gland cells and parenchyma nuclei. This is the "mundnapf" of the German investigators. During contractions of the body it is shifted backwards and forwards, and its walls contracted by means of the layers of diagonal muscle fibres lying in its limiting wall (figs. 41 and 43, *mc.*).

In the oldest specimens of Bucephalus which I obtained, a slight pressure forced out a rounded plug of transparent, viscid material (fig. 23). This complex of glands, I believe, produces the material from which the cyst is formed.

It is interesting to note that the large cells composing this organ have an appearance similar to that of the gland cells of the body, and that their contents have a like staining reaction.

The Digestive Tract.

At about the middle of the ventral side of the body there is a slight depression, at the bottom of which lies the mouth (fig. 41). This passes immediately into the pharynx, which is a thick-walled muscular organ with an outer and an inner set of circular muscles, an outer and an inner set of longitudinal muscles, and many radial muscles extending from the outer to the inner surface of the organ.

The short œsophagus passes obliquely forward from the pharynx to the gut. The wall of the gut consists of a single layer of cells. Its shape in younger stages is extremely variable. It may be simple, i. e. a rounded sac, or its walls may be pushed out into numerous cæcas. Ultimately the structure becomes rounded up, and in the oldest cercariæ is seen only as a subspherical cavity.

Lacaze-Duthiers (**20**) mentions a yellowish colour in the gut which I have never been able to observe.

So far as I have observed the gut is empty at this stage. It is probably not functional until later.

The Water Vascular System.

On examination with a low magnification the somewhat **S**-shaped contractile sac of the water vascular system may usually be seen without difficulty. Upon watching it for a time it may be seen to contract quickly, when its contents are forced back into the middle piece of the tail, and thence into the lumen of the tails. The contraction takes place rhythmically, and at every contraction of the water vascular sac there is an almost simultaneous expansion of the membranous portion of the tail.

The wall of the sac is thin, formed of a single layer of greatly flattened cells whose nuclei bulge into the cavity (fig. 37).

In fresh unstained specimens little more than this sac may be seen. In living specimens which are slightly stained with methylene blue, the longitudinal trunks of the water vascular system may be seen. Their arrangement is the same as that described by Ziegler for B. polymorphus. From about the middle of each side of the bladder a short duct is given off, which divides almost immediately into an anterior and a posterior lateral trunk. These trunks extend forwards and backwards respectively, receiving, from time to time, branches at whose extremities lie flame-cells (fig. 39).

The flame-cells are seen with difficulty. I was able to observe them most readily when the slightly stained Bucephalus had remained under a coverslip for about twenty minutes. In such preparations they might be watched for but a short time, their activity ceasing within a few minutes.

The excretory pore on the ventral surface may be seen in the living animal. Sections reveal a somewhat strange arrangement of outlets. From the duct passing from the contractile sac to the cavity in the tail, two short canals are given off, one of which passes to the dorsal surface, where it opens through a very fine pore, and one to the ventral surface at the junction of the body with the median portion of the tail (figs. 24 and 25).

When I first noticed this peculiar condition I attributed it to some abnormality, but an examination of several series convinced me that it is the usual arrangement.

A layer of sphincter muscles surrounds the outlet of the sac and prevents the return of liquid forced into the tail.

The Genital Organs.

In Bucephalus the reproductive organs are rudimentary. By comparison with the adult stage, Gasterostomum, we are able to identify an elongated mass of nuclei which stain deeply

and are smaller than the parenchyma nuclei, as the rudiment of the penis sheath, and a rounded mass of slightly larger nuclei, as the rudiment of the testes (figs. 1 and 41).

The Nervous System.

Immediately behind the cystogenous organ lies the brain, consisting of halves united by a broad commissure. The brain consists of a fibrous mass in which lie a few scattered ganglion-cells (figs. 35, 36, 43).

The cells or nuclei which Ziegler has described as ganglion-cells I believe to be simply parenchyma nuclei which serve as a sort of incomplete sheath for the nervous system (figs. 43, *n.s.*; 35 and 36, *par.n.*). My observations on this point confirm those of Schwarz (**35**) on Cercaria armata, C. echinata, etc.

From the brain are given off anteriorly four comparatively large nerves, composed of many fibrils. The nerves rapidly subdivide, the branches being distributed to the anterior end of the body. The cystogenous organ is especially richly supplied. Posteriorly, two main trunks are given off from the brain (fig. 43). These pass backward along the ventral side of the body, giving off branches to the pharynx and continuing to the tail region. Scattered ganglion-cells may be seen throughout the course of these nerve-trunks.

The rudiment of the brain appears in the embryo soon after the beginning of elongation from the germ-ball stage (fig. 13). At first it is simply a nearly solid aggregation of nuclei apparently no different than the usual parenchyma nuclei. From this mass of tissue fibrous elements and ganglion-cells are gradually developed.

The use of methylene blue as an intra-vitam stain confirmed observations which I had previously made on ganglion-cells in material stained with iron hæmatoxylin. Time has not permitted the use of other and more modern methods.

The Tail of Bucephalus.

Ziegler's description of the tail of B. polymorphus applies approximately to that of B. haimeanus. While my observations agree in general with Ziegler's careful description, and are, in fact, only supplementary to it, it is, perhaps, better to describe the structure in B. haimeanus rather than to merely consider the differences between the two forms.

As already noted the tail consists of a middle piece, from each side of which is given off an appendage, the middle piece being in direct connection with the posterior end of the body (fig. 29). The basal portion of the middle piece has firm thickened walls whose structure is the same as that of the body wall. Posteriorly these walls pass out into a bladder-like structure, which is constricted in the middle, and laterally into the appendages. In the walls of this bladder, and on one side of the appendages, are seen in the living cercaria many clear, highly-refractive, spherical bodies. When stained these bodies become uniformly blackened, appearing to be solid, homogeneous structures.

In the appendages they extend in two definite rows (fig. 32), one on each side of the median line, from the base of the appendage to its tip (fig. 1).

It seems probable that they are for the purpose of furnishing rigidity to the membranous portion of the middle piece and to the appendages. In the appendages they are so arranged that the compound rows become simple as the tails elongate.

I was at first inclined to the view that they are the products of excretory activity. Similar structures have been noted in other Trematode larvæ by various workers and their origin thus described. As I have not found them free in the lumen of the middle piece or of the appendages, but always apparently embedded in the wall, I am led to doubt my first impression, and to regard them as bodies formed for the purpose of support. Cells which may give rise to these

bodies are seen in the tail during developmental stages (fig. 34).

As already noted, the contractile sac of the water vascular system opens into the lumen of the middle piece, and this, in turn, into the lumen of the appendages. When the water vascular sac contracts, its contents are forced into the middle piece and its membranous wall distended; when this wall contracts, the contents are forced into the appendages, which, as a result, become rigid.

When the appendages are broken off, as frequently happens in free-swimming stages, though never within the sporocysts, the bladder-like portion of the middle piece collapses, and the definite form of the more solid portion may be seen (figs. 26, 27, and 28).

This portion is extremely muscular, and may undergo a great variety of changes of form. Each side of the middle piece receives muscles from its own and also from the opposite side of the body (fig. 30).

In B. haimeanus the "wülste" or "scheiben" described in B. polymorphus are not present.

The appendages are covered with a thin homogeneous cuticle similar to that covering the body. The appearance of "lasso cells" described by McCrady is caused by the refractive bodies already mentioned which lie upon the inside of each tail.

Sections show a lumen situated just beneath these tracts of spherical bodies (figs. 32 and 33), the space between the lumen and the muscle layers being filled with a nucleated parenchymatous tissue. Closely applied to the outer wall are six double longitudinal muscle bands. Outside of these is the layer of circular muscle fibrils.

From the study of the section of a tail the great improbability of Pagenstecher's idea that the tails become sporocysts is at once apparent. In the sporocyst there is nothing corresponding to the supporting spherules or to the double muscle bands.

Transitional Stages.

From the Silverside (Menidia menidia) I obtained forms which are intermediate between Bucephalus and Gasterostomum.

Encysted Forms.

The encysted forms were within a rather clear capsule, through which the outlines of the body and its structure could be seen (fig. 48). The contractile sac of the water vascular system was greatly distended and filled with refractive granules. The cystogenous organ appeared as an opaque mass in which nuclei were scattered.

Free Forms.

Many of the free forms appeared similar to Bucephalus from which the tails had been removed.

In other forms the sucking disc had taken the place of the cystogenous organ, the penis sheath had assumed definite form, and the spines were very noticeable (fig. 49).

Gasterostomum gracilescens.

The body is about 1·0 mm. long and 0·5 mm. broad. It tends to be cylindrical in form, the anterior half of the body being of a rather uniform diameter with the anterior end rounded; the posterior half tapers gently to a pointed end (figs. 46 and 47).

In the living worm comparatively little of the anatomy may be made out. It is easily seen that the body is covered with minute spines, arranged in longitudinal rows. Through the integument may be seen the penis sheath, the testes and the ovary, the vitellaria and the pharynx and gut. In specimens in which the uterus is filled with eggs absolutely nothing of the internal structure may be seen, the uterus

being so greatly distended by the mass of yellowish eggs that it surrounds and conceals the other organs of the body.

The body is covered by a transparent cuticle from 0·002 to 0·003 mm. in thickness, in which are embedded the rather broad, flat spines. The striated appearance of the body observed in Bucephalus is no longer noticeable.

The innermost layer of the cuticle stains the more deeply.

Musculature.

The body-wall has the usual muscle layers, composed of circular, longitudinal, and diagonal muscle fibres. The arrangement of these muscles is best seen in a tangential section (fig. 50).

The circular muscle fibres, although larger than those in Bucephalus, are still extremely fine, being only about 0·0005 mm. in diameter; the longitudinal muscles are somewhat larger, varying in diameter from 0·00075 to 0·001 mm.; the diagonal muscles, of which there are two sets, are about the same size as the longitudinal.

The Sucking Disc.

The sucking disc opens ventrally at the anterior end of the body (fig. 54). The arrangement of the muscles is the same as that described by Ziegler (7, p. 548) for G. fimbriatum.

The Digestive Tract.

The mouth opens on the ventral surface in the anterior half of the body. It is succeeded by a short pre-pharynx, which passes into the pharynx. This is similar, except in size, to that of Bucephalus. The muscles are much larger than in the cercaria; the ring muscles are oval in cross section, about 0·002 mm. long and 0·001 mm. wide. The radial muscles are spindle-shaped, about 0·001 mm. in diameter in their thickest part (fig. 53).

The pharynx is followed by the œsophagus, the walls of

which are provided with a lining resembling the cuticle covering the surface of the body. This ceases abruptly at its contact with the intestinal epithelium.

The wall of the gut consists of a single layer of vacuolated cells, which lie on an extremely thin basement membrane. These cells are irregular in outline, and remind one of the endoderm cells in Hydra. A deeply-staining nucleus may be seen toward the base of each cell, while the vacuoles occupy a position toward the opposite end.

Ziegler (**7**), in describing the gut in G. fimbriatum, mentions an irregular row of nuclei and the vacuolated appearance of the intestinal epithelium, but was not able to observe cell boundaries.

In my sections there is present in every instance a definite layer of lightly-staining material extending from the ends of the digestive cells into the cavity of the gut. This layer seems to consist of exceedingly fine fibres or threads (fig. 53, *ppr.*).

Looss (**47**, 1894) described a similar condition of the intestinal epithelium in several distoma. Juel (**48**, 1889), for Apoblema species, and Lander (**49**, 1904), for Hemiurus crenatus, describe a portion of the gut as being so modified.

Sommer (**50**, 1880) describes and figures the inner ends of the gut cells of Distomum hepaticum as extending either in a network of coarse pseudopodia or in fine threads.

The appearance in G. gracilescens is probably due to the same cause, i. e. is caused by the protrusion of the substance of the gut cells into exceedingly fine protoplasmic processes, which function in the absorption of food.

In the wall of the gut are two sets of diagonal muscles, crossing each other at right angles.

The Nervous System.

The brain lies just behind the sucking disc. As in Bucephalus, it consists of a fibrous mass in which a few scattered ganglion cells are embedded (fig. 57). The cell bodies,

usually spindle-shaped, are seen more easily than in Bucephalus. The distribution of nerves is much the same, a number passing forward and two main stems passing laterally and ventrally, innervating the pharynx and the posterior part of the body.

The Water Vascular System.

In but a few of the living Gasterostoma, which I have studied, have I been able to make out any trace of the excretory system. In the few fortunate instances I have observed scattered flame cells and the outline of the end bladder. In no case have I seen the bladder contract.

Longitudinal sections show that the sac opens to the exterior through an excretory pore which is situated at the posterior end of the body near the dorsal surface (fig. 56). The duct connecting the water vascular sac with the exterior is short and surrounded by large sphincter muscles.

The Genital Organs.

The reproductive organs, of which only the rudiment was present in Bucephalus, had reached their full development in most of the Gasterostoma which I have examined. The arrangement is that of the typical Trematode (figs. 46 and 47).

Female Reproductive Organs.

The ovary is situated a little to the right of the median line, slightly anterior to the middle of the body. It is oval in outline, slightly longer than broad, with its long axis parallel to the long axis of the body (fig. 52).

From its posterior end it gives off an oviduct which widens into a flask-shaped enlargement. At the posterior end of this enlargement Laurer's canal originates. This passes through a convoluted course to open on the dorsal surface of the body. After leaving the enlargement (seminal receptacle)

the oviduct bends in nearly a right angle and receives the median vitelline duct, which has been formed by the union of the two lateral vitelline ducts.

Immediately beyond the junction of the oviduct with the vitelline duct, the oviduct passes into a second enlargement which is surrounded by the shell-glands; from thence it emerges as the uterus, which passes through an irregular course to open into the genital atrium.

The course of the uterus may be traced only in sexually immature animals. As previously noted, when filled with developing eggs, it is greatly distended, and appears as a large sac almost filling the body. In the living worm the shell-glands appear as an aggregation of rounded cells, which is closely applied to the oviduct. In sections, they are elongated in outline, with a deeply staining nucleus and with coarsely granular contents.

The vitellaria, as a rule, thirty-two in number, sixteen on each side, are situated in the anterior dorsal part of the body, immediately behind the sucking disc. On each side of the body they are united by fine ducts, which may be seen in the living worm, with the main vitelline duct of that side. The two vitelline ducts pass backward, uniting into a single duct before passing into the oviduct (fig. 47, *vit.*).

Each vitellarium consists of a rounded accumulation of cells, in which the nucleus is very distinct (fig. 44). Packed closely within the cell and surrounding its nucleus are the yolk granules. Before these globules pass down the vitelline ducts, the cellular structure breaks down, the yolk granules passing outward into the ducts while the nuclei remain behind. In sections passing through the yolk-laden ducts I have never found nuclei, while sections through the vitellaria, after the yolk has passed out, show many nuclei.

The Male Reproductive Organs.

The testes, lying one slightly behind the other, are situated posterior to the ovary (figs. 46, 47, 52). From each testis

runs a vas deferens. These unite with one another to form a common duct, which enters the seminal vesicle on its dorsal side (fig. 56, *sv.*).

The seminal vesicle opens into an ejaculatory duct whose walls in part are lined with an epithelium of coarsely granular cells. I can find no nuclei in this layer. This epithelium extends through about two-thirds of the length of the ejaculatory duct, where it is succeeded by a thin membrane which continues to the genital sinus (fig. 56).

The space between the wall of the penis sheath and the ejaculatory duct is filled with parenchyma cells (fig. 45). The character of this tissue changes in the portion of the penis which lies within the sinus, becoming here much reticulated and spongy in appearance (fig. 56).

The genital sinus, lined by a cuticle which appears similar to that covering the body, opens to the exterior through a short duct which is surrounded by a layer of circular muscles.

I have been able to make no observations as to the manner in which the male organ functions. The surface of that part lying within the sinus is much folded. It seems probable that this portion is a true penis, i. e. is protrusible.

Molin (**11**, 1861) gives a figures of G. armatum in which the penis is extended.

Though self-fertilisation may be possible, the arrangement of the genital apertures and the structure of the genital atrium seems to render it improbable. Spermatozoa set free in the genital atrium might make their way into the uterus.

Spermatozoa are to be found in all parts of the uterus, though nowhere in such great abundance as in the seminal receptacle, and in that part of the uterus just after the shell glands. They are also present in Laurer's canal, through which, it is probable, they are simply making their way to the exterior.

The eggs, seen in section in the oviduct, are irregular in outline, giving the impression of being amœboid bodies. Fertilisation probably takes place before the egg passes into the ootype.

My time has not permitted a completed study of the segmentation and development of these eggs. Limited observations seem to show that the process is similar to that described by Schauinsland (**51**, 1883) for D. tereticolle.

Note.—When I commenced the anatomical and histological work on the marine forms of Bucephalus and Gasterostomum, an account of which has been given above, I had no idea that the forms would prove so similar to the fresh-water forms of the same name described by Ziegler in 1883.

To me one of the chief points of interest in my work has been the proof of the practical identity of these forms.

So far as possible I have carefully avoided a restatement of facts which have already been established by Ziegler. In the instances when this has been done it has been for the purpose of furnishing a basis for the comparison of the marine and fresh-water species.

An Account of Experiments for Determining the Life-History of Bucephalus Haimeanus.

In September, 1902, I began a systematic effort to obtain the completed life-history of B. haimeanus. The work was carried on simultaneously along various lines. I shall first describe—

Experiments on Oyster Infection.

It has been noticed, and my observations confirmed the reports of others in this respect, that the oysters in certain areas were badly infected with the parasite, while in other localities cases of infection were comparatively rare.

One of the problems presented by this condition of affairs concerns the manner in which infection is spread. The question presented itself, is infection direct, that is, do cercariæ, or portions of the sporocyst escape from one oyster and make their way directly to another oyster?

As a means of solving this problem I selected oysters from

beds in Newport River on which I knew that about 25 per cent. of the oysters were infected, and, after marking them, placed them in wire cages with oysters from Fort Macon, where I had never found the parasite. The cages were then sunken in the water beneath the wharf at the laboratory.

The cages used for this purpose were made of galvanised wire netting, and were about two feet square and six inches deep.

In cage No. 1 were placed twenty-four Newport River oysters and eighteen Fort Macon oysters.

In cage No. 2 were placed twenty Newport River oysters and twenty Fort Macon oysters.

Cage No. 1 remained in the water until November 1st (about six weeks) when it was taken up and its contents sent to me at Baltimore. Four of the Newport and three of the Fort Macon oysters had died during the experiment. Examination showed that of the twenty Newport oysters remaining, five were badly infected. None of the Fort Macon oysters showed any signs of infection.

Cage No. 2 was sent to me on February 1st. In this cage two Newport and two Fort Macon oysters had died during the experiment. Of the eighteen Newport oysters, four were infected, while none of the Fort Macon oysters showed any trace of infection.

In June, 1903, I began a modification of this experiment. From a large number of infected oysters I selected specimens in which there was a great abundance of sporocysts, and consequently a great number of embryos in all stages of development. These oysters were torn to pieces in a dish of sea water and a considerable quantity of cercariæ and sporocysts thus obtained. By slightly separating the valves of the shell of uninfected (Fort Macon) oysters with a knife, the tip of a pipette containing cercariæ and sporocysts could be inserted, its contents ejected into the mantle cavity of the oysters, and the valves then allowed to close.

Thirty of these oysters were immediately placed in a cage

and anchored in the water beneath the wharf. The remainder were allowed to remain out of water for twenty-four hours, and were then placed sixteen in one cage and eighteen in another and anchored beside the first cage.

On August 24th the oysters were removed from cage No. 1. Ten of the oysters had died. Of the remaining twenty none were infected.

On September 2nd the oysters were removed from cage No. 2. Two of the oysters had died. Of the remaining fourteen none were infected.

On September 16th the oysters were removed from cage No. 3. One of the oysters had died. Of the remaining seventeen none were infected.

The results of the foregoing experiments I regard as negative. While I do not believe that infection is communicated from one oyster to another, I do not regard these experiments as furnishing conclusive evidence that such transmission does not take place.

Feeding Experiments for the Purpose of Determining the Further Life-history of B. haimeanus.

My second set of experiments was carried on in August and September, 1903, when I attempted to obtain the adult form of the parasite by feeding infected oysters to fish which were kept in aquaria. These experiments were tried on six species of fish. Oysters were opened and the infected ones selected, torn in pieces, and thrown into the aquaria. They were eaten greedily.

Thirty minutes after feeding I removed the stomach and intestines from one of each of the species fed and found that although most of the cercariæ were dead, some were still alive.

Thirty minutes later, or an hour after feeding, I removed the stomach and intestines from one of each of the species fed and could find no living cercariæ.

A diet which consisted of infected oysters exclusively was

continued for a month, during the first week of which I daily examined the viscera from one of each of the six species of experiment fish. During the last three weeks the examinations were made at intervals of a week.

In a black bass (Centropristis striatus) which I killed on the fourth day I found ten Gasterostoma in an advanced stage of development. Later experiments convinced me that these were not a result of my feeding experiments.

The failure of this feeding experiment gave me the choice of two alternatives:

1st. Either the cercariæ were not at such a stage of development that they were able to resist the action of the digestive juices of the intended host, or—

2nd. The fish which I experimented upon were immune toward this particular parasite, a conclusion which did not seem correct since one of the experiment fish contained Gasterostoma, although, out of forty-six black bass examined during this and the preceding summer, I had found none which contained Gasterostoma.

I felt justified, therefore, in concluding that the host of the adult form of Bucephalus was not necessarily an oyster-eating fish.

In the meantime, as has been already noted, I had been attacking the problem from another direction.

The Search for Gasterostomum.

In 1902, while the first set of experiments was being carried on, I attempted to find the host of Gasterostomum, which the concensus of opinion of the various workers on Trematodes, based on the similarity in structure of Bucephalus and Gasterostomum, suggested as the probable adult form.

This search was carried on rather blindly, and during that season resulted in nothing. Many fish were obtained by seining in the vicinity of the oyster-beds, but although they served as the hosts of innumerable parasites, Gasterostomum

was not of the number. The same was true of the crustacea and annelids examined. During the winter of 1902, at my request, Mr. Coker kindly procured and examined the viscera of birds and terrapins which live in the marshes in the vicinity of the oyster-beds, but without result. I also examined various water plants in the hope of finding encysted forms of Bucephalus.

In June, 1903, I continued the examination of the viscera and gills of fishes. On June 25th I procured two gars (Tylosurus marinus), and found in the lower part of the intestine of each Gasterostoma in abundance.

Here, then, was a fish which might furnish a clue to the solution of the problem.

At this time other work made it necessary for me to leave Beaufort for a few weeks, so the further study was delayed for a time. At Woods Hole, in discussing the matter with Dr. Edwin Linton, whose work on fish parasites is so well known, he informed me that during the earlier part of the previous summer, in his work on the 'Fish Parasites of Beaufort,' he had found Gasterostomum in abundance in the gar (Tylosurus marinus), and also in Menidia menidia and Stolephorus Brownii, which I had not yet examined.

On my return to Beaufort in August I took up the work where I had dropped it six weeks previously. On this occasion I devoted my attention for a time exclusively to the gar, making special note of the stomach contents. The food for a time consisted chiefly of shrimp, although the almost completely digested remains of small fish were sometimes present.

Shrimp being abundant at the time I obtained a large quantity, which I examined carefully for parasites. Although these were abundant none could be found which bore any resemblance to Bucephalus or to Gasterostomum, most of the parasites observed being larval cestodes.

Finally I succeeded in obtaining gars in which recently captured fish were found. These proved almost in every instance to be Silversides (Menidia menidia).

My attention was then turned to this fish. Upon examining

the gills and viscera with the naked eye no parasites of the Gasterostomum type could be seen. After slitting open the stomach and intestine and spreading it out upon a glass plate I carefully washed the contents free from the walls with sea-water. Examining these washings with a magnification of about 150 diameters I found present, besides numbers of cestode larvæ, great numbers of small transparent cysts of the appearance shown in fig. 48, as well as undoubted Gasterostomum forms like those shown in fig. 49.

The encysted forms were surrounded by a rather transparent capsule through which the structure of the enclosed form could be seen.

The free forms were but slightly different in appearance from Bucephali which have lost their tails. But I had no proof, as yet, that these forms were related to the forms found in the gar. While there was a general resemblance between the two I did not feel justified in concluding that the one developed to the other.

Recourse was had to further feeding experiments.

Experiments for determining the relationship between the Gasterostomum found in the Silverside and the Gasterostomum found in the Gar.

As a further experiment I decided upon feeding the viscera of the Silversides to fish in which Gasterostomum did not normally occur as a parasite. For this purpose I selected four species of fish, the white perch (Morone americana), black bass (Centropristis striatus), croaker (Micropogon undulatus), and pin-fish (Lagodon rhomboides).

Each species was placed in a separate aquarium. The experiment lasted a week, during which, on alternate days, I fed the fish, viscera from the Silverside, giving no other food.

Twenty-four hours after the first feeding I examined the stomach and intestine of one specimen of each species. No Gasterostoma of sufficient size for observation with a hand lens were present, but in washings from the intestine many small Gasterostoma identical to those from the Silverside were seen. The encapsuled forms had disappeared.

Twenty-four hours later I again made a similar examination with similar results. Two days later the results were the same.

Three days later (at the end of one week's time) I made the last examination. Unfortunately, by the breaking of an aquarium, one set of experiment fishes had been lost. In the three remaining species small Gasterostoma were found in abundance. On this occasion it was possible to notice a difference in the size of the Gasterostoma present. Some had nearly doubled in size.

It is interesting to note that, even in the short time during which the experiments had been conducted, the Gasterostoma had grown to a greater size in the perch and the black bass than in the pin-fish.

The experiment should have been continued for a longer time, for a month at least, but the time during which I could stay at Beaufort had passed, and it became necessary to discontinue the work.

Sufficient had been shown by the experiment to convince me that the Gasterostoma found in the Silverside could resist the action of the digestive juices of other fish. There were present in the intestine of the gar many specimens only slightly different in appearance, chiefly in size and sexual maturity, from those which I obtained at the end of the week from the experiment fishes.

Since I had already determined that the Silverside was one of the gar's sources of food I could but naturally conclude that it was also its source of Gasterostoma.

How and where the Silverside obtains its cercariæ I do not know. The food of the Silverside consists chiefly of small crustacea. An examination of many of these failed to

show that they served in any way as temporary hosts for Bucephalus.

It is quite possible that the Silverside obtains them during their free-swimming stage. A careful examination of many of the smaller fish (Dr. Linton has already found encapsuled forms in the anchovy [Stolephorus brownii]) will, I think, reveal the presence of larval Gasterostomum forms.

That it is necessary for Gasterostomum to undergo a portion of its life-history within the Silverside or a small fish of similar habit I doubt greatly.

Giard (**30**) found encysted stages of Bucephalus in Belone vulgaris, a gar fish. I can see no reason why the gar may not obtain some of its Gasterostoma first hand, i. e. that it may obtain cercariæ during its feeding, the cercariæ encysting within the gar's body, as Giard has described, and, after reaching its adult sexual condition, breaking from its cyst to attach itself to the wall of the intestine of its host.

The hypothesis proposed by Giard that Gasterostomum larvæ in Belone vulgaris probably reach their adult condition in some fish which preys upon this gar does not seem to me necessary.

The Genus Gasterostomum.

It would be preferable, for several reasons, if the matter of the present section were placed at the end of the historical account of Gasterostomum; but inasmuch as it has its foundation in the observations and experiments detailed in the present paper, it seems better that it should follow the account of these experiments.

After studying the form which is the subject of this paper, and seeing the many changes of appearance which it is possible for a single specimen to assume, considerable doubt arises in my mind as to the genuineness of the many species which have been described.

Wagener's (**3**) beautiful figures of young G. fimbriatum

from the "Hecht" show a form exactly identical with the immature Gasterostoma which I have found in Tylosurus. In his description of the older stages he described a complicated structure of the anterior end of the body. Ziegler (7) has shown where he was in error in this description.

Even a most casual comparison of the form which I have studied, with Ziegler's figures of G. fimbriatum impresses me with the great similarity between the two forms, and when one enters into a more careful comparison, comparing section with section, the impression deepens to a conviction that the two forms are identical. But G. fimbriatum is the parasite of fresh-water fishes, while the Gasterostomum of the present paper finds its host among marine forms. This fact might be regarded as sufficient warrant for placing the forms in separate species. An additional reason for such a classification might be found in the fact that B. Polymorphus, the larval form in the one case and B. haimeanus, the larval form in the other, are somewhat different, chiefly in the length and shape of the tails.

As I have gone over the literature, comparing the descriptions and the figures published by various investigators, and bearing in mind the results of my own observations, I have been gradually forced to the conclusion that the species described by these numerous investigators are simply physiological varieties of the same species.

It is possible, in fact eminently probable, that changes of various sorts may have been brought about by differences in environment, and that all of the species which have been described may have come from similar larval forms which have reached different hosts, and have there been subjected to influences which have been more or less conducive to their further development.

In such a supposition I believe that I am warranted. The encysted Gasterostoma, differing from Bucephalus only in the absence of the tails, which I obtained from Menidia, as well as the free forms which I found in the intestine of the same host, had reached at the end of a week, after being fed

to different fishes, noticeable differences in appearance, those in the perch and the bass, as noted above, having progressed further in their development than those in the pin-fish. That these changes should continue seems most probable.

Since this is true, is it not possible that all of the forms of Gasterostomum which have been described have come from similar larval forms, and that the differences which may exist have come about during a single generation?

When one compares the published figures of—

G. gracilescens, Wagener, 1858;
„ armatum, Molin, 1858. Levinsen, 1881; Olsson, 1867;
„ crucibulum, Rud;
„ triglæ, Van Beneden, 1871;
„ ovatum, Linton, 1899;
„ sp. Lt. (from Tylosurus marinus), 1899;

(the form which I have studied), he is impressed with their great similarity. The differences as shown by these investigators is slight.

From a limited region Van Beneden described four new species, and also found individuals which he referred to two old species.

I have tentatively held the form under consideration as G. gracilescens, not only because the more immature forms found in Tylosurus agree with the figures of young G. gracilescens published by various investigators, but also because I believe that most of the species described are physiological varieties of this form. The described species of Gasterostomum are all to be found in regions from which Bucephalus has been described.

The Effect of the Parasite on the Oyster.

From an economic standpoint a consideration of the effect of the presence of the sporocysts in the oyster is of importance.

The distribution of this parasite is undoubtedly wide.

McCrady found it in oysters at Charleston; it is found in abundance at Beaufort; it is plentiful in Pamlico Sound; it occurs in Louisiana; it was present in oysters which I purchased in Baltimore, and which were said to have come from the Rappahannock.

The extent of infection varies. Of the Pamlico Sound oysters which I examined thirty were from Stumpy Point Bay; of these six or 20 per cent. were infected, none of them badly. From Juniper Bay Point I had twelve oysters, of which four or 33 per cent. were badly infected.

In February, 1902, of seventy-two Rappahannock (?) oysters which I examined, four or about 5 per cent. were infected.

As to the percentage of infected oysters at Cameron, I can give no data. They are said to be badly infected.

At Beaufort, in 1901—1902, the percentage in several hundred oysters examined was from 23 per cent. to 25 per cent.

Through the kindness of Dr. Grave I was enabled, in 1903, to make use of the beds which he had established in Newport River in his experiments on oyster culture.

In June, 1903, one bushel of oysters from bed No. 6 gave 32·5 per cent., while the other beds in the same region gave 21 per cent. of badly infected oysters.

In September, 1903, upon tonging upon bed No. 6 many oysters were found to have recently died. (Many shells, the valves of which were still attached, were tonged up.) Examination of two bushels of the remaining oysters showed but 3 per cent. infected. About one bushel was left in a heap in the water at one side of the wharf. On the day following it was noticed that the shells of some of the oysters were separated, and that small crabs were present in large numbers and were feeding upon the dead oysters. Twenty-eight of these crabs were taken. Examination of their stomachs showed that twenty-six contained cercaria oysters, the cercariæ being dead in every instance. (Huet's observations that the cercariæ seem to cause the death of the host and then escape into the surrounding water does not seem to be confirmed.)

No living cercariæ were found in any of the dead oysters which I was able to obtain.

At the end of three days all of the remaining oysters were opened. None were found to be infected.

During July and August heavy rains in the country had flooded the marshes, and the water in Newport River had become extremely fresh, furnishing conditions which were trying to even the healthy oyster.

The above observations seem to show that the presence of the cercariæ seems to render the oyster less capable of withstanding adverse conditions. While conditions that are conducive to the well being of the oyster prevail, the presence of the cercariæ does not seem to cause any great mortality, but, when circumstances arise during which the latent vitality of the oyster is called forth, the infected oysters are unable to meet the requirements.

Even during the best of conditions the parasite must be considered as injurious, since it prevents the formation of the sexual products.

From my observations I feel reasonably certain that the cercariæ thrive best in slightly brackish water, such as usually prevails in Newport River.

It will be noted that similar conditions prevail in the localities mentioned above as reporting infected oysters.

The oysters from the salt water at Fort Macon are without the parasite.

In oysters from the Mullet Pond on Shackelford Bank the growth of the cercariæ in the oysters which I examined seems to have been inhibited.

It is interesting to note that the water of this pond, by reason of its location, is subject to considerable variations in salinity. It is normally fed by fresh-water springs, but during an extremely high tide or during storms salt water may be swept into the pond.

It is my belief that, during a period when the water was somewhat fresh, the cercariæ obtained a foothold. Later, when by reason of the incoming of salt water and subsequent

evaporation, the salinity of the pond was greatly raised the growth of the cercariæ was inhibited.

Summary.

The observations detailed in this paper may be summarised as follows:

1. Germ-cells which arise within the wall of the sporocyst of G. gracilescens give off small cells, which may be considered of the nature of polar bodies, segment, and develop to the cercaria B. haimeanus.

2. The cercaria B. haimeanus, Lacaze-Duthiers (1854) = B. cuculus, McCrady (1868), parasitic in the oyster, differs structurally from B. polymorphus, von Baer, parasitic in fresh water mussels, only in the shape of the tails.

3. G. gracilescens, Wagener (1852) parasitic in Lophius piscatorius = Gasterostomum sp., Linton, 1899, parasitic in Tylosurus marinus, differs from G. fimbriatum, von Siebold, only in habitat (one is a marine, the other a freshwater, form), and slightly dissimilar larva.

4. B. haimeanus, as shown by experimental evidence, is a larval stage of G. gracilescens.

5. Experimental evidence shows that supposedly different species are physiological varieties of the same species.

6. The presence of the sporocysts and cercaria of G. gracilescens in the oyster prevents the formation of reproductive elements, and also renders the host incapable of withstanding adverse conditions.

7. B. haimeanus thrives best in oysters growing in brackish water. Its growth is inhibited by increased salinity.

It will be noticed that there is one gap in my account of the life-history of Gasterostomum. I have not yet proved the infection of oysters by embryos developed from the eggs of the adult. I hope to make this the object of experimental work within the near future.

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DESCRIPTION OF PLATES 39—42,

Illustrating the paper by Mr. David Hilt Tennent on "A Study of the Life History of Bucephalus haimeanus, a Parasite of the Oyster."

Abbreviations.

aln. Anterior lateral nerve. *blp.* Bladder-like portion of middle piece. *br.* Brain. *cerc.* Cercaria. *c.sac.* Contractile sac. *ctg.* Cystogenous gland. *ctgo.* Cystogenous organ. *cst.* Cyst. *ctl.* Cavity in middle portion of tail. *cu.* Cuticle. *cw.* Wall of yolk cells. *d.* Duct. *da.* Anterior duct. *dc.* Digestive cell. *dg.* Sperm duct. *dp.* Dorsal pore. *dp'.* Posterior duct. *dvc.* Dorso-ventral canal. *ejd.* Ejaculatory duct. *ep.* Epithelial cells. *exp.* Excretory pore. *fl.* Flame cell. *frb.* Formation cells of refractive bodies. *g.a.* Genital atrium. *g.b.* Germ ball. *g.c.* Germ cell. *gl.c.* Gland cell (sunken epithelial cell). *gp.* Genital pore. *gpw.* Glandular muscular fold. *grl.* Granular layer. *gt.* Gut. *g.t.* Germ tube. *inv.* Three-lipped invagination. *kl.* Keimlager. *Lc.* Laurer's canal. *Lcp.* External opening of Laurer's canal. *lm.* Longitudinal muscles. *l.m.f.* Longitudinal muscle fibres. *ln.* Lateral nerve. *lum.* Lumen. *lts.* Left testis. *mb.* Muscle band. *mc.* Constrictor muscles surrounding cystogenous organ. *mp.* Mucous plug. *myb.* Myoblast. *n.* Nucleus in membrane. *nc.* Nerve cell. *ndc.* Nuclei gut cells. *n.s.* Neural sheath. *nyc.* Nucleus yolk cell. *o.dr.* Oil droplets. *œs.* Œsophagus. *om.* Oblique muscles. *o.ut.* Opening of uterus to atrium. *ovd.* Oviduct. *ovy.* Ovary. *oyst.* Oyster tissue. *p.* Penis. *par.* Parenchyma. *par.n.* Parenchyma nuclei. *pb.* Polar bodies. *ph.* Pharynx. *ppr.* Protoplasmic processes. *ps.* Penis sheath. *pt.* Disintegrated material between wall of germ tube and tissues of host. *rb.* Refractive bodies. *rdm.* Radial muscles. *ret.p.* Reticulated parenchyma. *rm.* Circular muscles. *rm'.* Inner layer circular muscles. *r.m.f.* Circular muscle fibres. *rps.* Rudiment of penis sheath. *rts.* Right testis. *sc.* Sub-cuticula. *sd.* Sucking disc. *sh.* Shell glands. *shgd.* Shell glands. *sm.* Sphincter muscles. *sp.* Spines. *sr.* Seminal receptacle. *sv.* Seminal vesicle. *tc.* Canal to tail. *tl.* Tail. *tr.* Rudiment of tail. *ts.* Testis. *ut.* Uterus. *uw.* Uterine wall. *vc.* Vacuole. *vd.* Vas deferens. *vit.* Vitelline ducts. *vitl.* Vitellaria. *vp.* Ventral pore. *wc.* Wall of cyst. *w.g.t.* Wall of germ tube. *wph.* Wall of pharynx. *wp.* Excretory pore. *w.vas.* Water vascular. *y.gr.* Yolk granules.

PLATE 39.

FIG. 1.—Bucephalus haimeanus viewed from the dorsal surface as a transparent object. Body slightly compressed by a cover slip. (Zeiss 4 D.) *m*. Mouth.

FIG. 2—Young sporocyst from gonad of oyster. (Zeiss 4 D.)

FIG. 3.—Slightly older sporocyst, through the transparent walls of which cercaria in various stages of development may be seen.

FIG. 4.—Germ tubes (branches of sporocyst) from an oyster in an advanced stage of infection.

FIG. 5.—Branching end of a germ tube.

FIG. 6.—Section of infected oyster tissue passing transversely through a germ tube. (Only a sector of the tube is shown.)

FIG. 7.—Longitudinal section of the growing end of a germ tube. Cells in this portion of the tube are undifferentiated.

FIG. 8.—Longitudinal section of old germ tube. (Only one side of tube is shown.)

FIG. 9.—Longitudinal section of developing cercaria (from lumen of germ tube) in which the tail rudiments have made their appearance. Structure of parenchyma nuclei is not represented.

FIG. 10.—Transverse section of the body wall of a mature cercaria.

FIG. 11.—Longitudinal section of the body wall of a mature cercaria.

FIG. 12.—Longitudinal section of an encysted form found in the gills and beneath the body-epithelium of the oyster.

FIG. 13.—Horizontal longitudinal section of a developing cercaria. (Older than that shown in Fig. 9.)

FIG. 14.—Section of infected oyster tissue. (Zeiss 4 A.)

FIG. 15.—*a*. Section of germ tube with germ cell protruding into lumen of tube.

a'. Germ cell with first polar body.

b. Germ cell with first polar body dividing.

c. Germ cell, first division. Three polar bodies may be seen on the surface.

d. Germ cell, second division (3-cell stage.)

e. Germ cell (4-cell stage).

f. Germ cell (5-cell stage).

g. Germ ball enclosed by a membrane which is formed by the union of two flattened cells.

FIG. 16.—Longitudinal section of germ tube, showing a germ cell giving off a polar body within the wall. The polar body has divided.

Fig. 17.—Transverse section through germ tube, in the wall of which an active differentiation of germ cells is going on. In the lumen of the tube lie germ cells and germ balls in various stages of development.

PLATE 40.

Fig. 18.—Section of germ ball.

Fig. 19.—Section of germ ball passing through the rudimentary pharynx.

Fig. 20.—Section of young germ ball.

Fig. 21.—Section through actively dividing germ ball.

Fig. 22.—Longitudinal section through the end of a germ tube in which a portion of the tube has become specialised as a germ-producing organ, "ovary."

Fig. 23.—Anterior end of Bucephalus haimeanus, in optical section, drawn from living cercaria. The mucous secretion of the glandular complex has been forced out into the invaginated portion of the anterior end of the body as a plug.

Fig. 24.—Dorso-ventral longitudinal section of the posterior end of Bucephalus haimeanus. The cuticle, etc., are not shown. The plane of the section passes slightly to one side of the median line.

Fig. 25.—Median dorso-ventral longitudinal section of the posterior end of Bucephalus haimeanus.

Figs. 26—28 represent individuals in which the tails have been lost. In such individuals the bladder-like portion collapses, and the muscular portion exhibits considerable changes of form.

Figs. 26—29 were drawn from clay models made for the purpose of showing the changes in form undergone by the posterior end of the body of Bucephalus.

Fig. 26.—Seen from dorsal surface. Middle piece elongated.

Fig. 27.—Seen from dorsal surface. Middle piece contracted and broadened.

Fig. 28.—Dorso-lateral view. The underside of the middle piece is seen to be concave, the concavity being caused by the collapse of the bladder-like portion.

Fig. 29.—Middle piece and a portion of the appendages in dorsal view.

Fig. 30.—Dorso-ventral longitudinal section of the posterior end of the body and middle piece, showing the muscles by means of which the movements of the middle piece are governed.

Fig. 31.—Surface view of cuticle of Bucephalus haimeanus, drawn from living specimen.

FIG. 32.—Transverse section of one of the tails of Bucephalus.

FIG. 33.—Longitudinal section taken slightly on one side of the median line of one of the tails of Bucephalus.

FIG. 34.—Horizontal section of the middle piece of the tail of an immature Bucephalus.

FIG. 35.—Transverse section through the anterior end of Bucephalus.

FIG. 36.—Transverse section through the anterior end of Bucephalus, slightly posterior to that shown in Fig. 35.

FIG. 37.—Transverse section through immature Bucephalus; cuticle not yet formed.

FIG. 38.—Transverse section through immature Bucephalus in region of pharynx; cuticle not yet formed.

FIG. 39.—The water vascular system of Bucephalus. Somewhat diagrammatic. Drawn from sections and from living specimens stained with methylene blue.

FIG. 40.—Section through shell glands of Gasterostomum gracilescens.

PLATE 41.

FIG. 41.—Dorso-ventral longitudinal section through nearly mature Bucephalus. The cercaria was slightly bent, so that the section is median from the mouth to the anterior end, and oblique posterior to the mouth. For median section of posterior end see Fig. 25.

FIG. 42.—Longitudinal section of portion of body wall of Bucephalus.

FIG. 43.—Horizontal longitudinal section of Bucephalus, showing brain and lateral nerves.

FIG. 44.—Section through a vitellarium of Gasterostomum gracilescens.

FIG. 45.—Transverse section through penis of Gasterostomum gracilescens.

FIG. 46.—Gasterostomum gracilescens in ventral view. R. Right.

FIG. 47.—Gasterostomum gracilescens in dorsal view. R. Right.

Figs. 46 and 47 are somewhat diagrammatic. The spines are not shown. Sketches were made from living specimens. After study of sections, details, which it had been impossible to make out in the whole mounts, were added.

FIG. 48.—Encysted form found in Menidia menidia.

FIG. 49.—Young Gasterostomum gracilescens found in Menidia menidia and in experiment fish at conclusion of feeding experiments.

Fig. 50.—Tangential section through body wall of Gasterostomum gracilescens, showing spines imbedded in the cuticle and the four sets of muscles lying below the body wall.

Fig. 51.—Longitudinal oblique section through one of tails of Bucephalus. The section passes through two longitudinal muscle bands.

Fig. 52.—The reproductive organs of Gasterostomum gracilescens, seen from the left side. Diagrammatic.

Fig. 53.—Longitudinal section through the digestive system of Gasterostomum gracilescens. *mth.* Mouth.

PLATE 42.

Fig. 54.—Dorso-ventral longitudinal section through the anterior end of Gasterostomum gracilescens.

Fig. 55.—Transverse section through penis and penis sheath of Gasterostomum at the level of the union of the uterus with the genital atrium.

Fig. 56.—Longitudinal section of the posterior end of the body of Gasterostomum gracilescens.

Fig. 57.—Transverse section of Gasterostomum gracilescens taken just behind the sucking disc.

Fig. 58.—Tangential section through wall of gut in Gasterostomum gracilescens.

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NEW SERIES.

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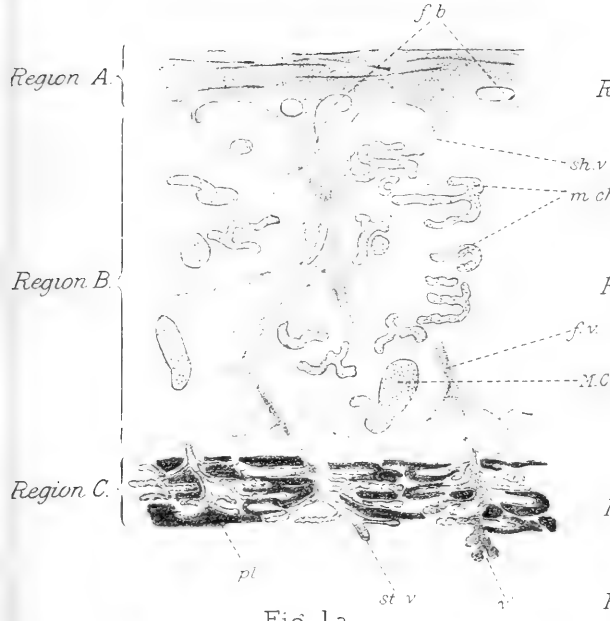

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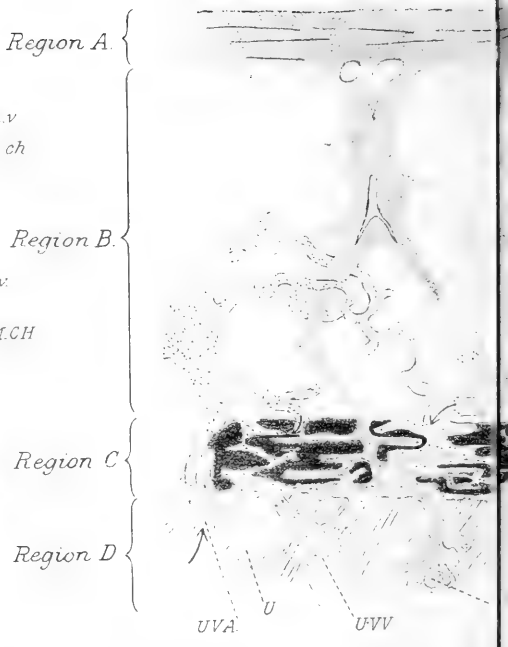

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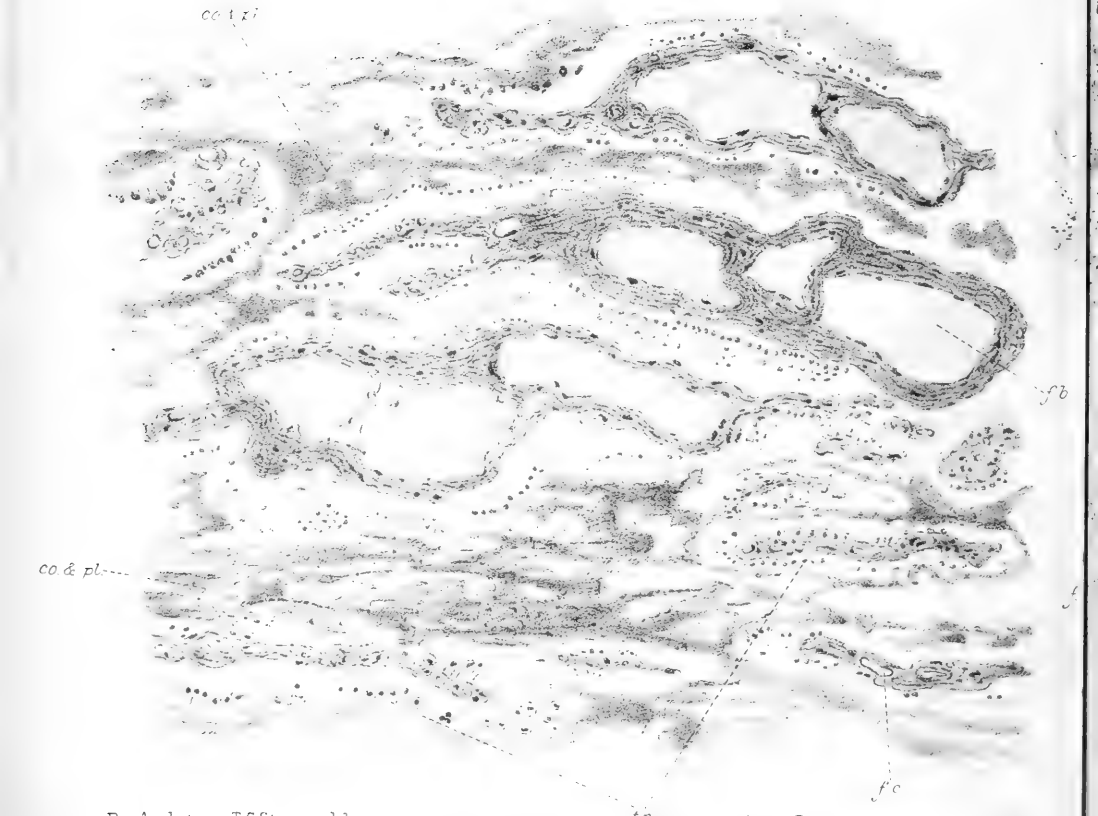

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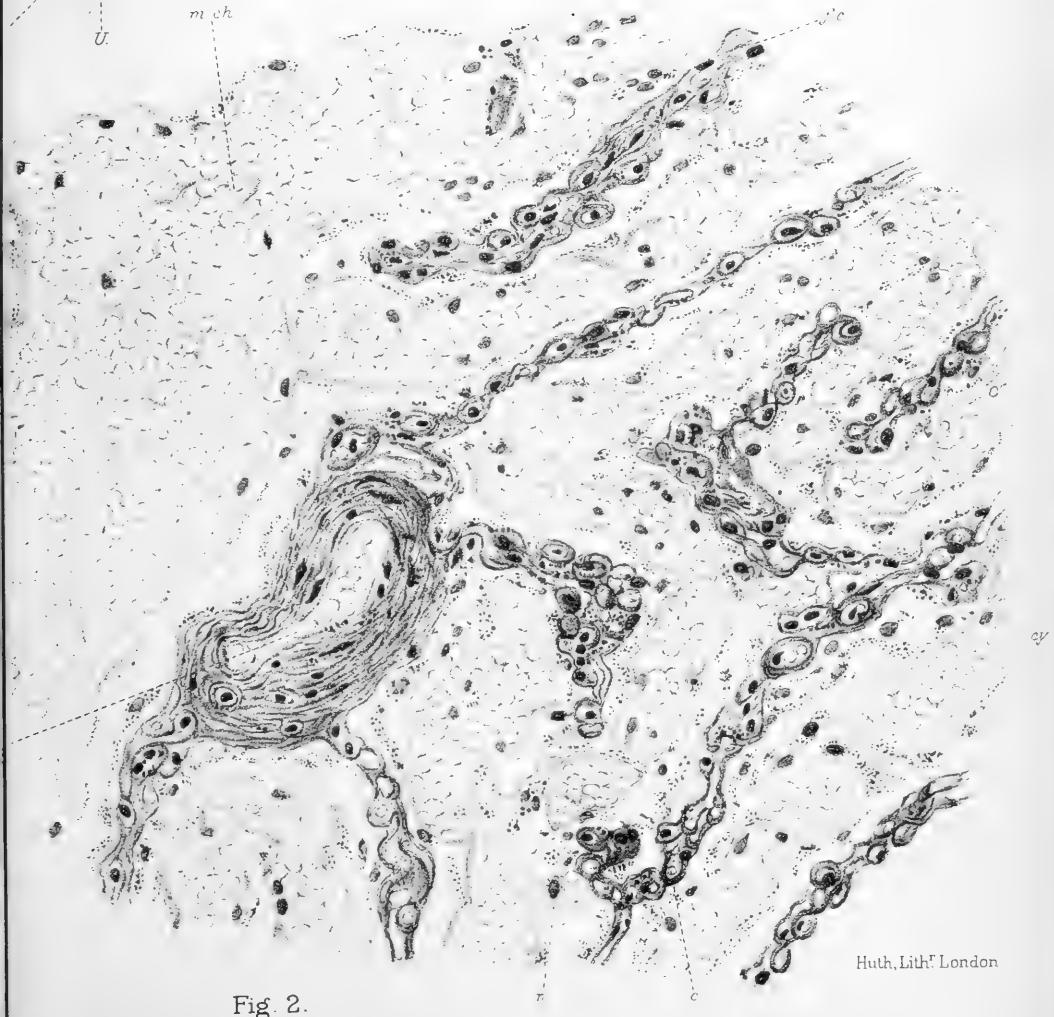

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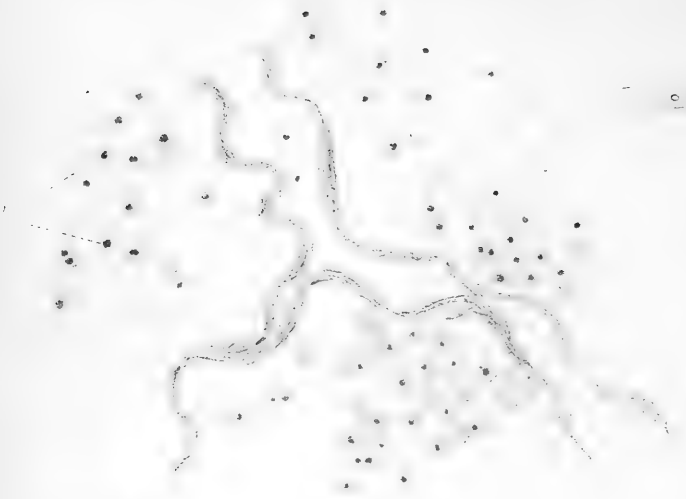

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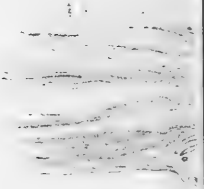

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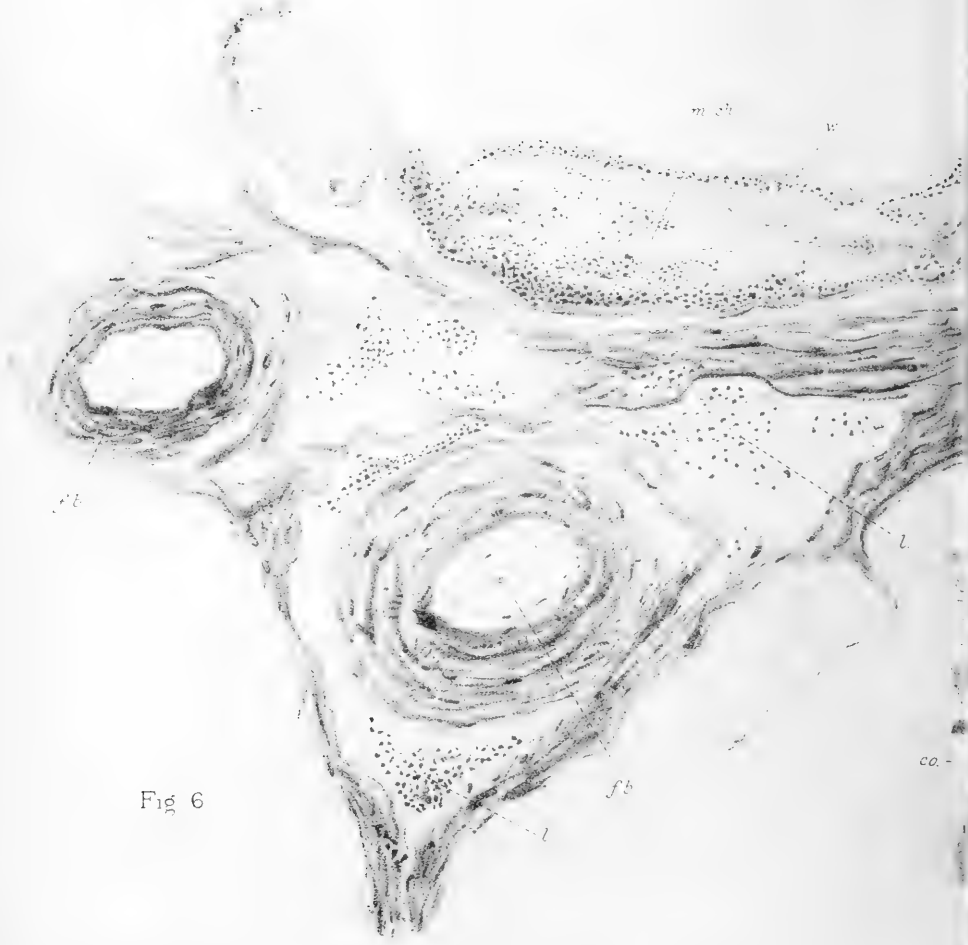

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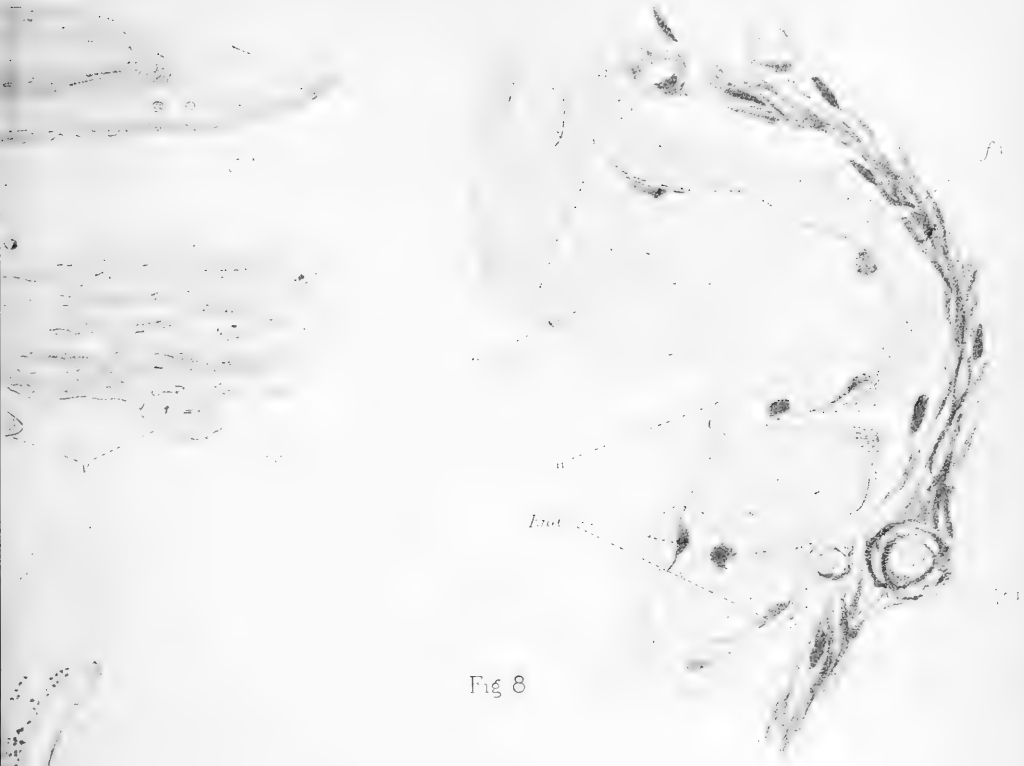

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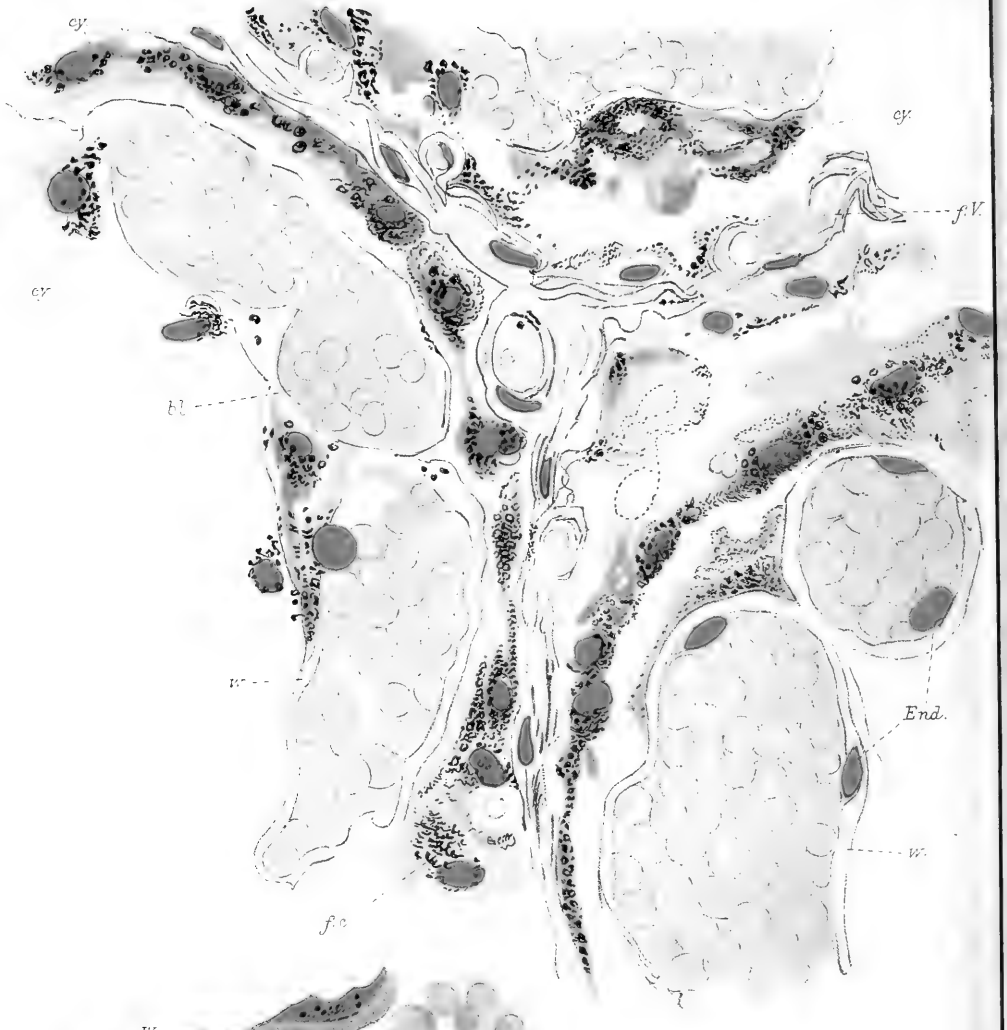

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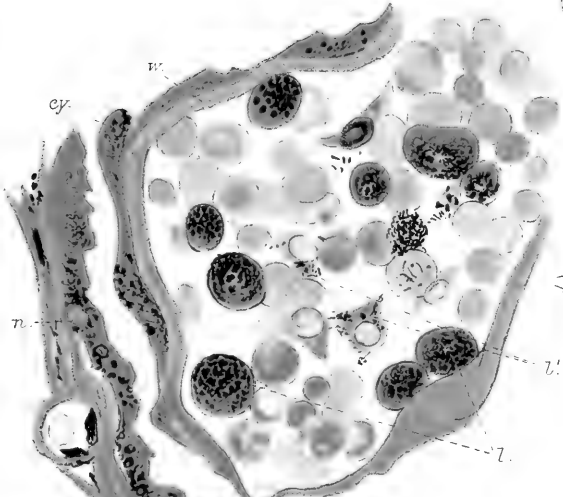

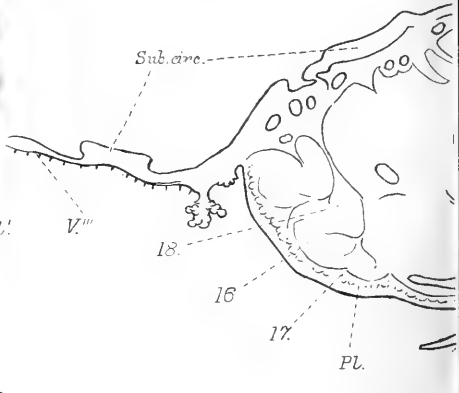

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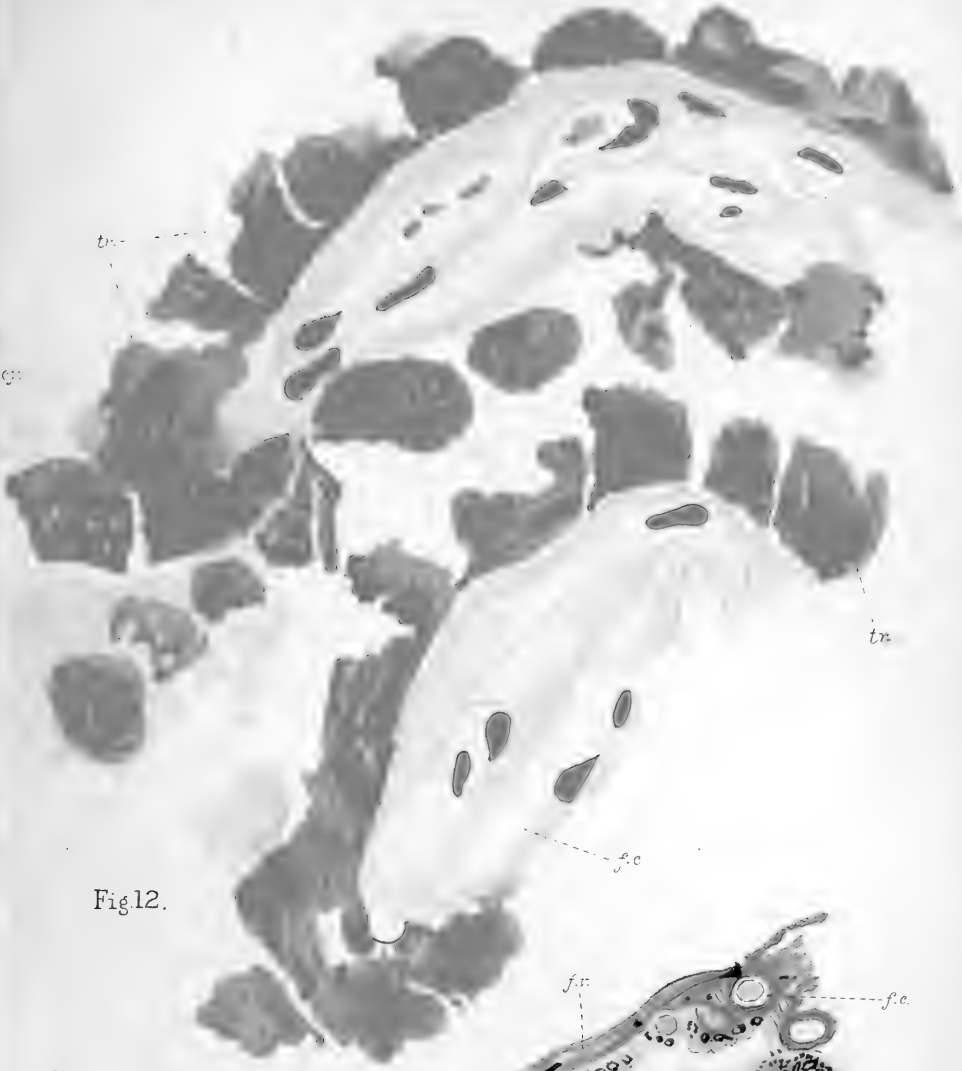

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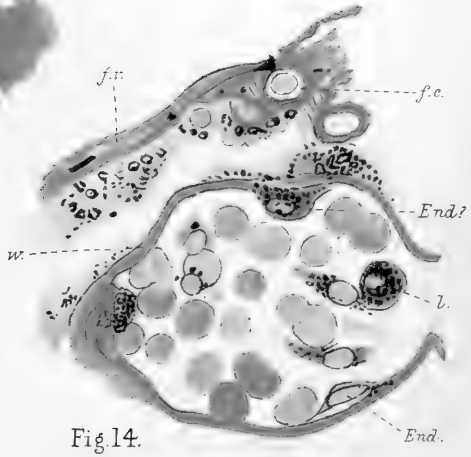

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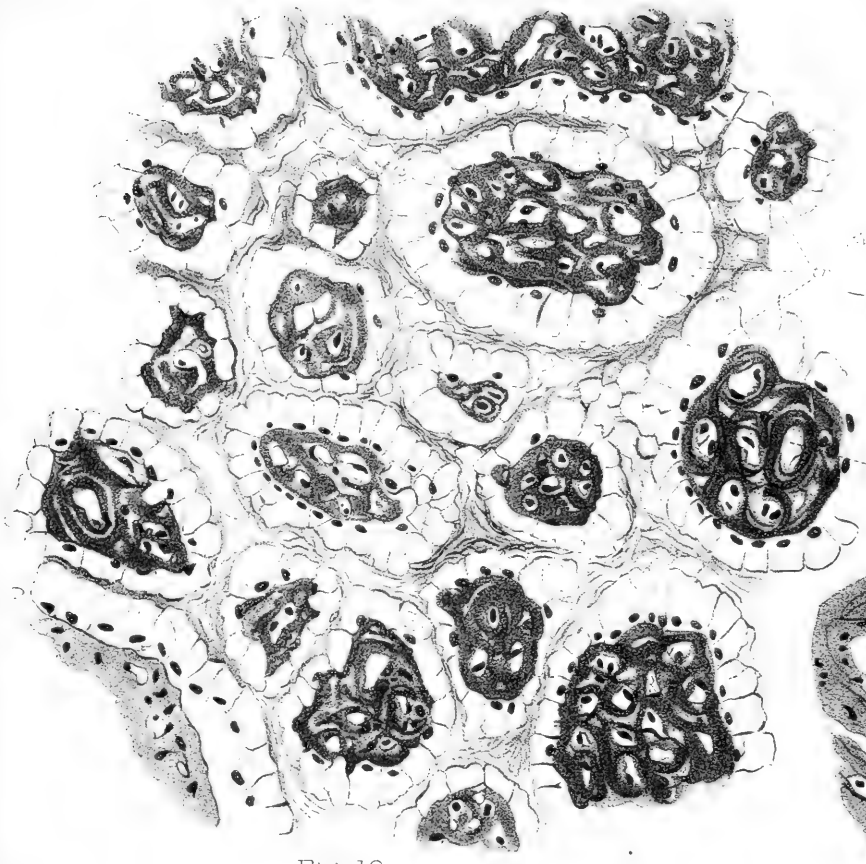

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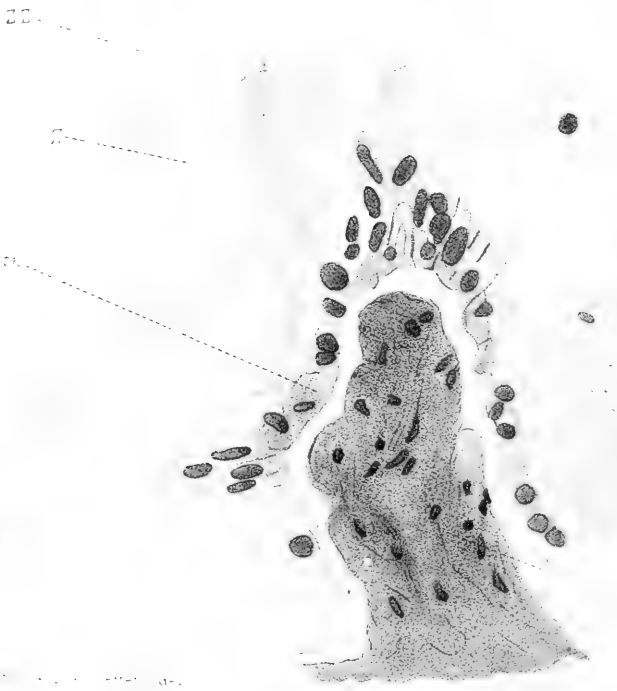

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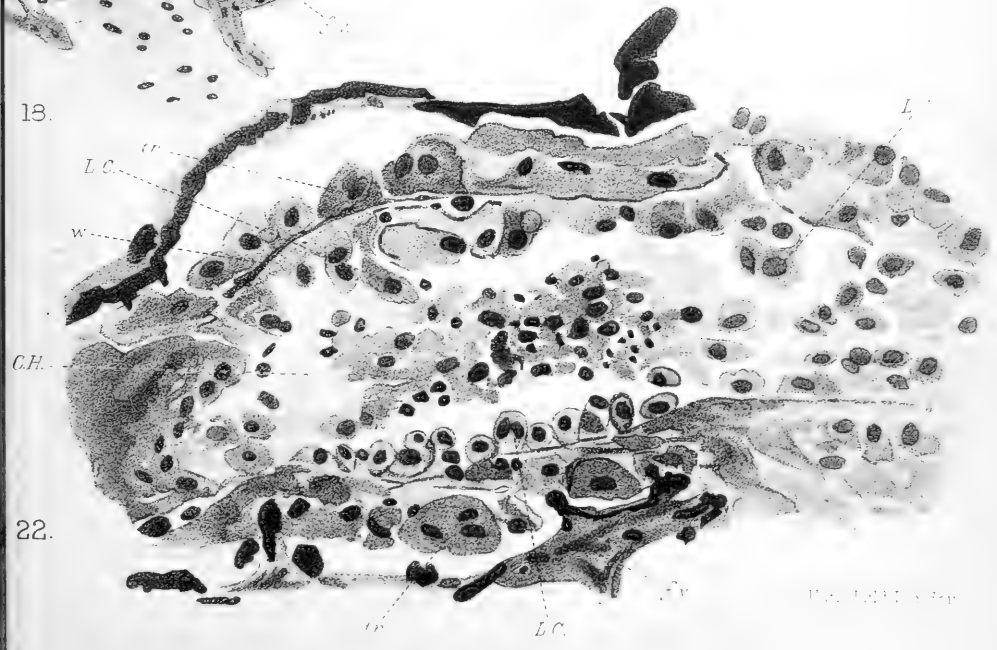

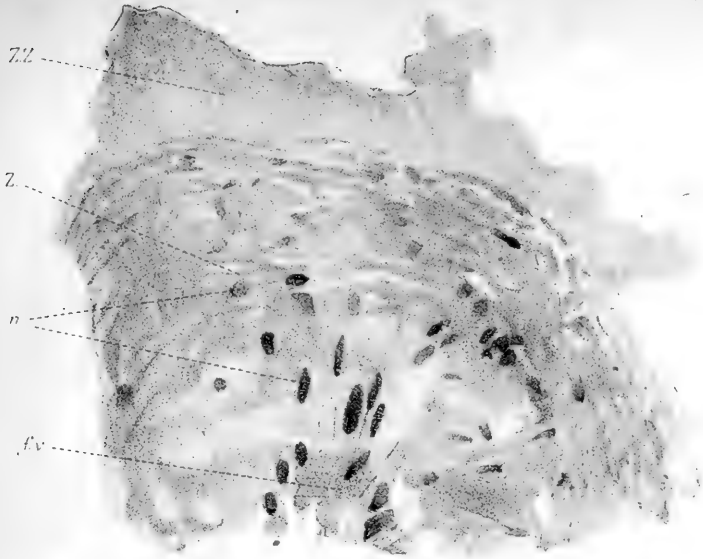

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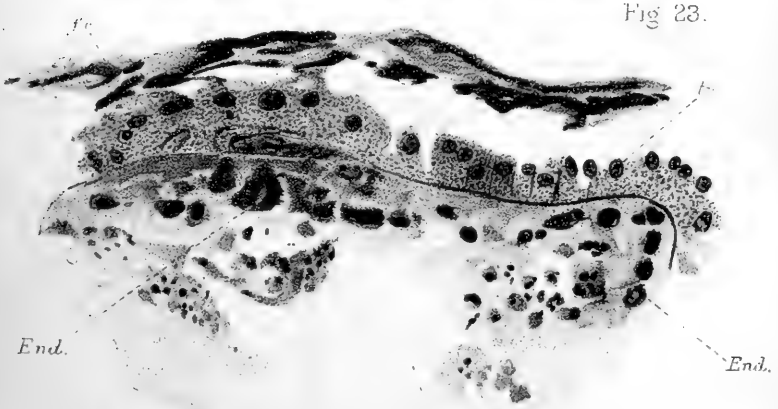

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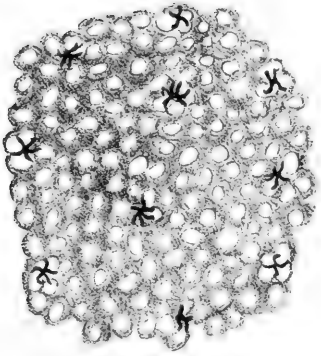

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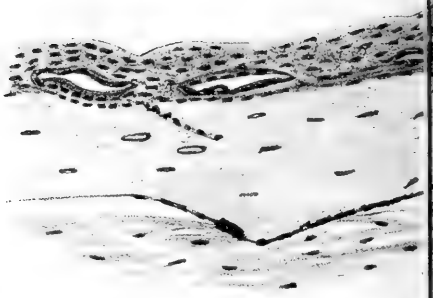

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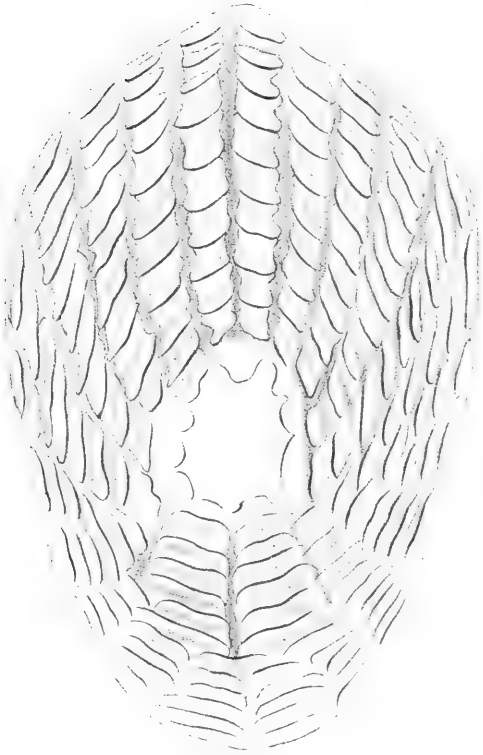

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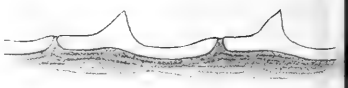

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Figs. 4–6. Ed. Wilson, del.

Fig. 2.

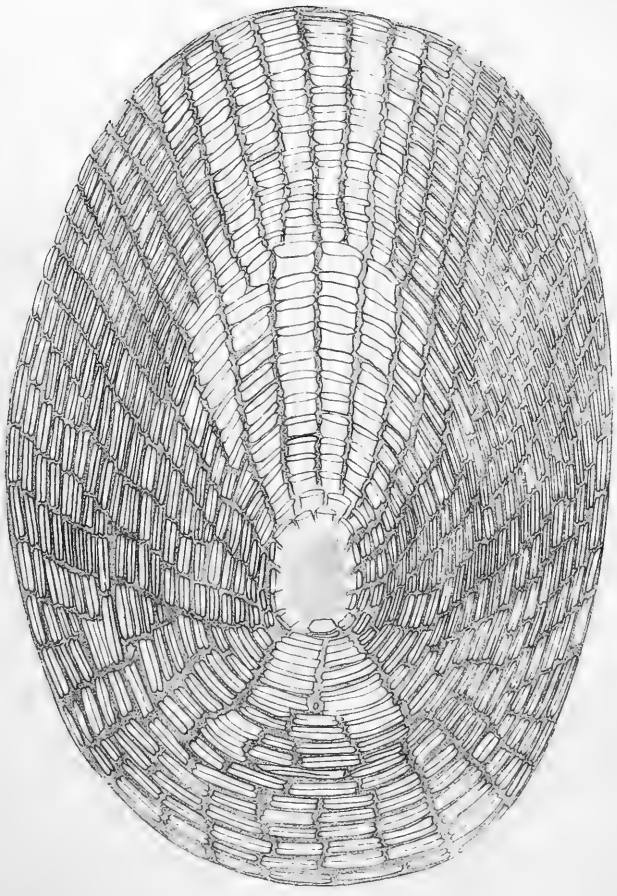

Fig. 6.

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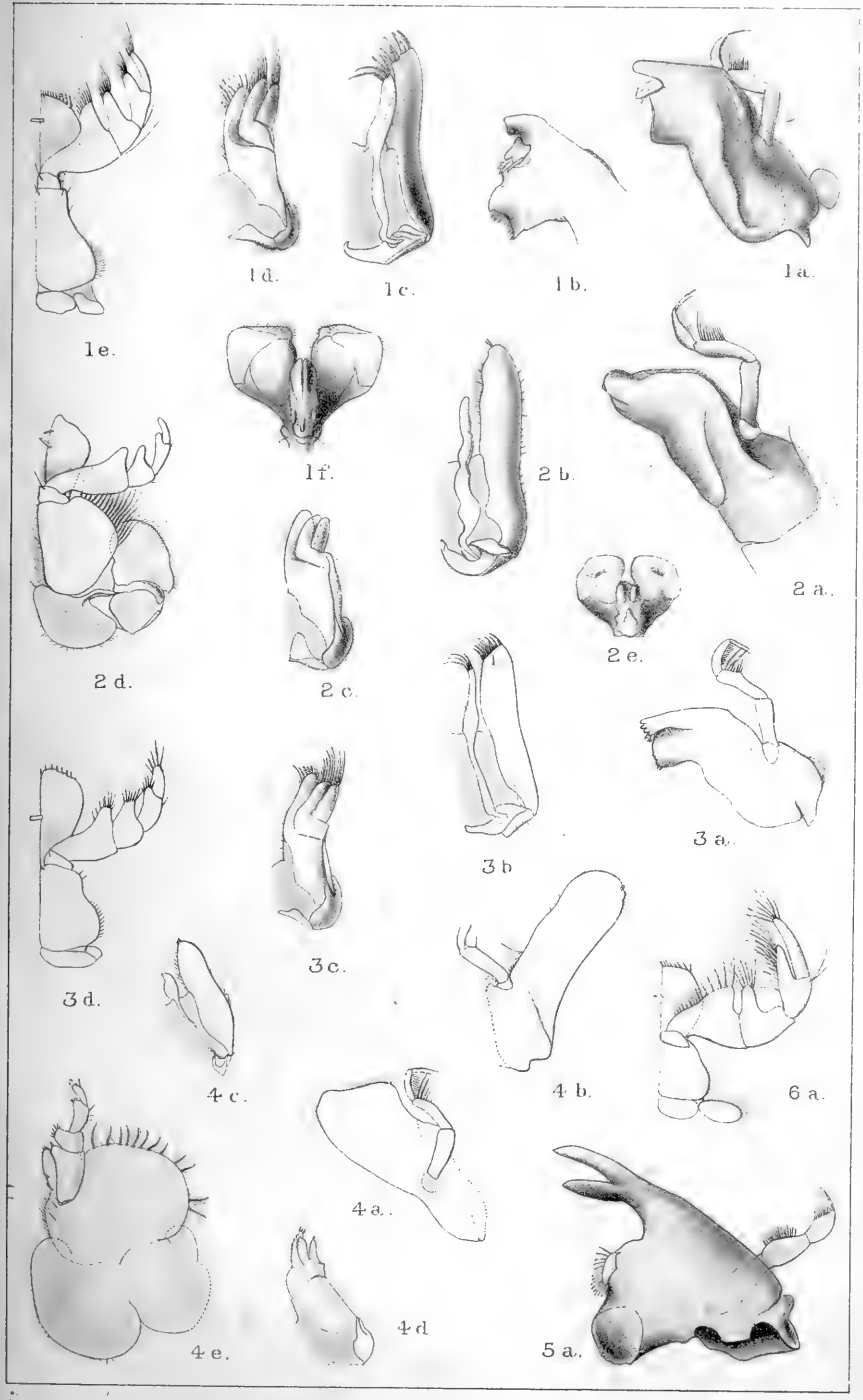


H. J. Hansen, del. Huth, sc.

Mouth-Parts of Sphæromidæ.

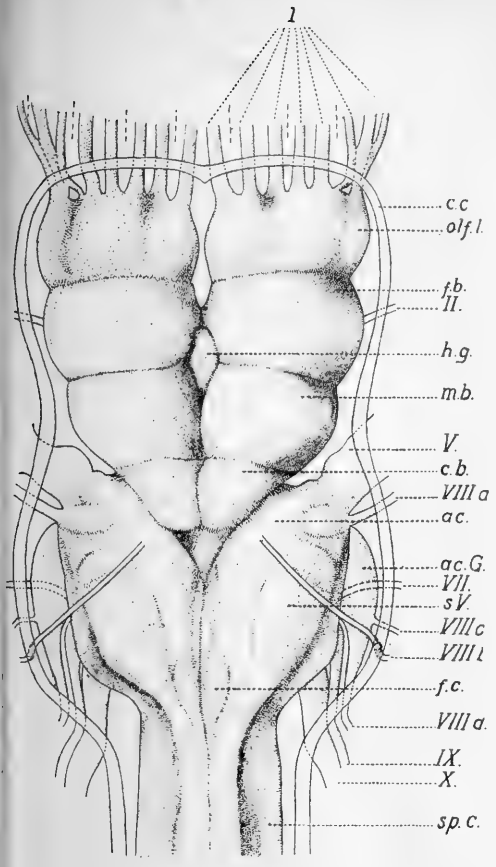


Fig. 1.

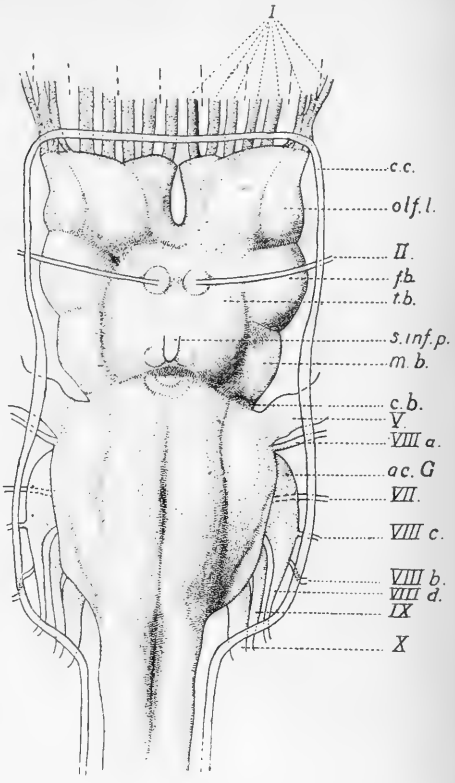
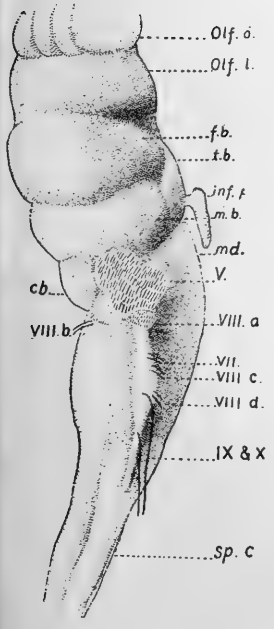


Fig. 2.



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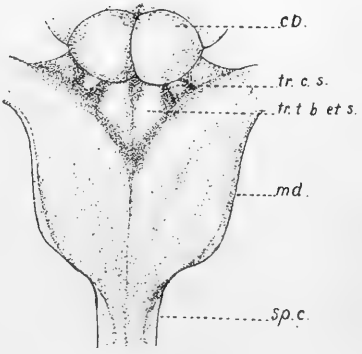

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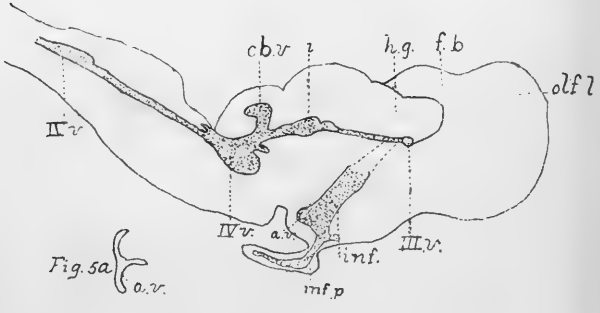

Fig. 5.

Adlard & Son, Impr.

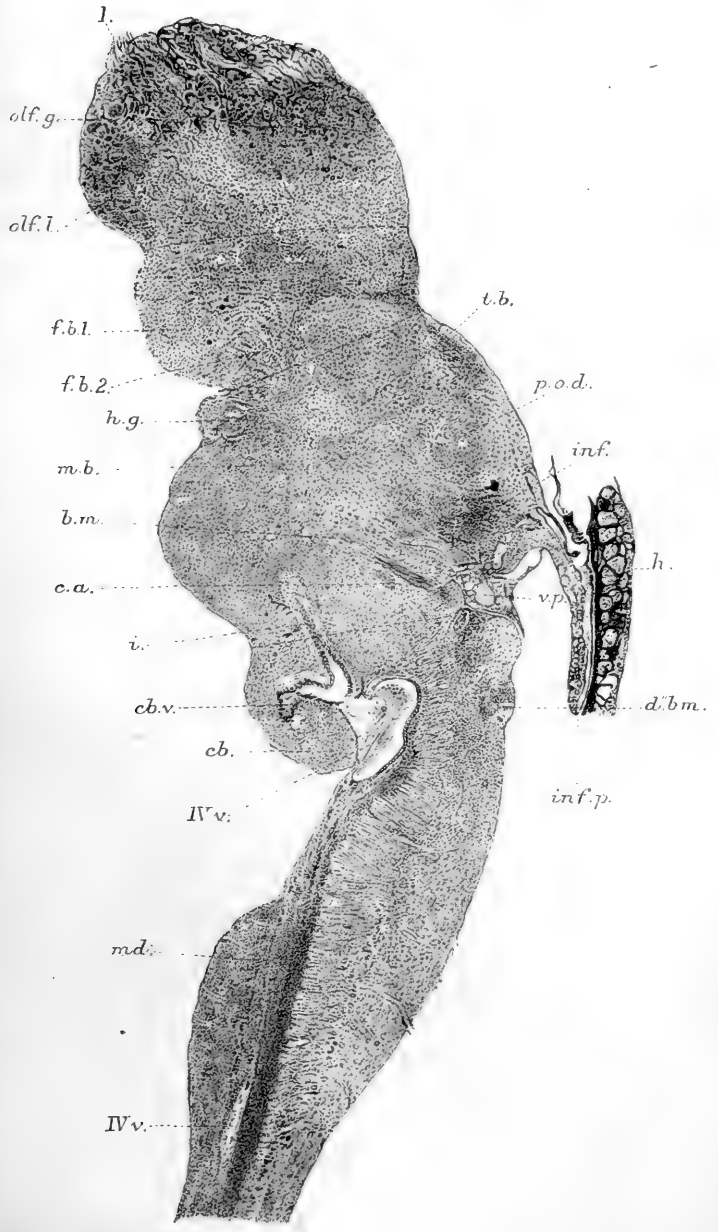

Fig. 6.

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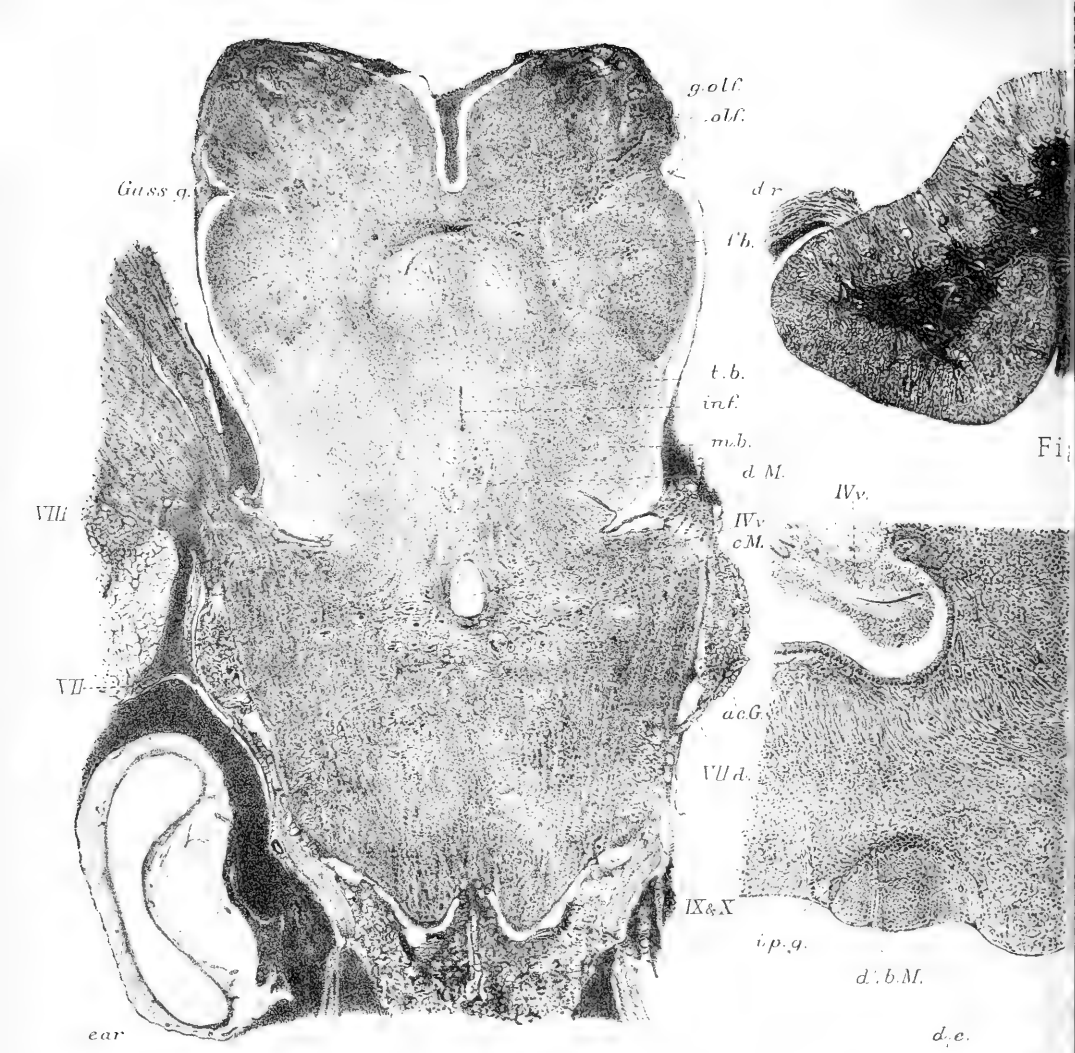

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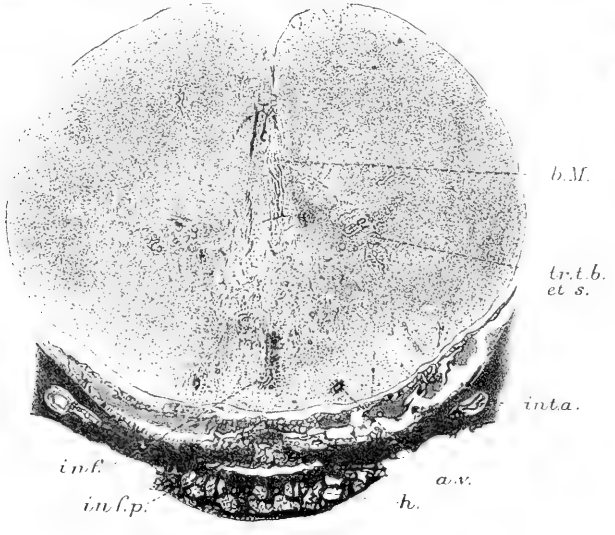

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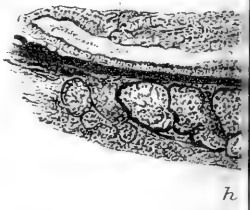

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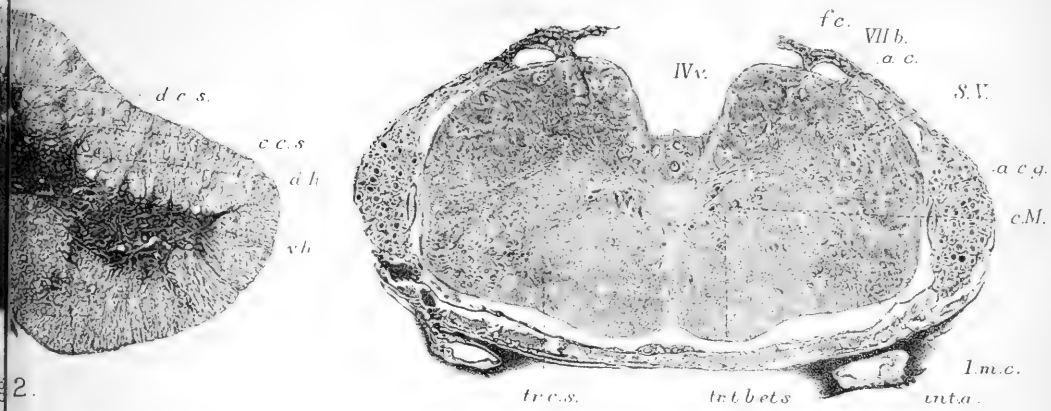

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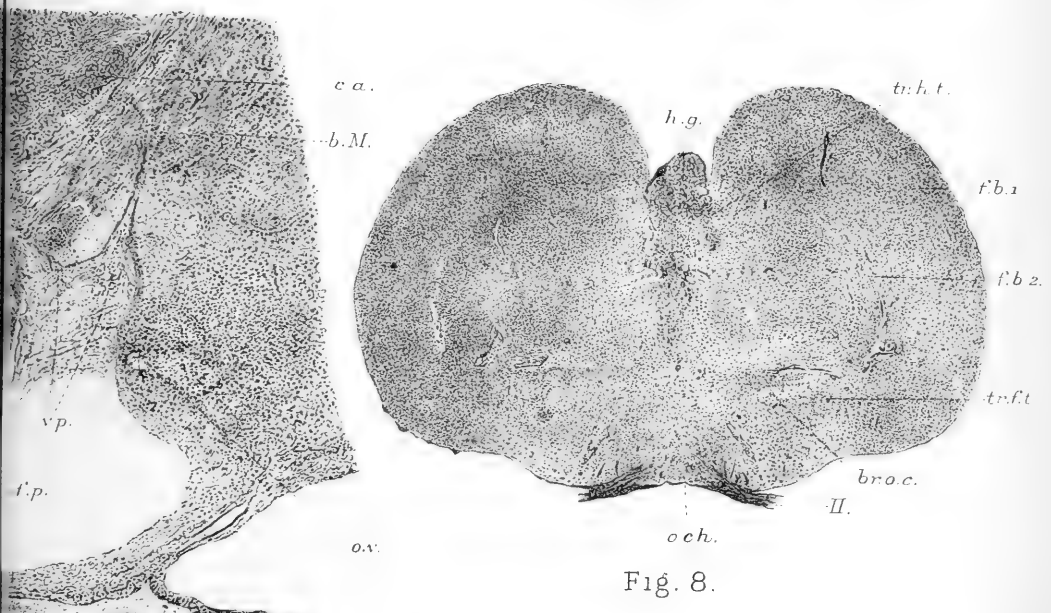

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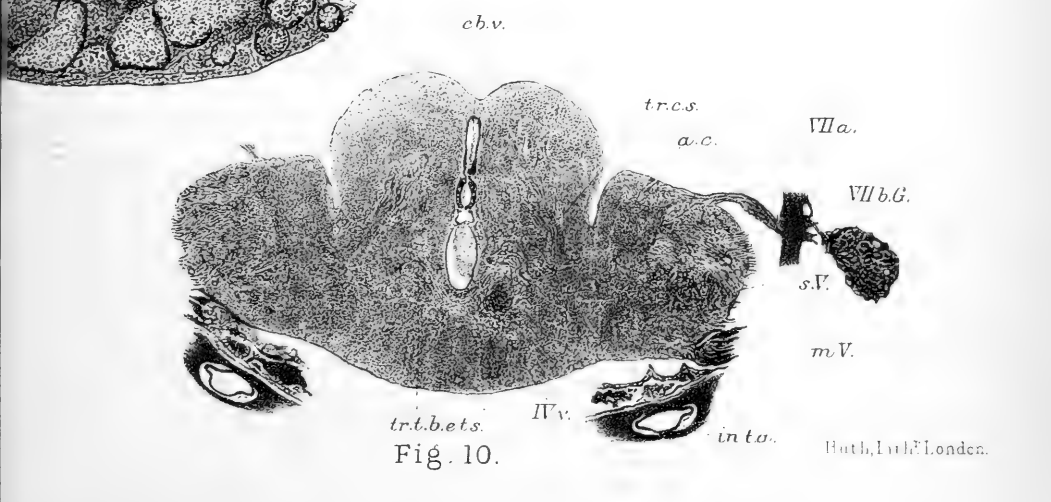

Fig. 10.

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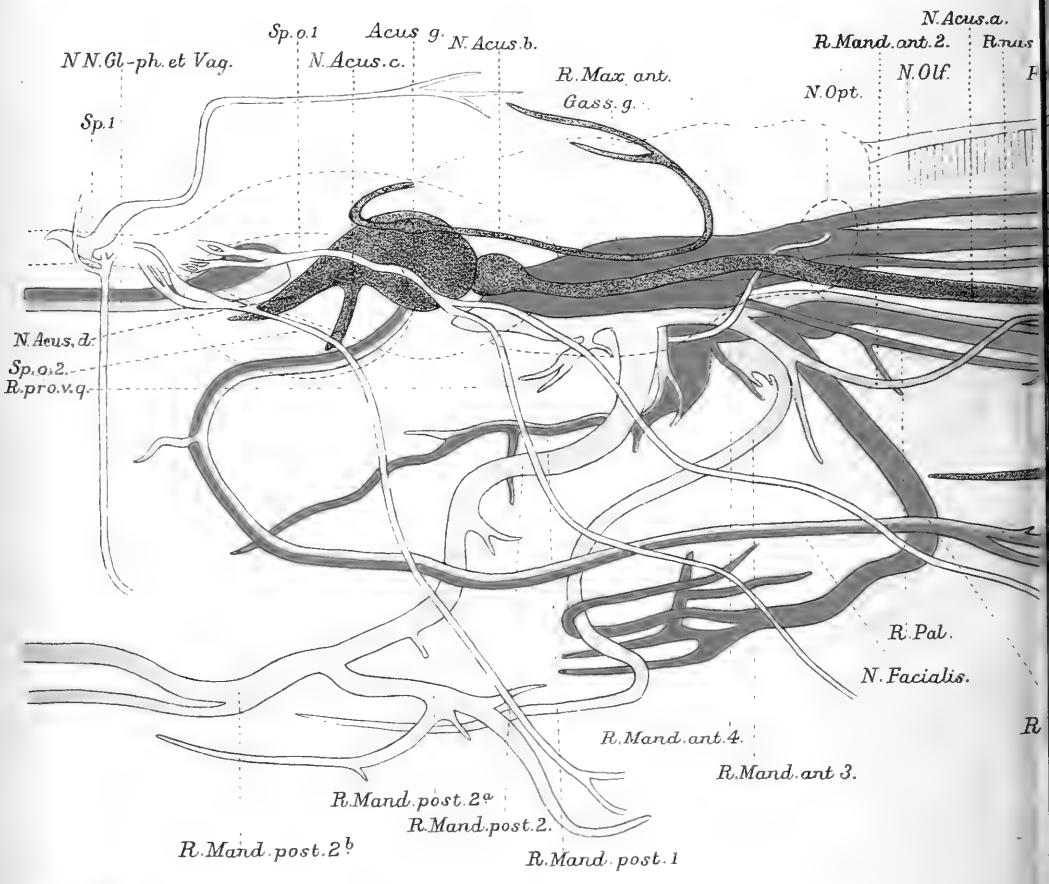
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R.Pal.
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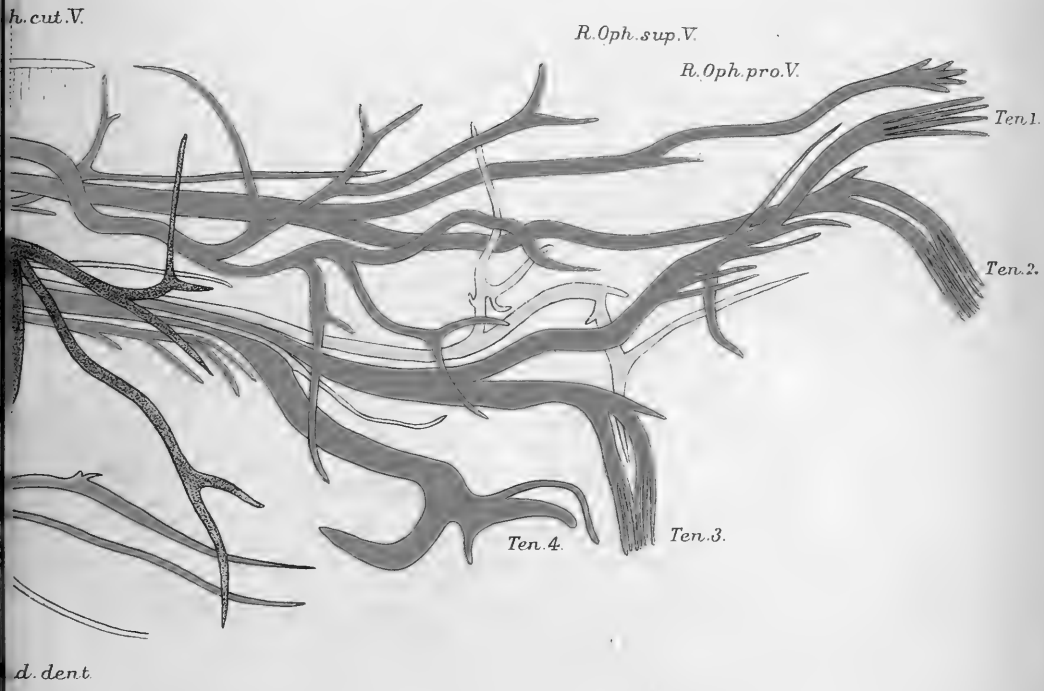
Fig



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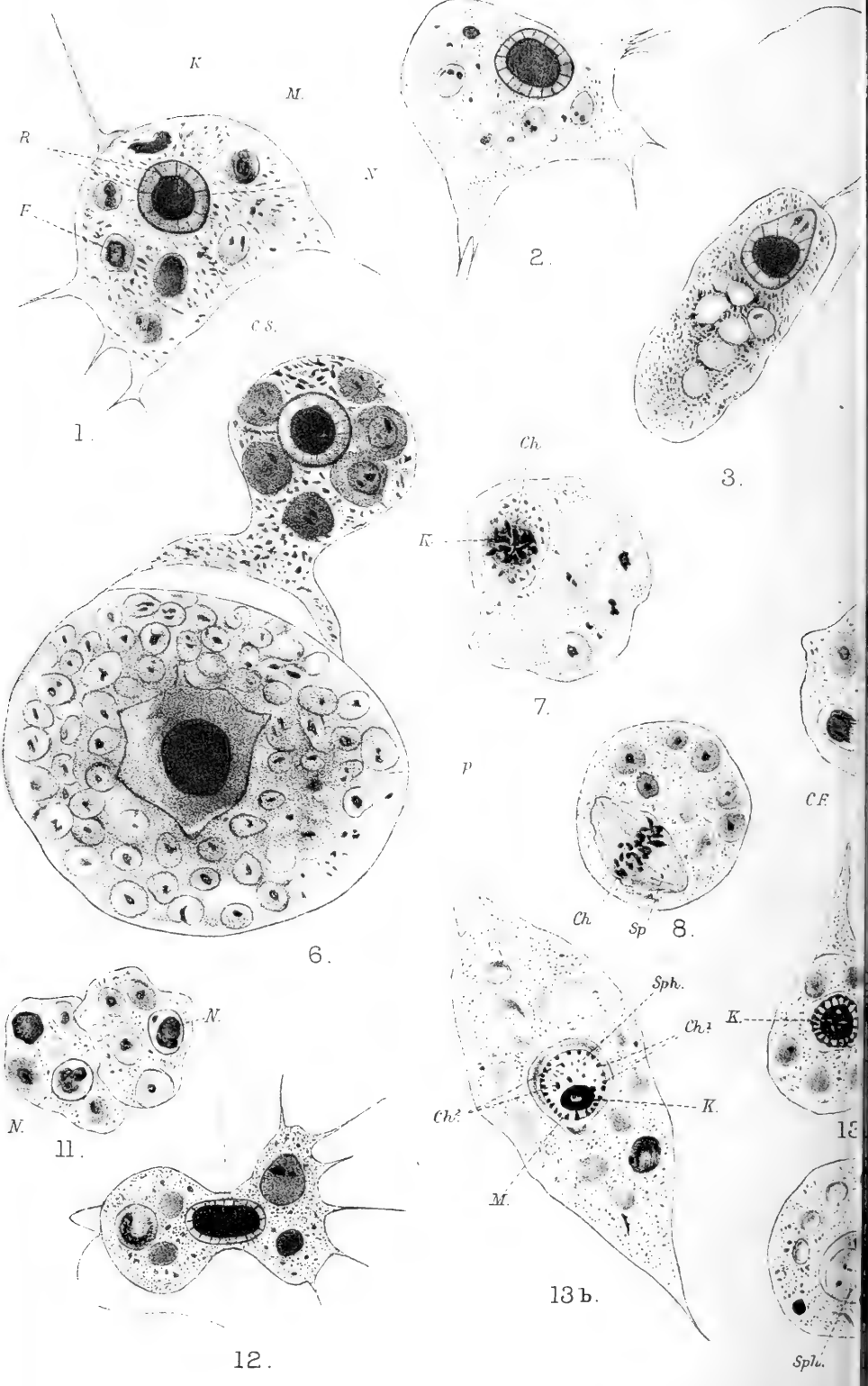
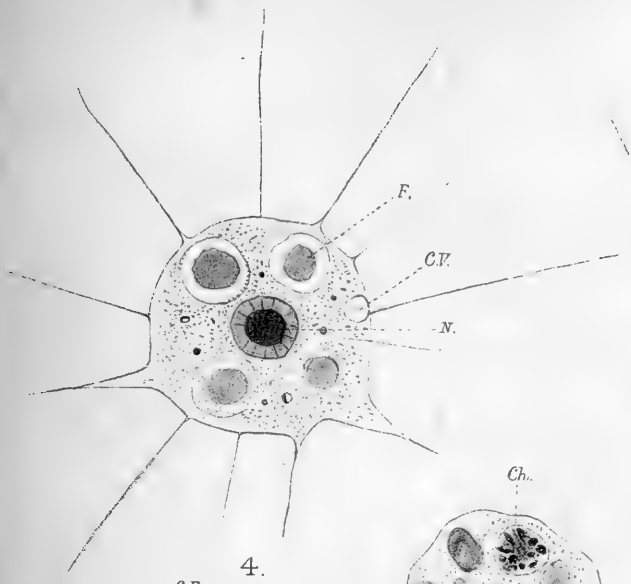


M. Robertson del



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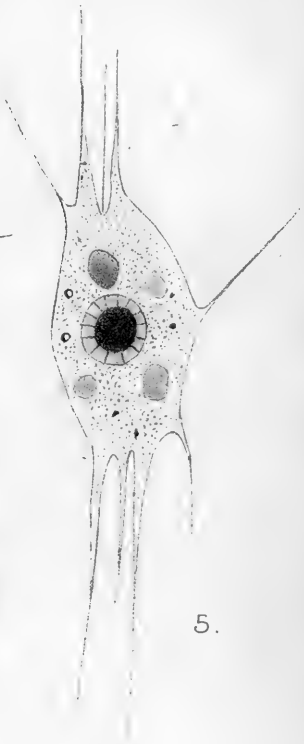

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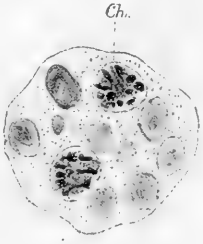

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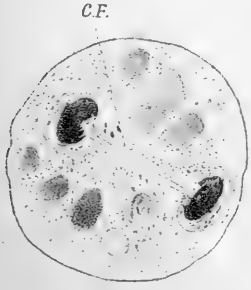

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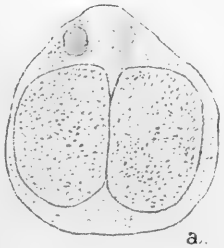

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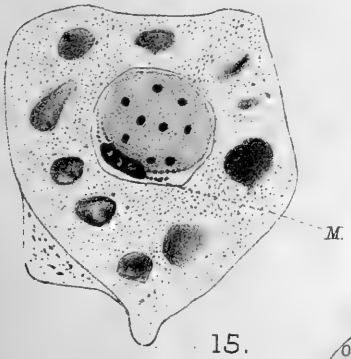

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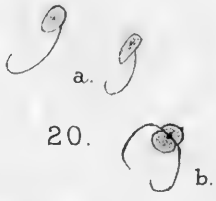

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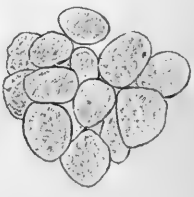

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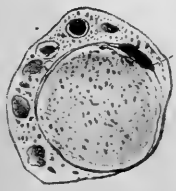

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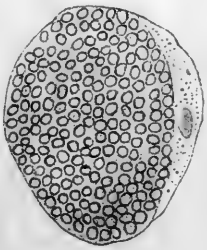

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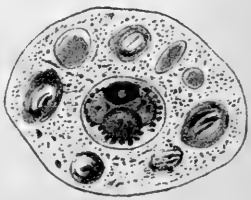

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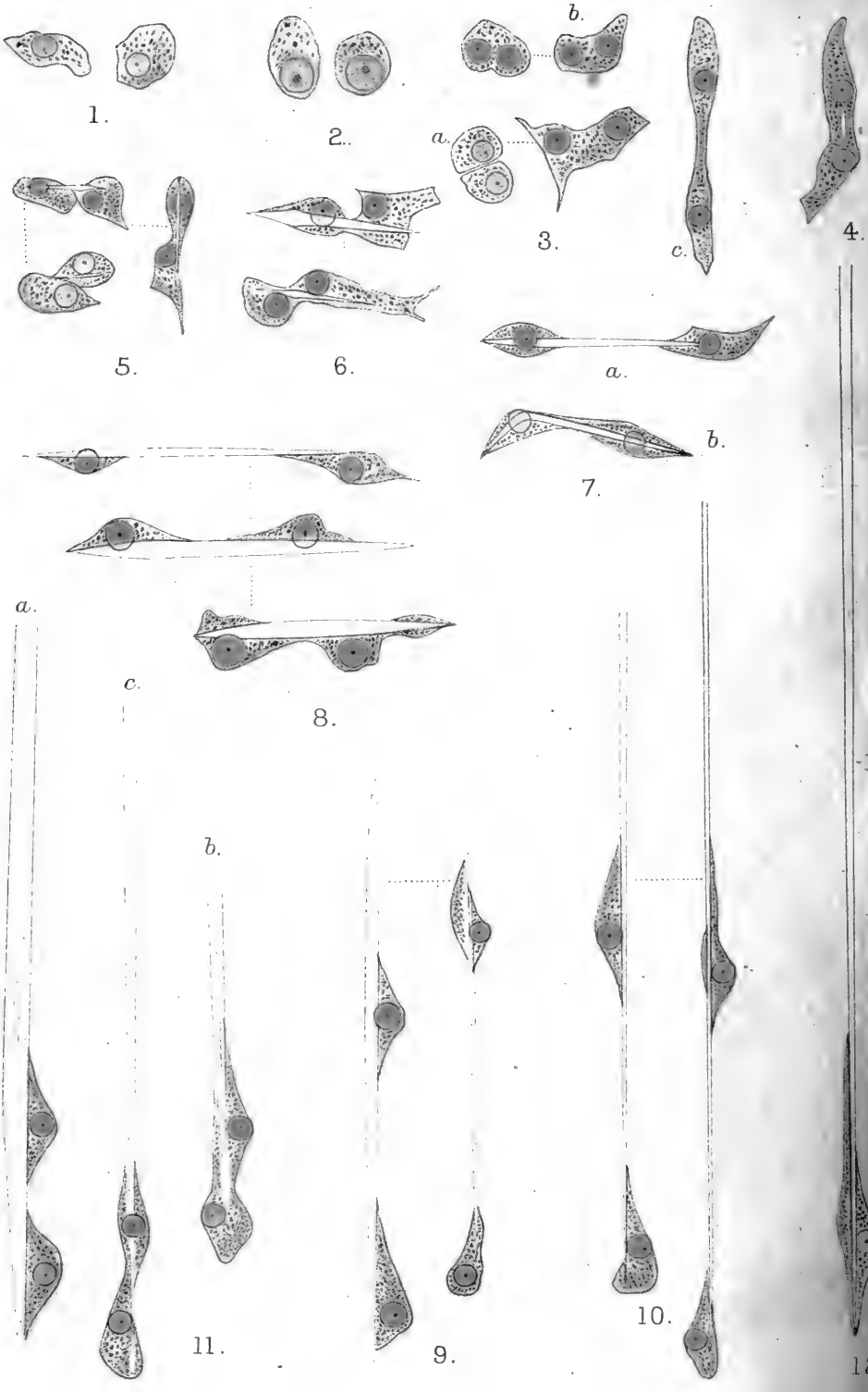
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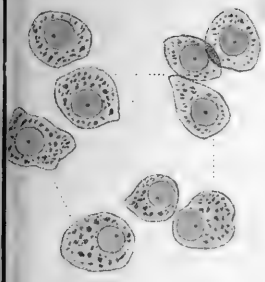

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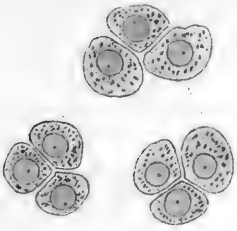

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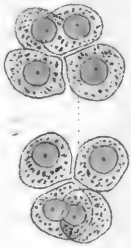

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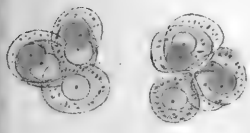

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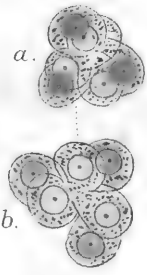

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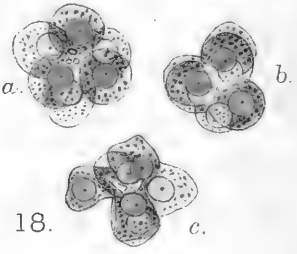

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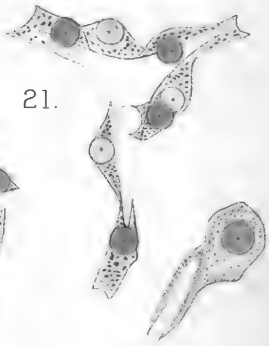

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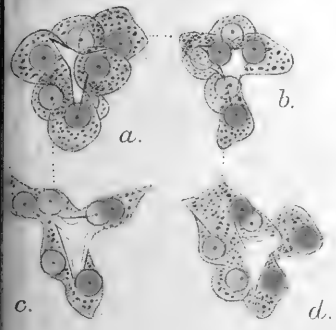

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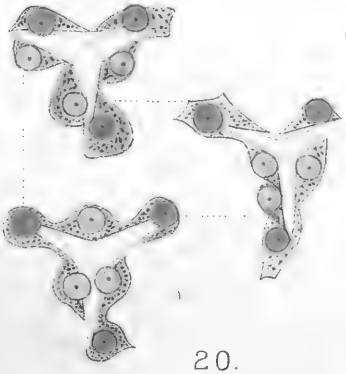

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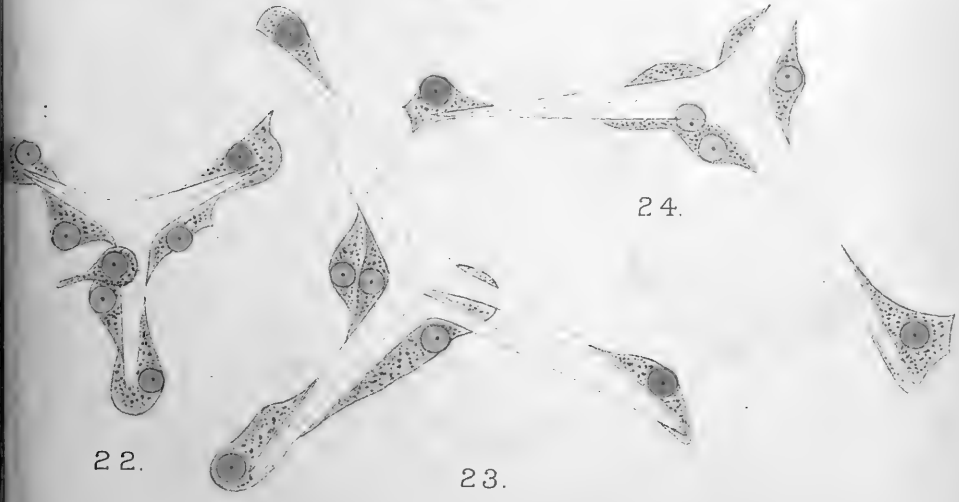

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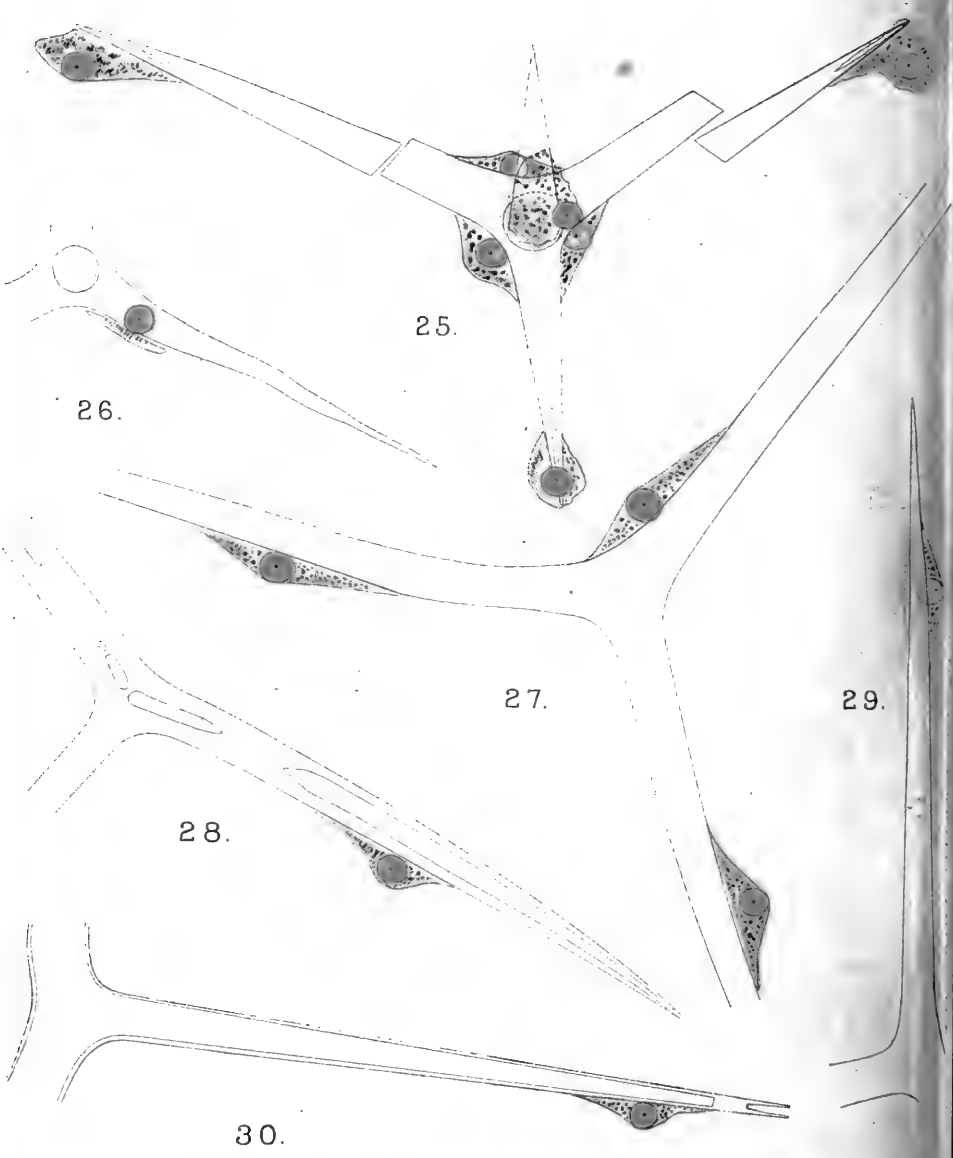
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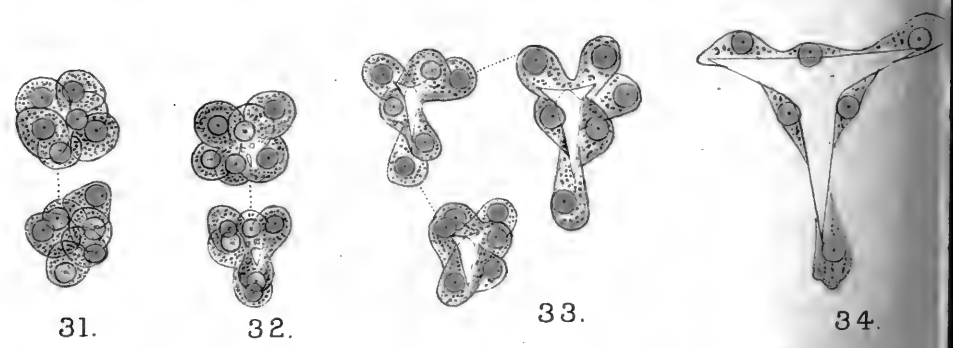
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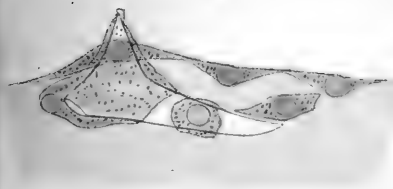

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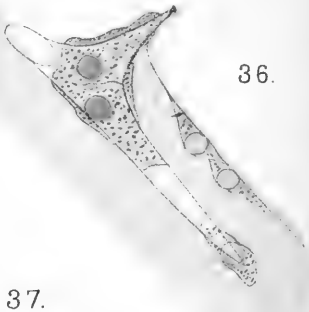

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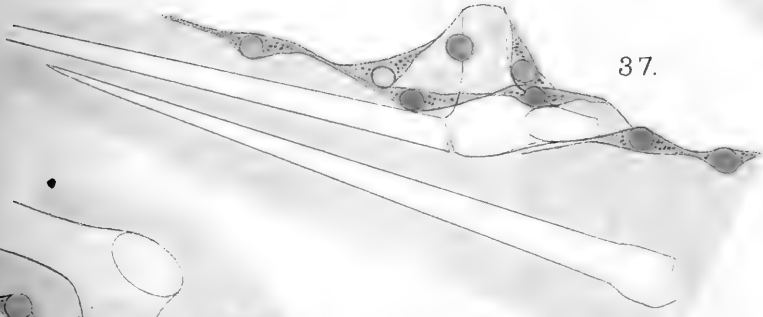

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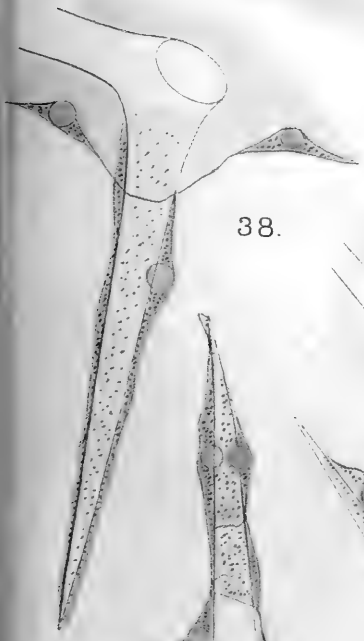

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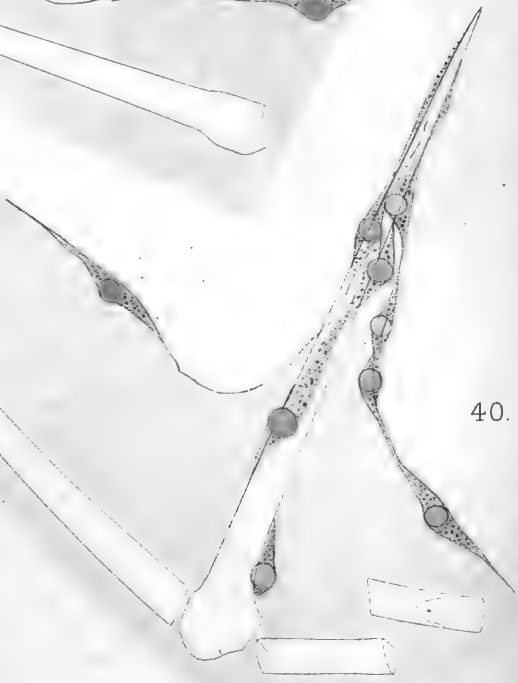

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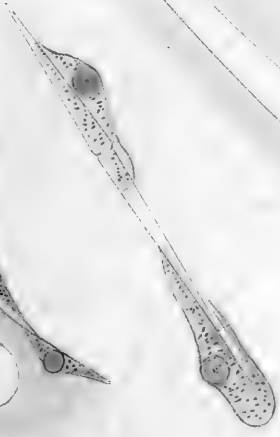

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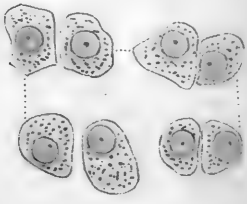

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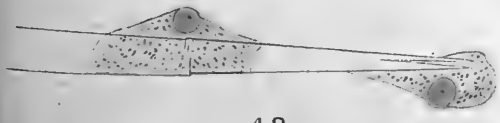

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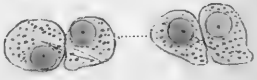

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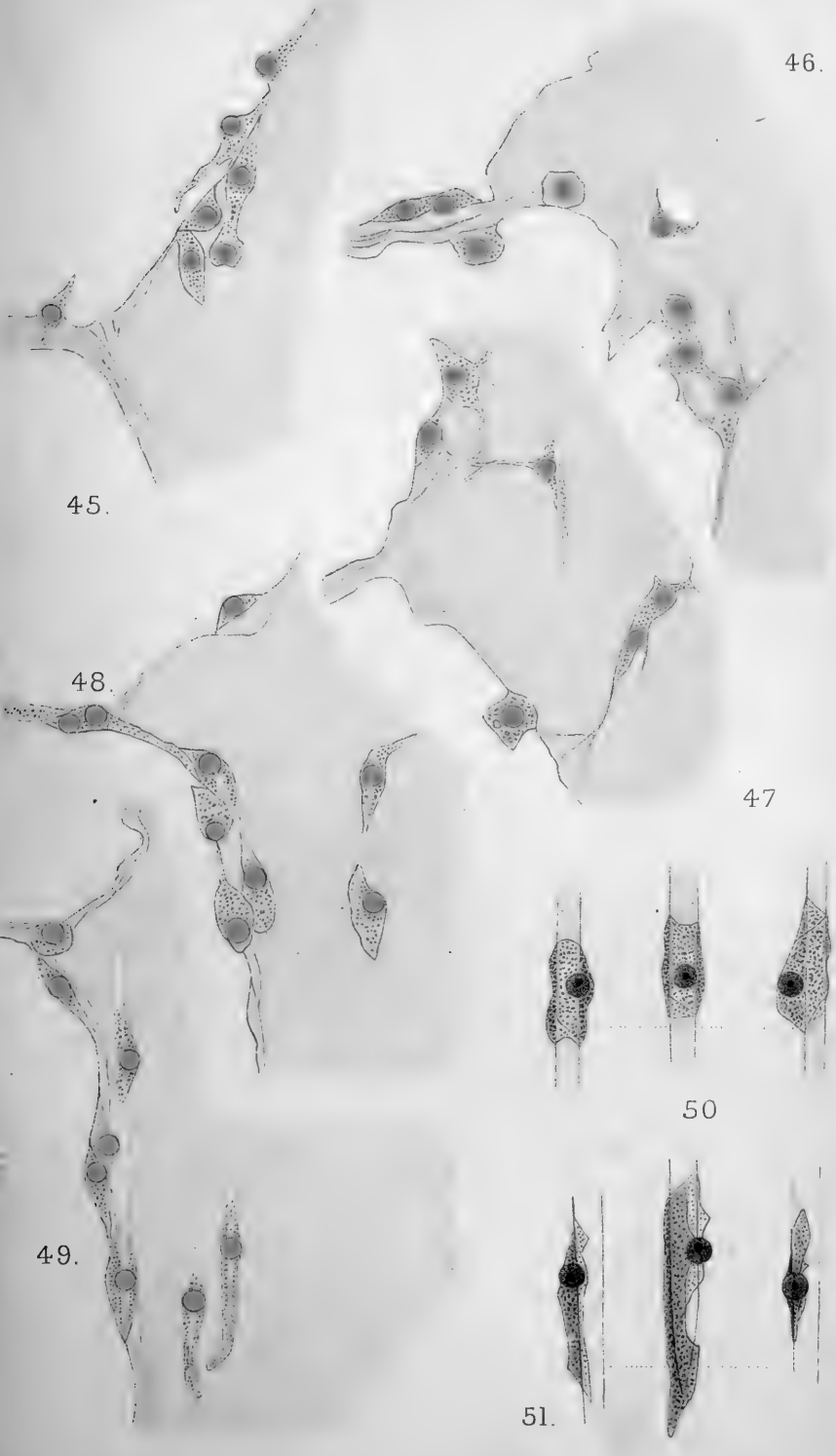

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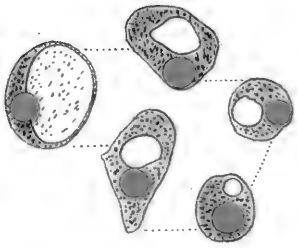

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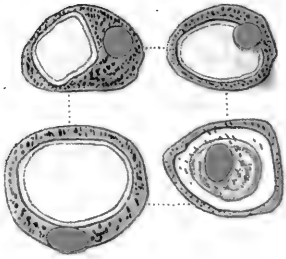

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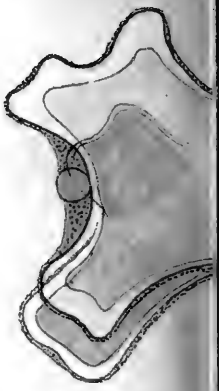

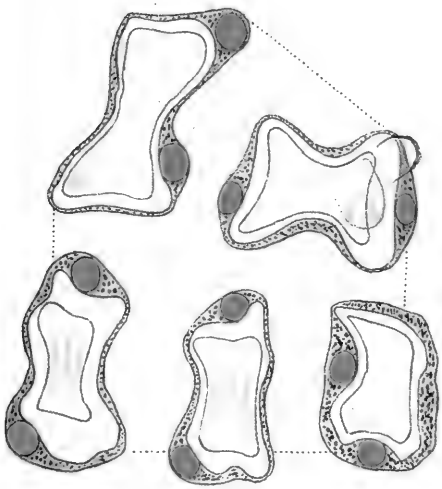

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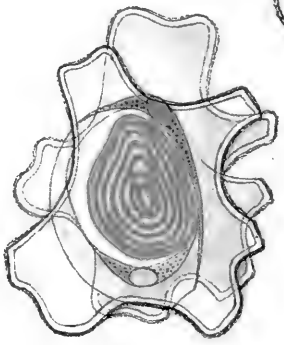

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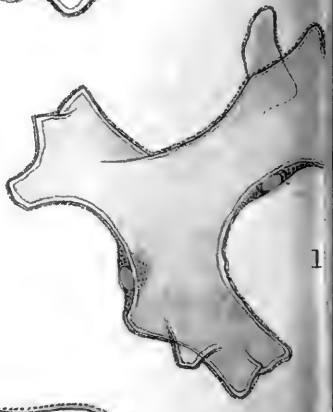

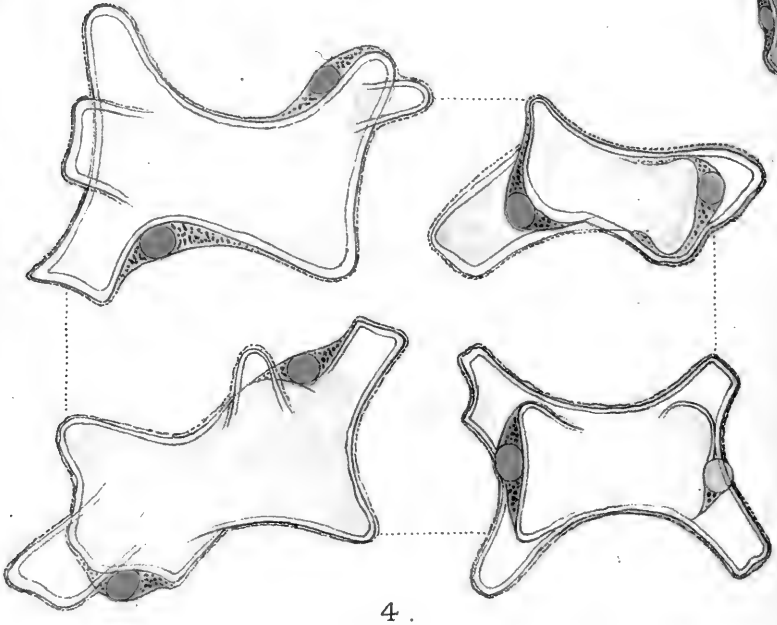

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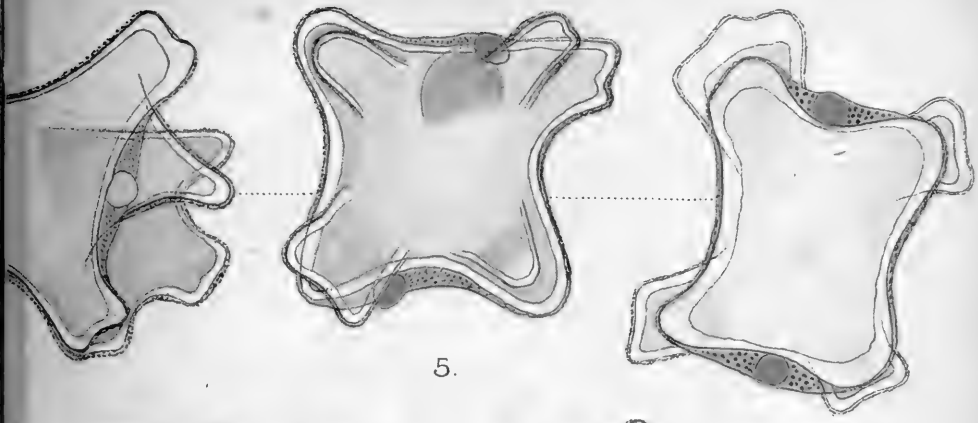

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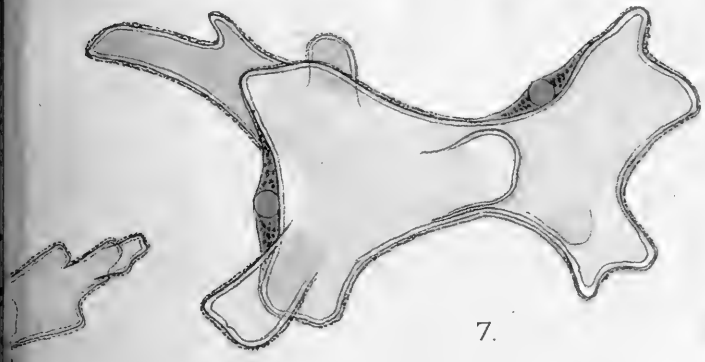

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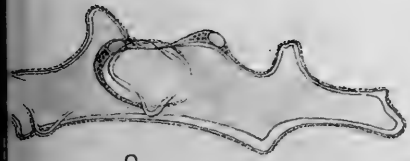

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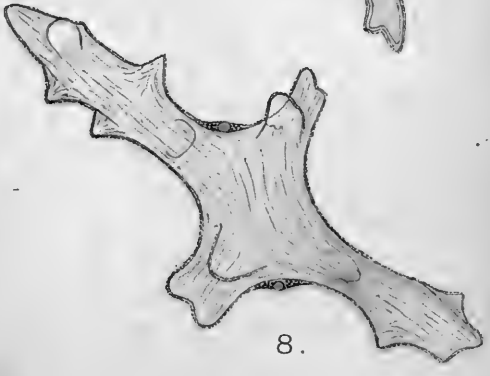

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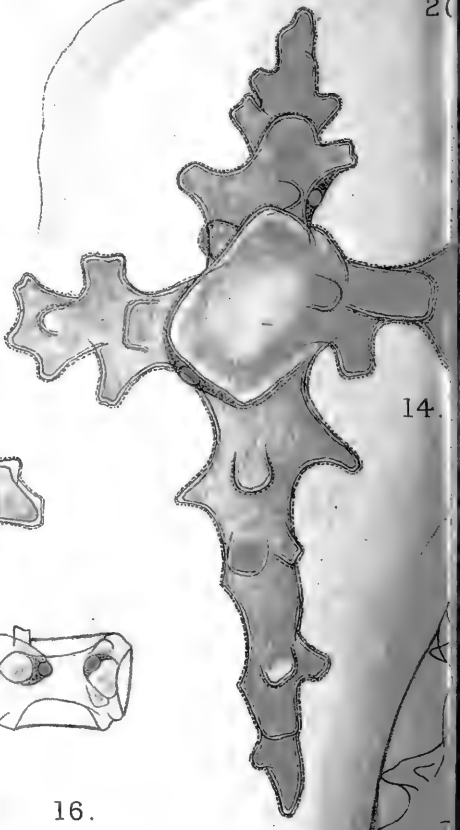

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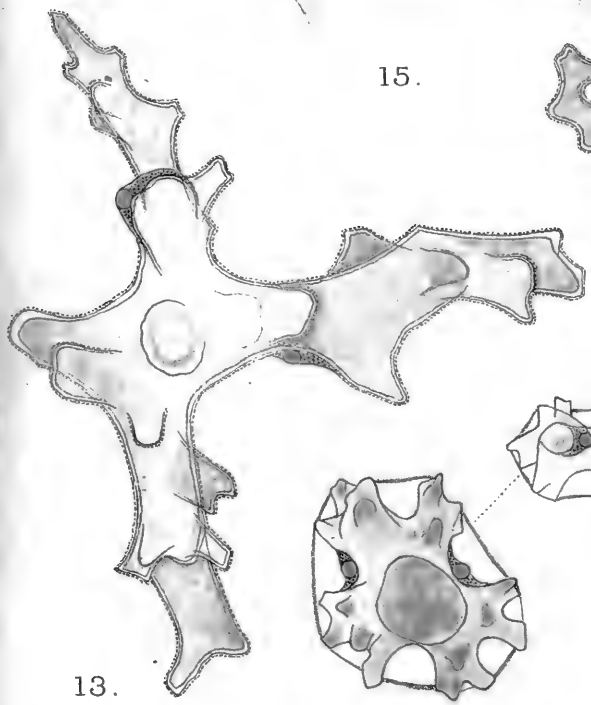

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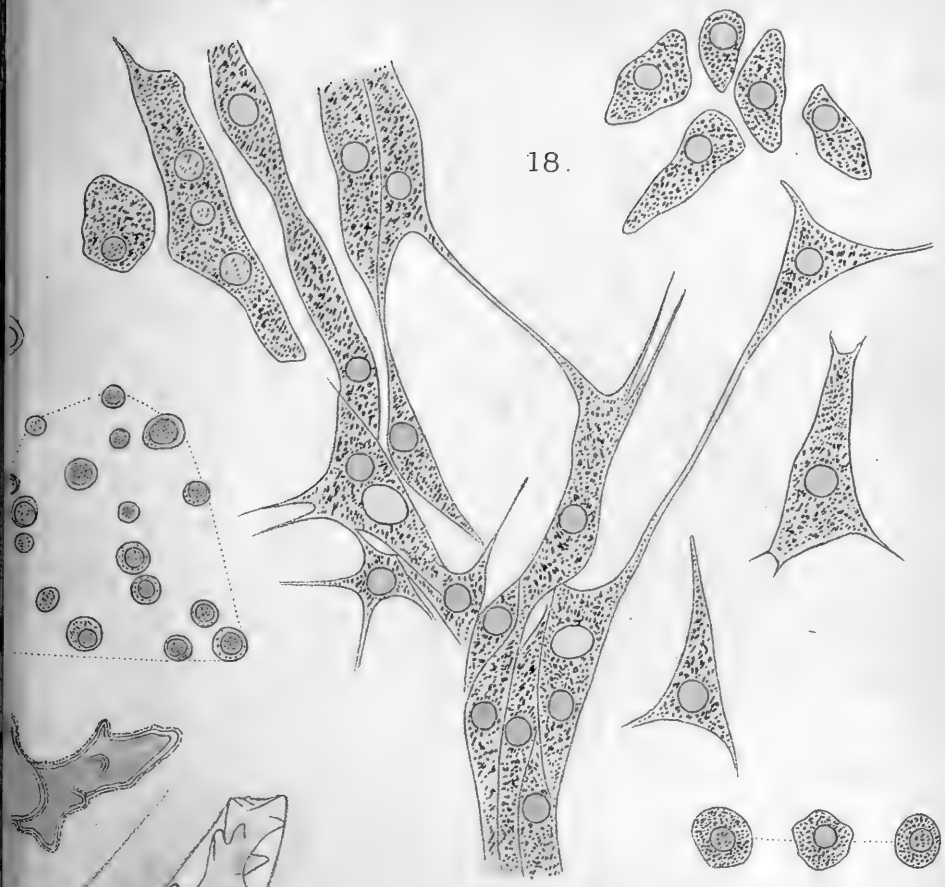

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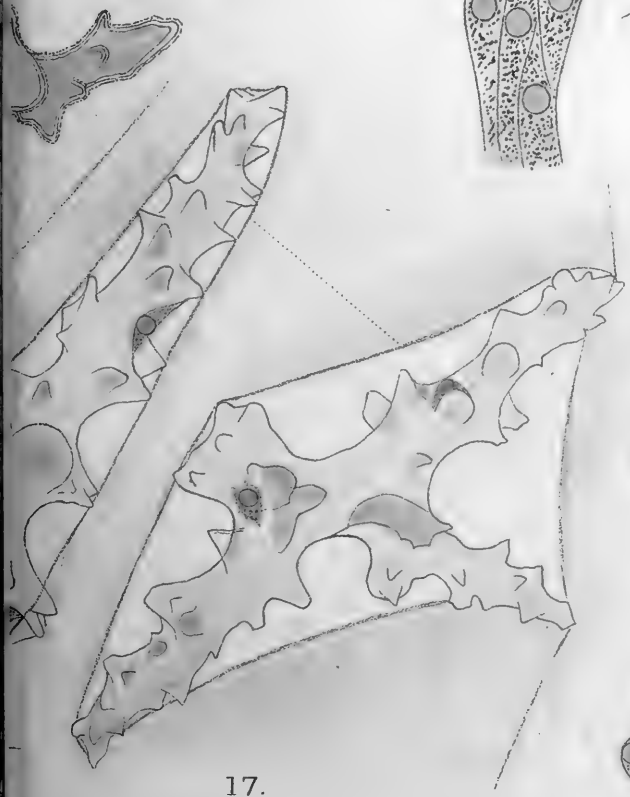

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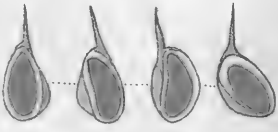

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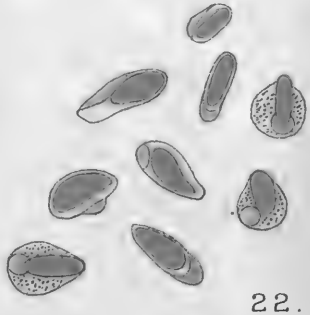

22.

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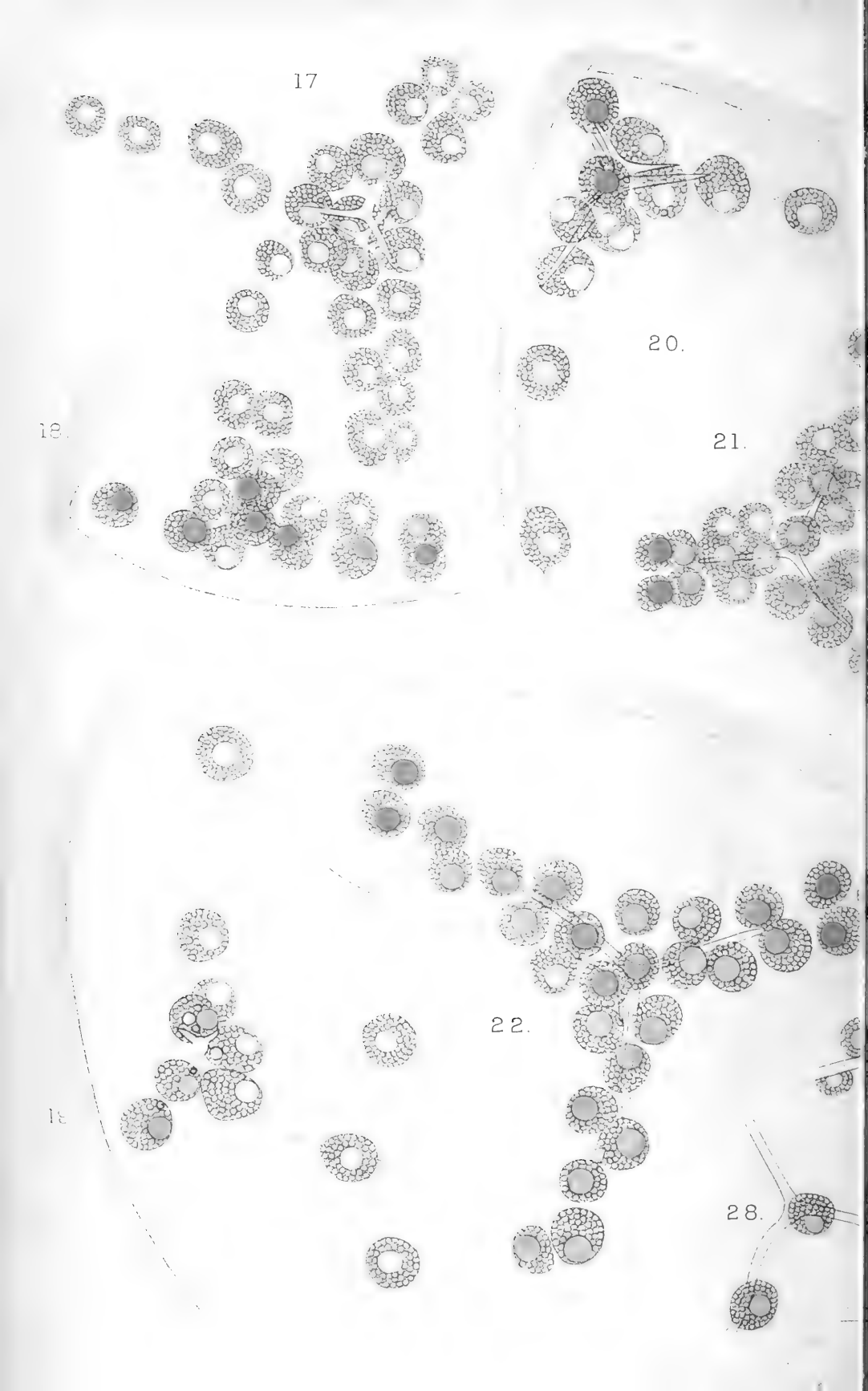
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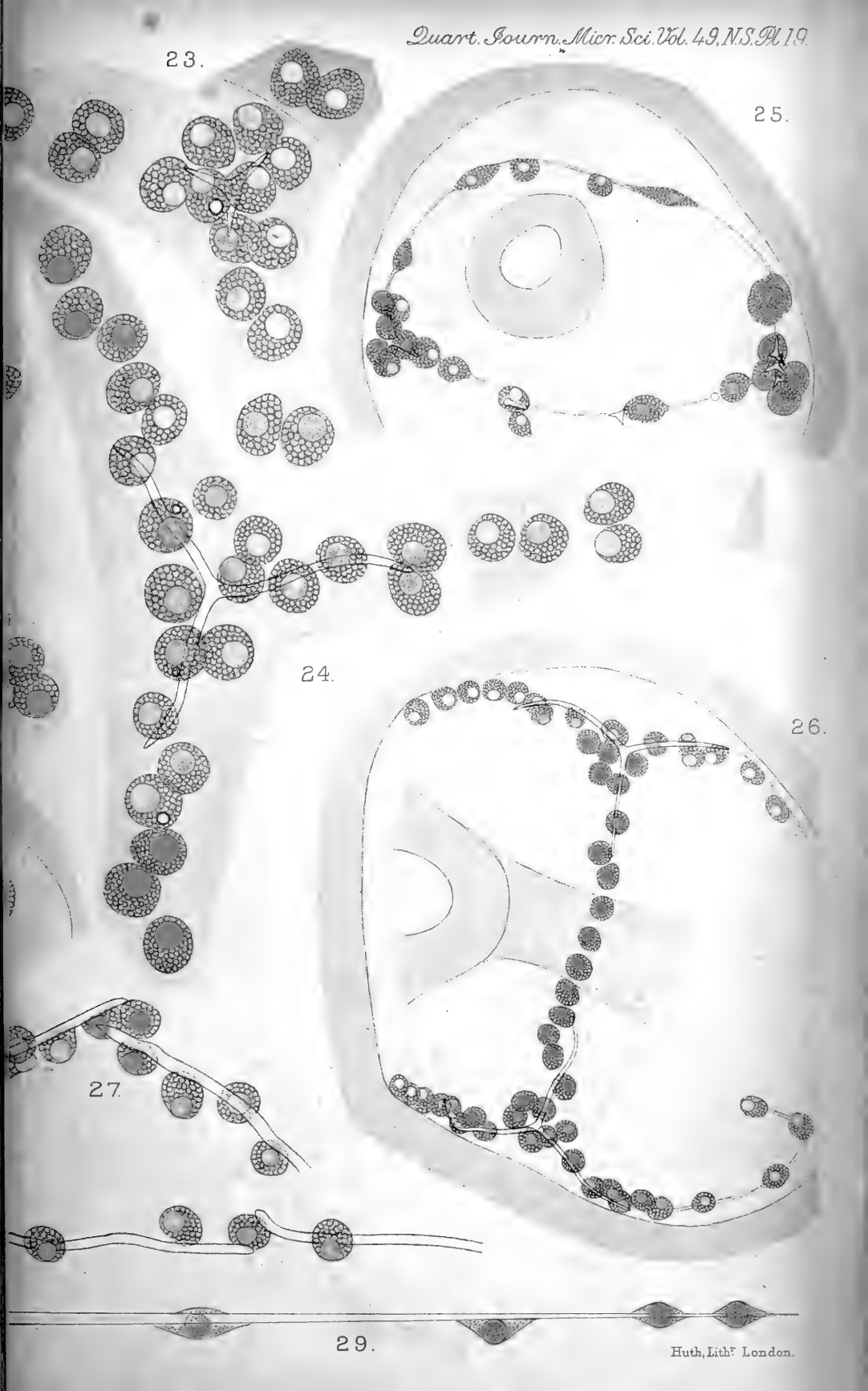

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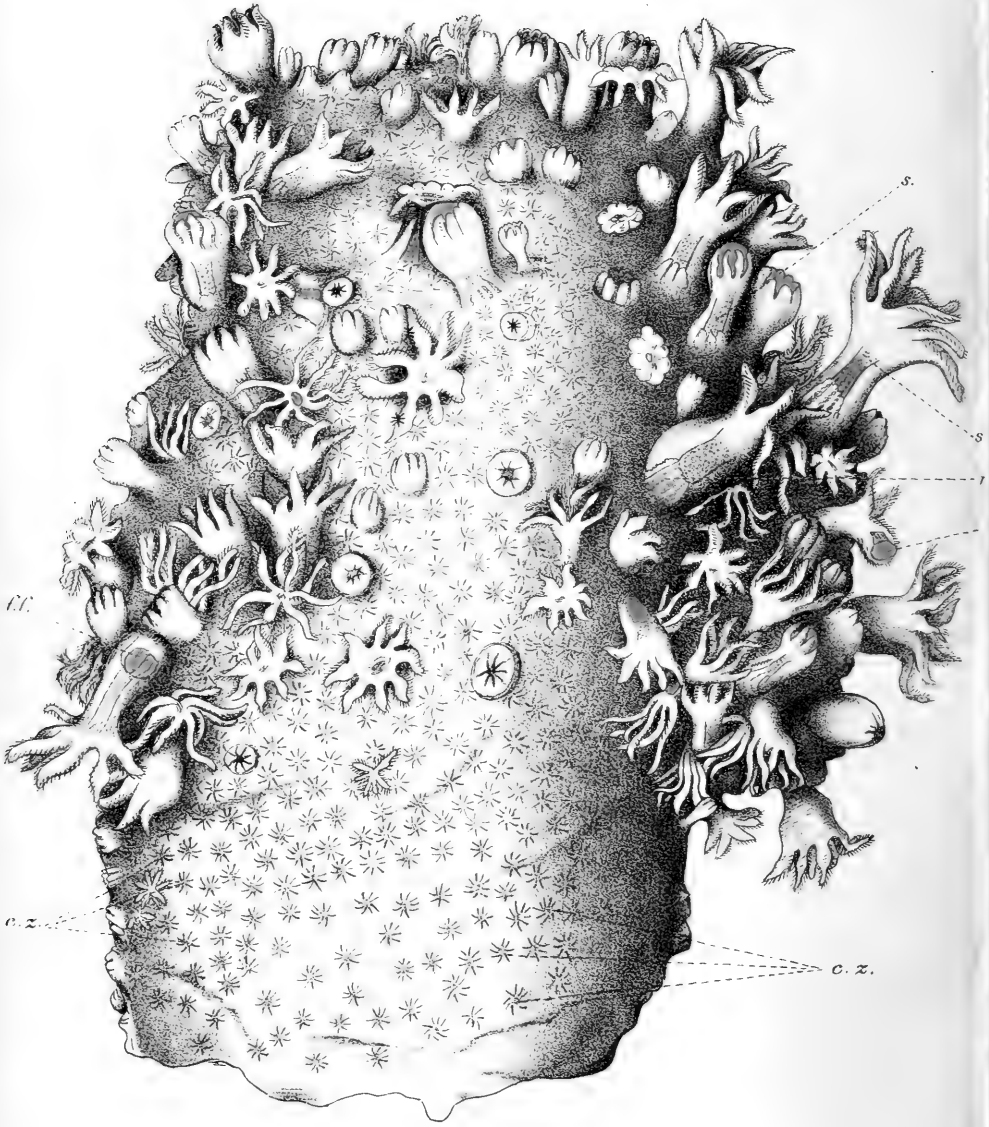

Fig. 1. × 4½

E. M. Pratt del.

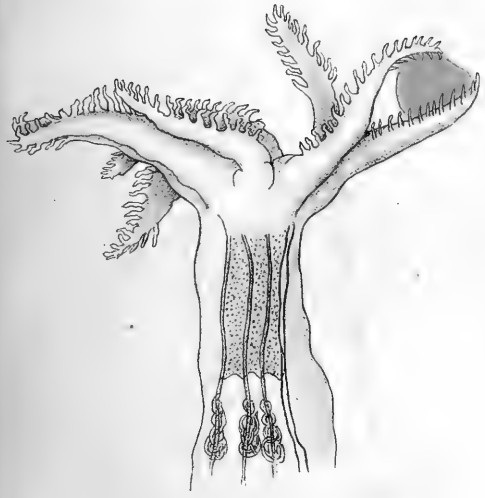

Fig. 2 a. x 10.

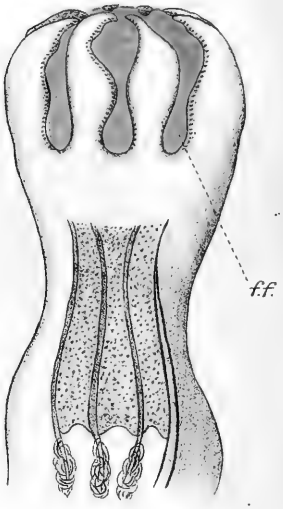

Fig. 2 b.

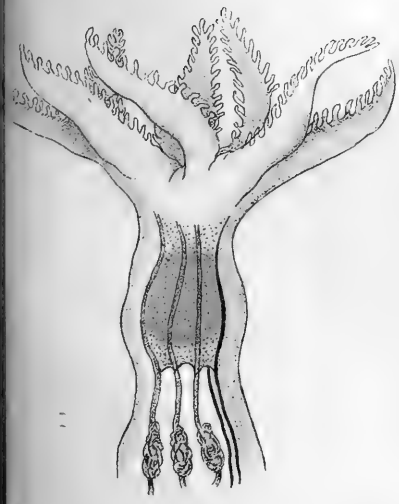

Fig. 2 c.

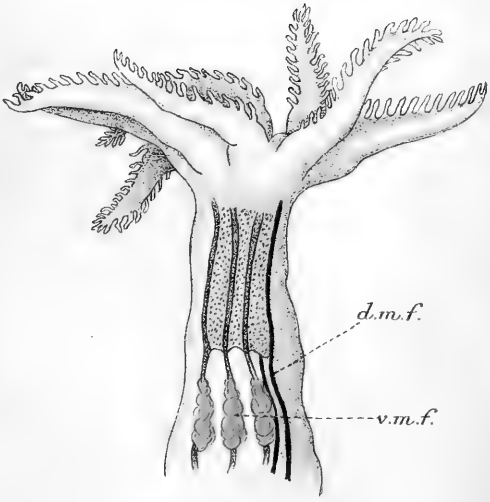

Fig. 2 d.

Huth, Lith^r London

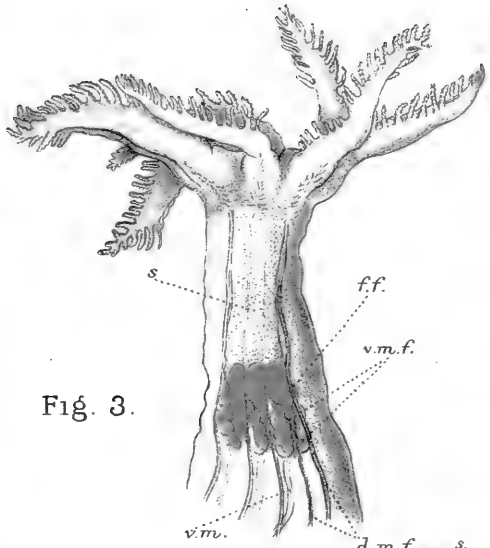

Fig. 3.

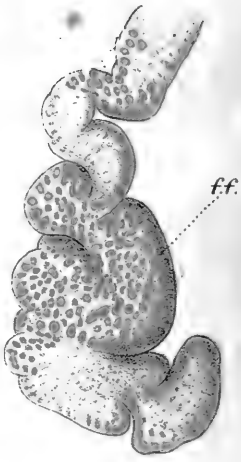

Fig. 4.

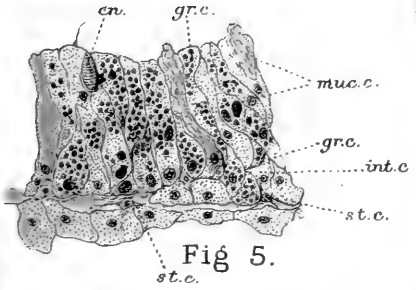

Fig 5.

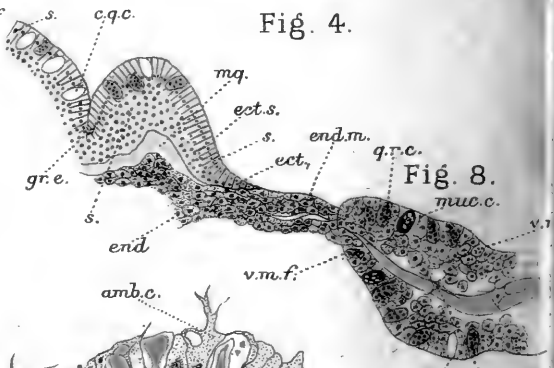

Fig. 8.

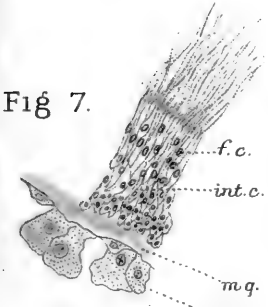

Fig 7.

Fig. 9.

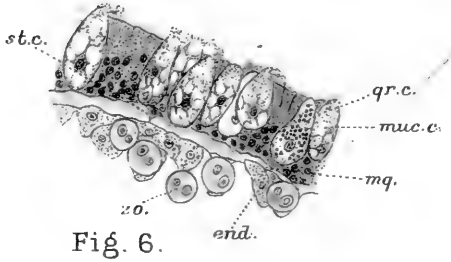

Fig. 6.

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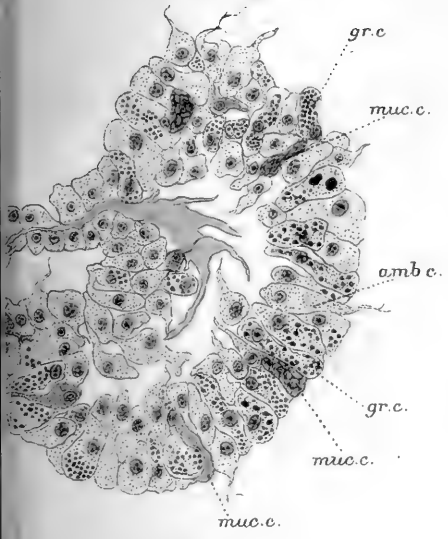
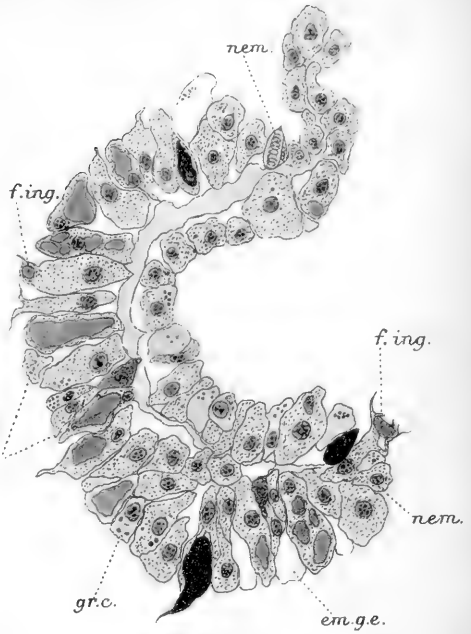


Fig. 10



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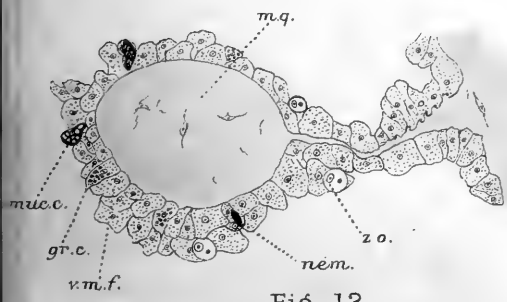


Fig. 12.

Fig. 13. $^{2}/_{3} \times 930$.

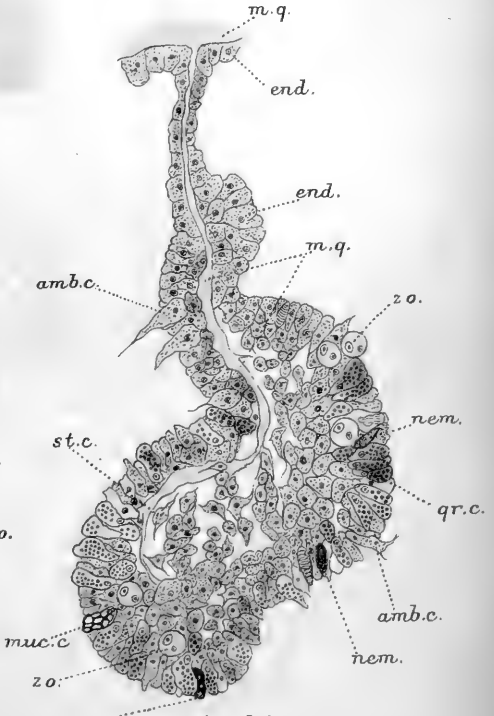
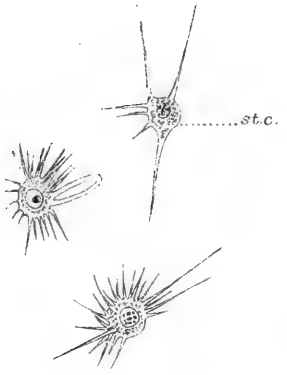


Fig. 14. × 416.

Huth, Lith^r London.



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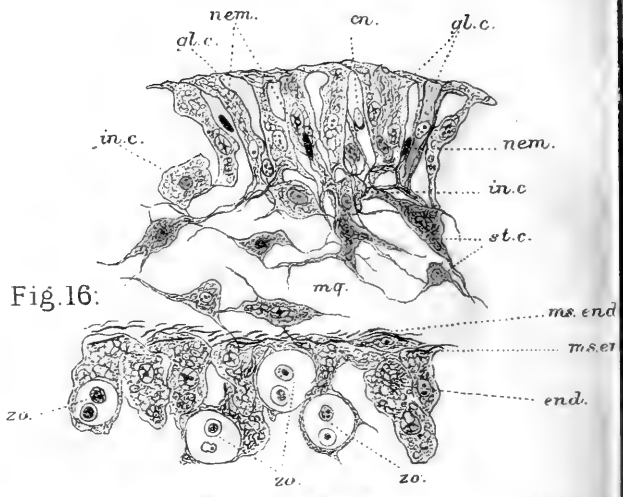
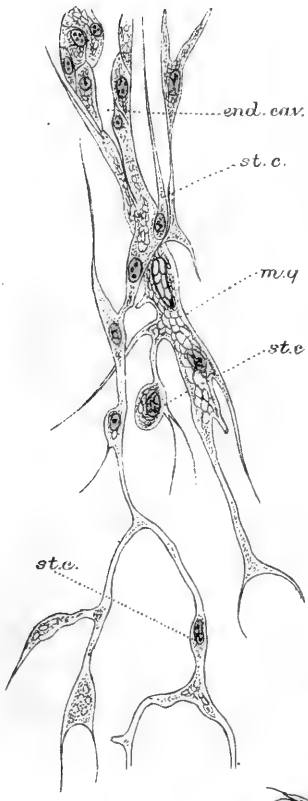


Fig. 16.



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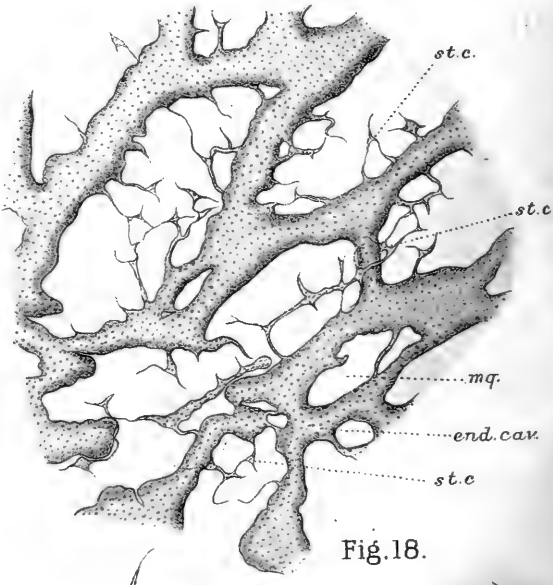

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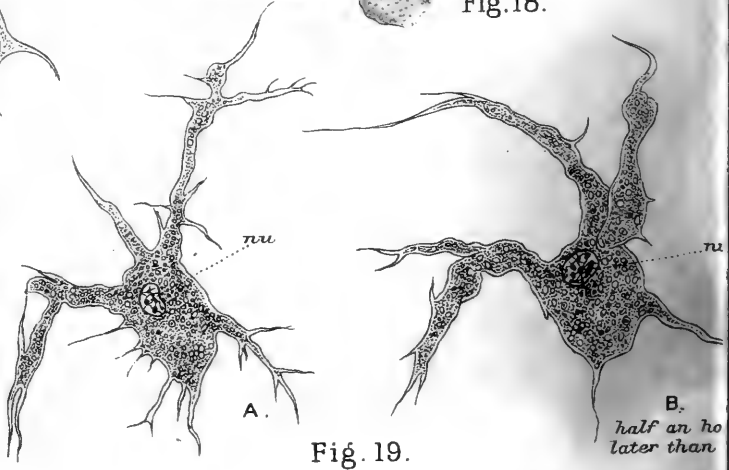

Fig. 19.

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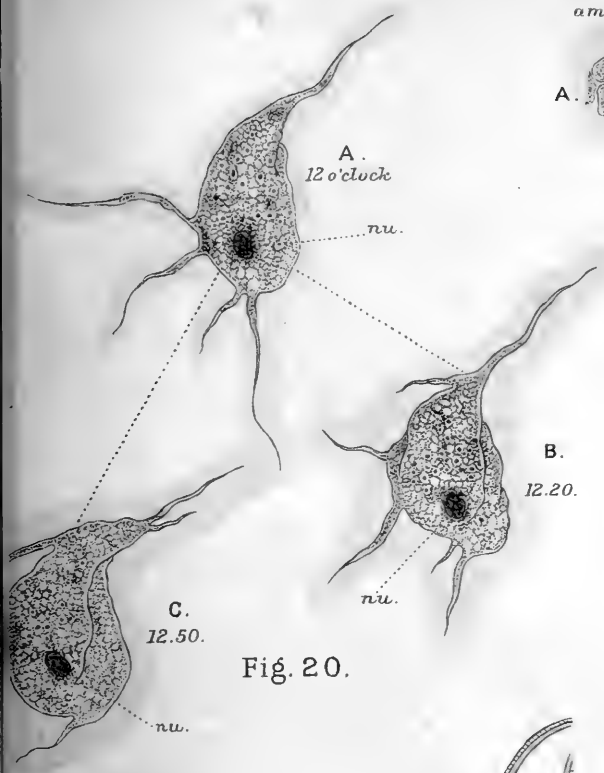

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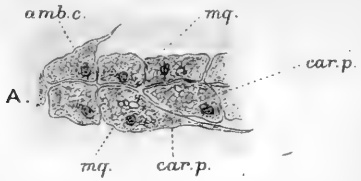

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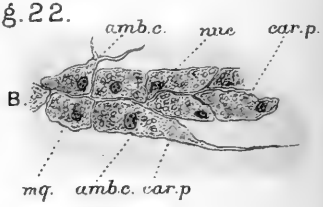

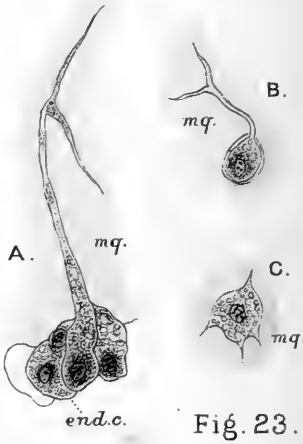

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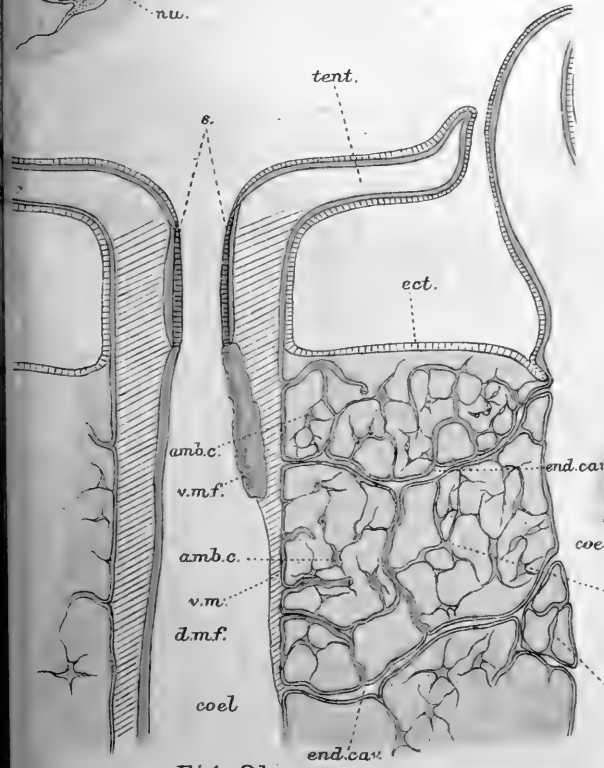

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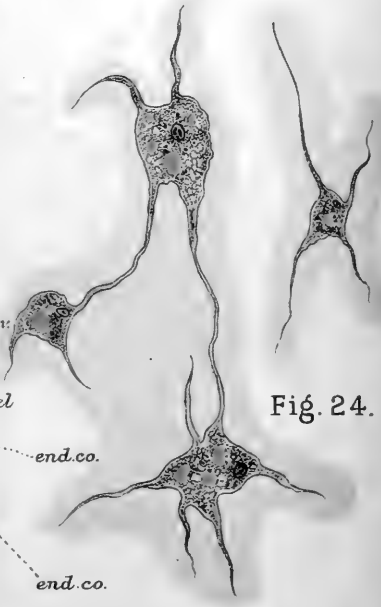
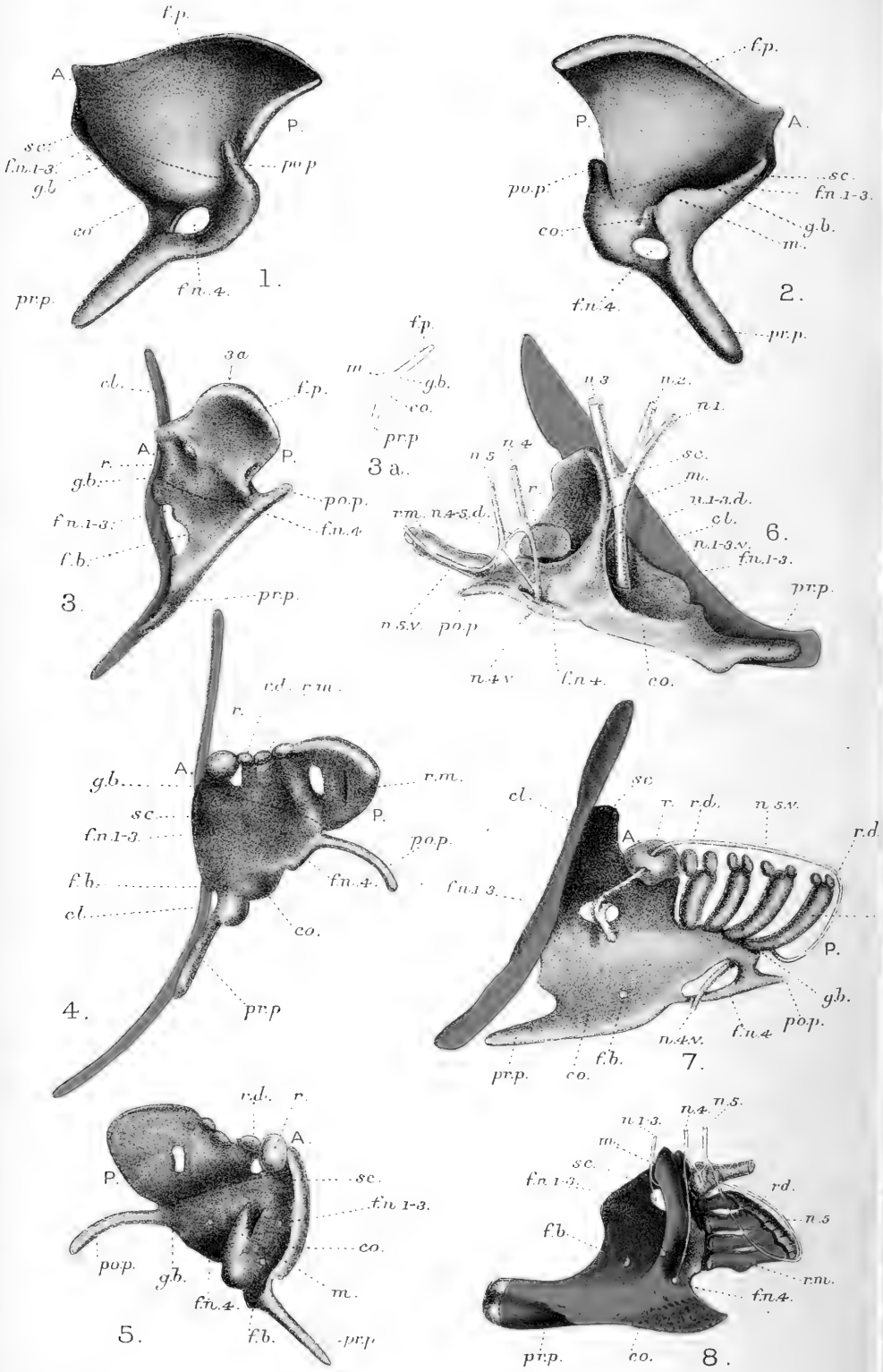


Fig. 24.

Huth, Lith^r London.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A.
P.
sc.
f.n.1-3.
g.b.
po.p.
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1.
f.p.
P.
A.
po.p.
sc.
f.n.1-3.
g.b.
co.
m.
f.n.4.
prp.
2.
3a
cl.
f.p.
A.
P.
r.
g.b.
po.p.
f.n.1-3.
f.n.4.
f.b.
prp.
3.
f.p.
m.
g.b.
co.
prp
3 a.
n.3
n.2.
n.1.
n.4
n.5
sc.
m.
r.
n.1-3.d.
rm. n.4-5.d.
cl.
n.1-3.v.
f.n.1-3.
prp.
n.5.v.
po.p
n.4.v
f.n.4.
co.
6.
cl.
r.d. r.m.
r.
g.b.
A.
r.m.
sc.
P.
f.n.1-3.
po.p.
f.b.
f.n.4.
cl.
co.
prp
4.
sc.
cl.
r.
r.d.
n.5.v.
r.d
A.
f.n.1-3.
P.
g.b.
po.p.
n.4.v.
f.n.4
f.b.
prp.
co.
7.
r.d.
r.
A.
P.
sc.
f.n 1-3.
co.
po.p.
g.b.
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f.n.4.
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5.
n.4
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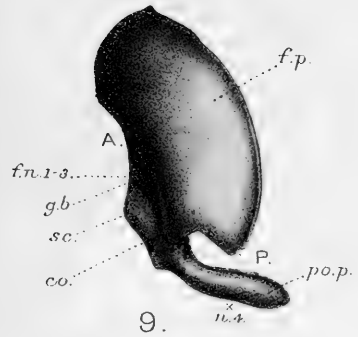
8.

H.H.S. del.



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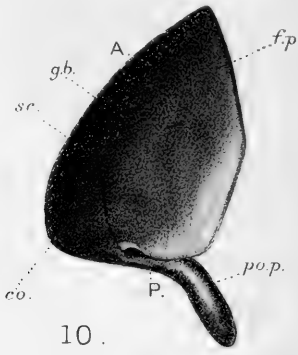

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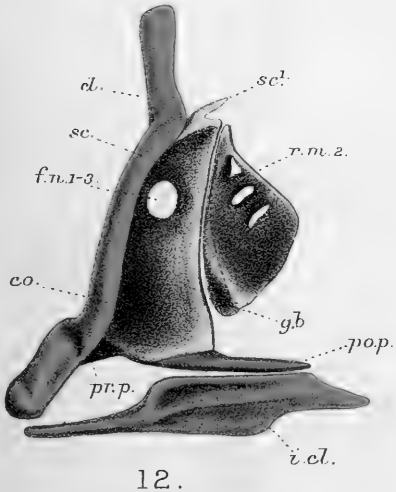

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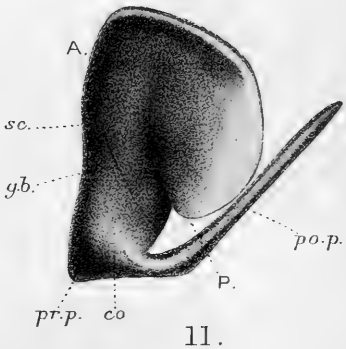

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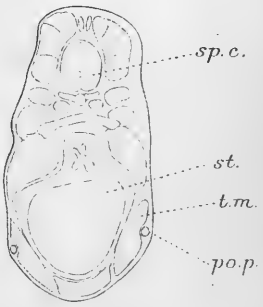

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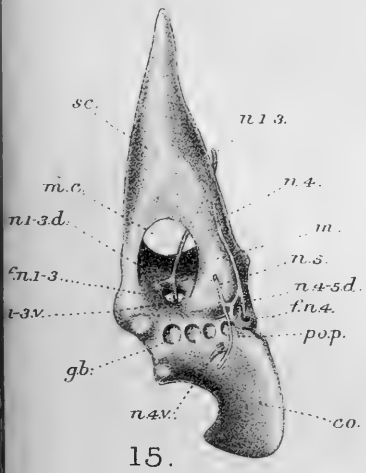

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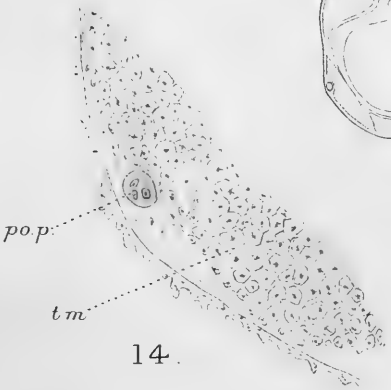

14.

Huth, Lith^r London.

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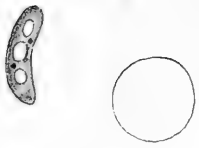


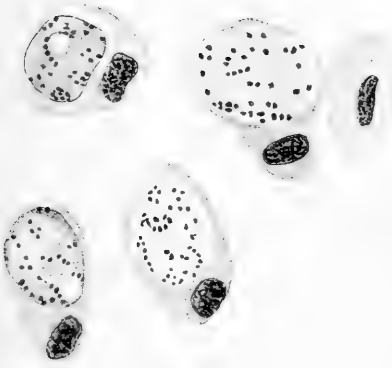
Fig. 2.

Fig 3.

Fig. 4.

Fig. 5.

Fig. 9.



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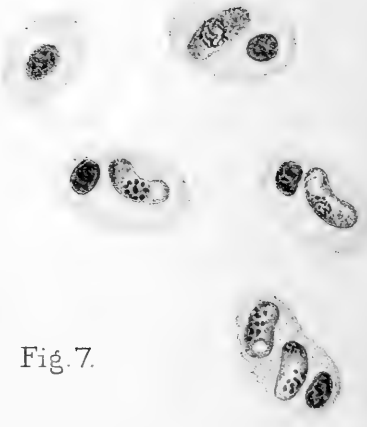
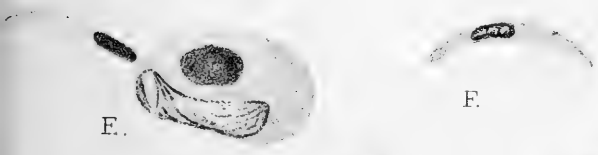


Fig. 7.

Fig. 8.



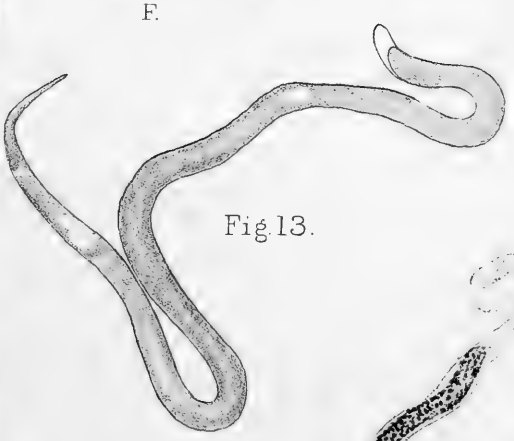


Fig. 13.

Fig. 10.

Fig. 11.

Fig. 12.

Huth, Lith. London.

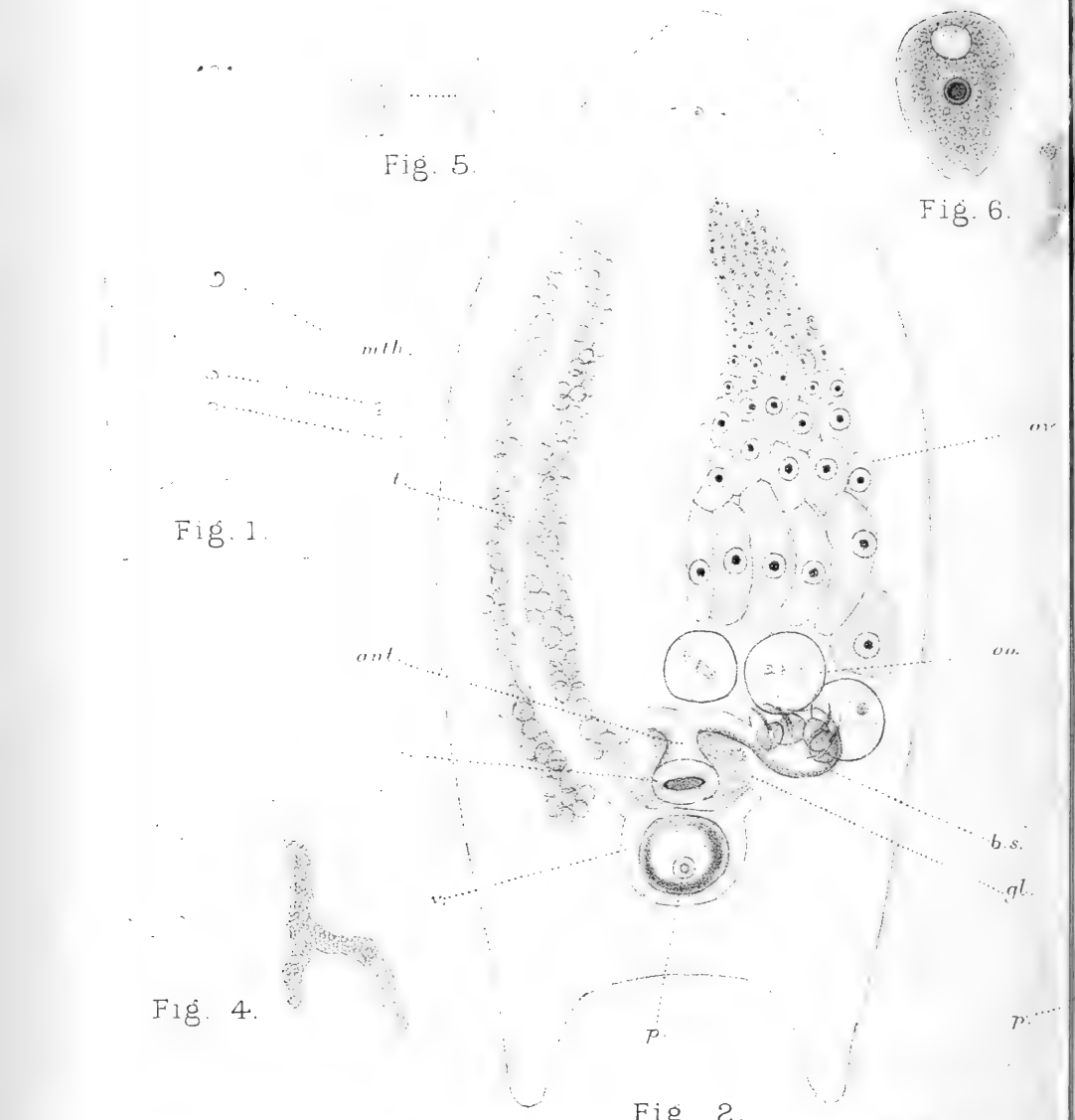


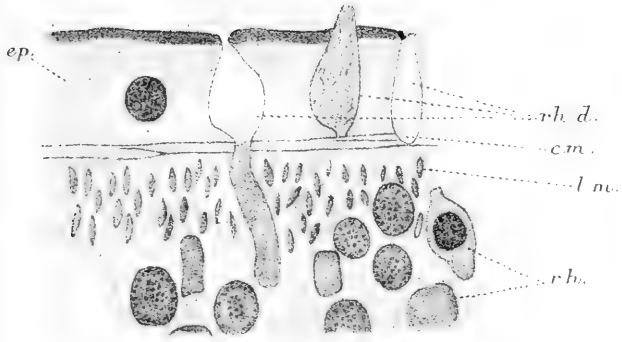
Fig. 1.

Fig. 2.

Fig. 4.

Fig. 5.

Fig. 6.



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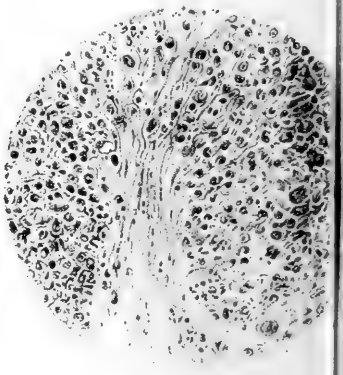
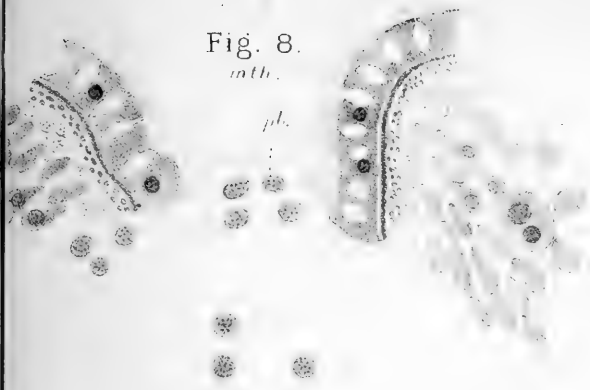


Fig. 7.

W.A.H. and A.C. del

Fig. 8.



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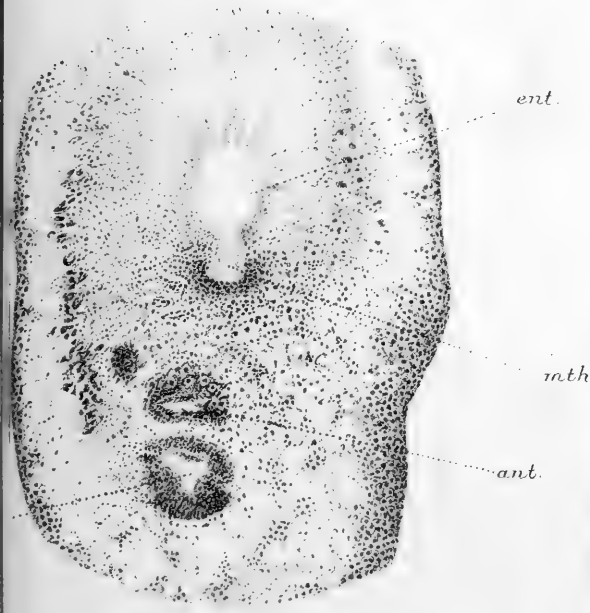

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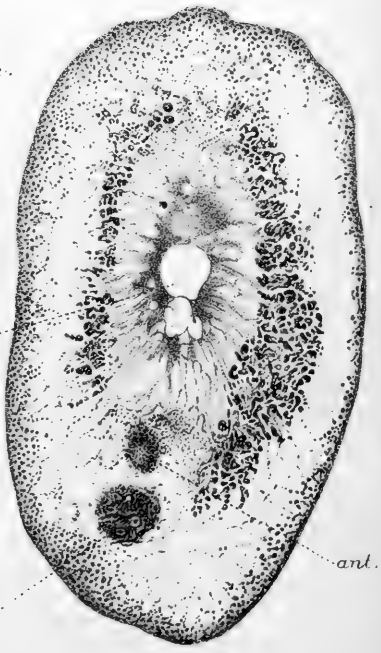

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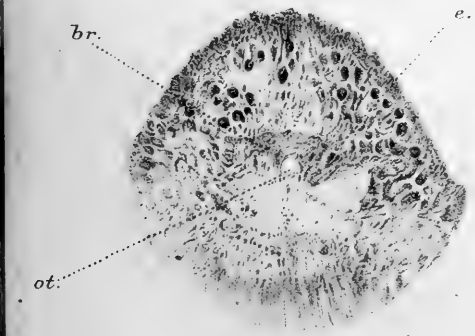

Fig. 12.

Huth, Lith^r London

Fig. 13.

Fig. 14.

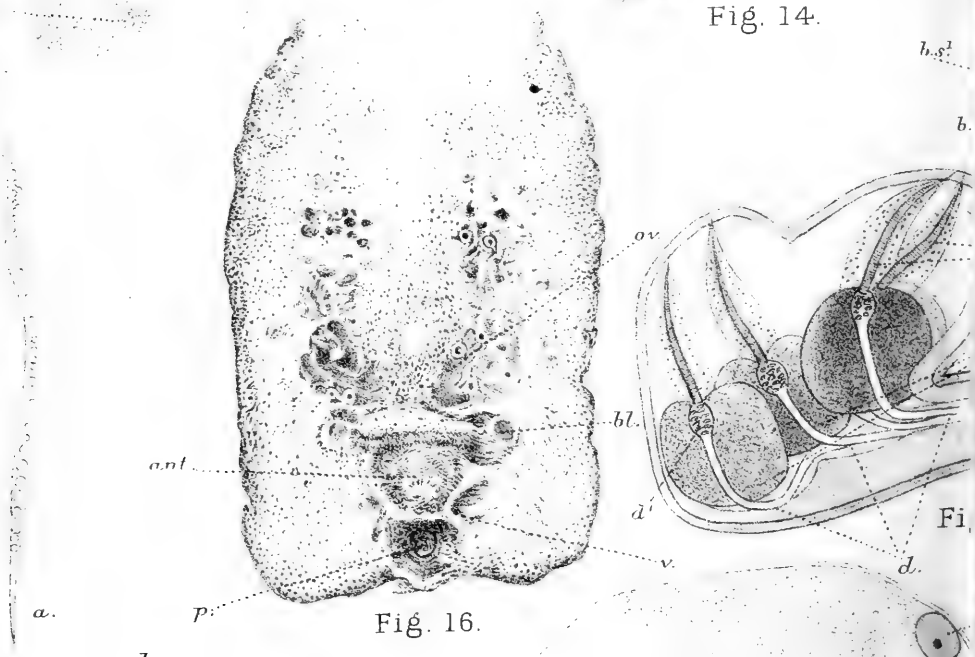

Fig. 16.

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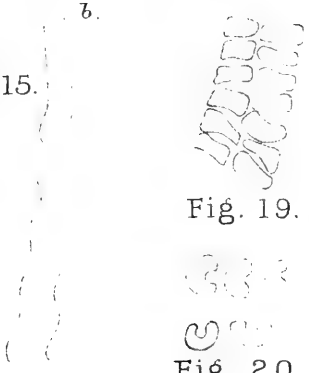
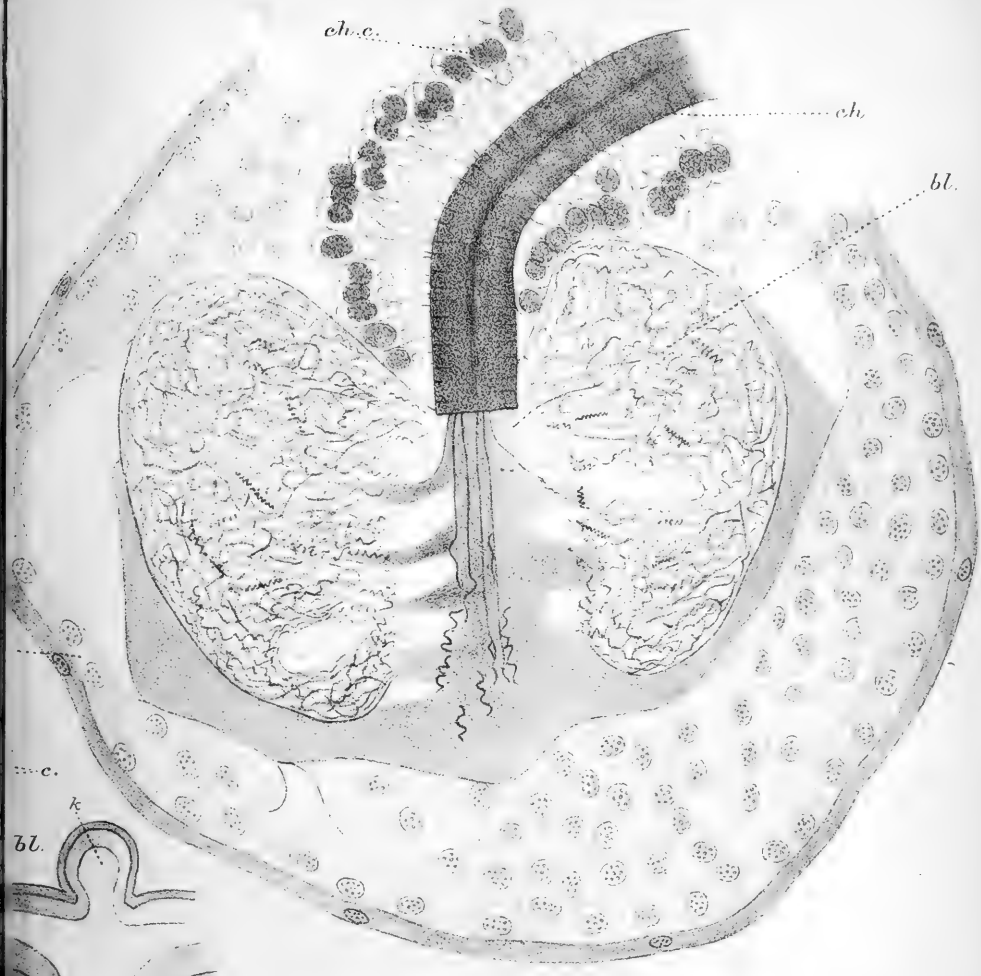


Fig. 19.

Fig. 20.

Fig. 23.

W.A.H. and A.C. del.



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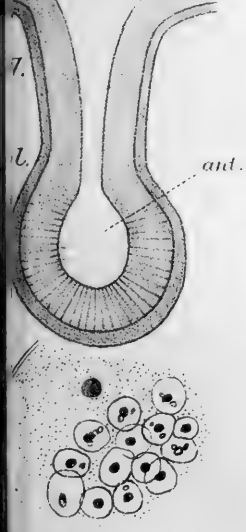


Fig. 24.

Fig. 21.

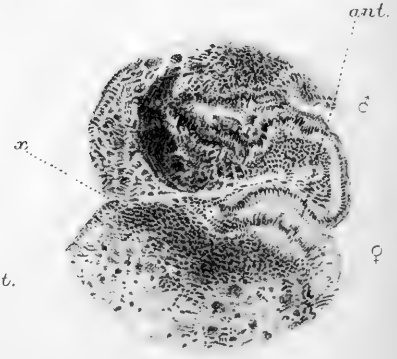


Fig. 22

Huth, Lith^r London.

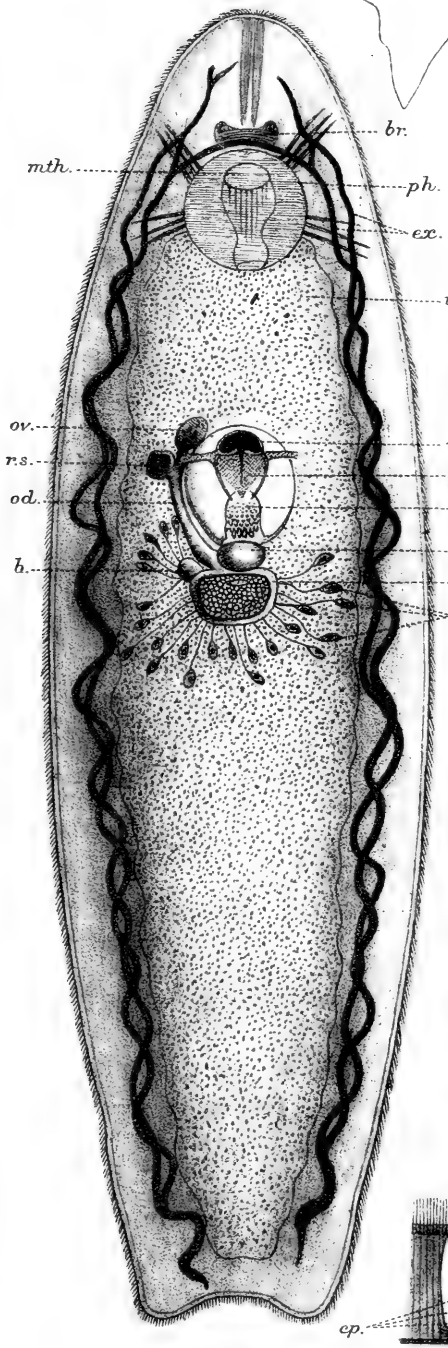
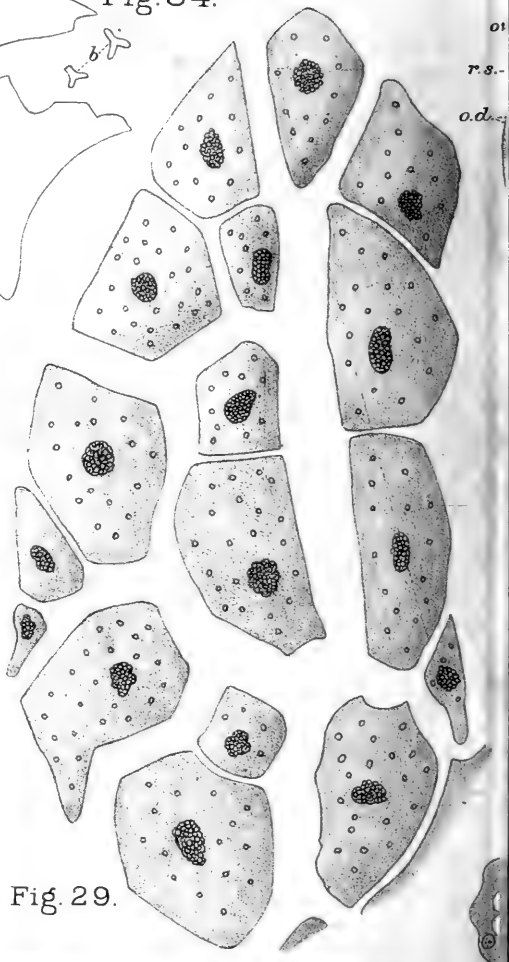


Fig. 25.

Fig. 34.



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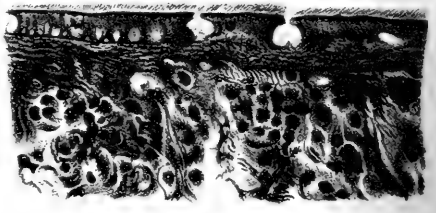

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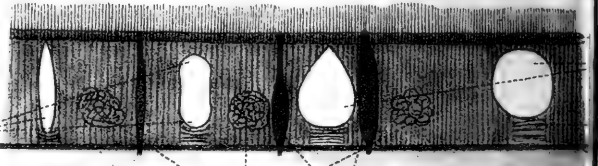
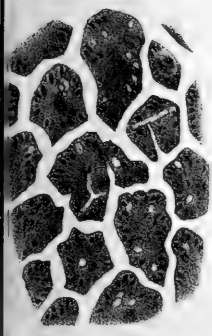


Fig. 27.

W.A.H. and A.C. del.

Fig. 32.



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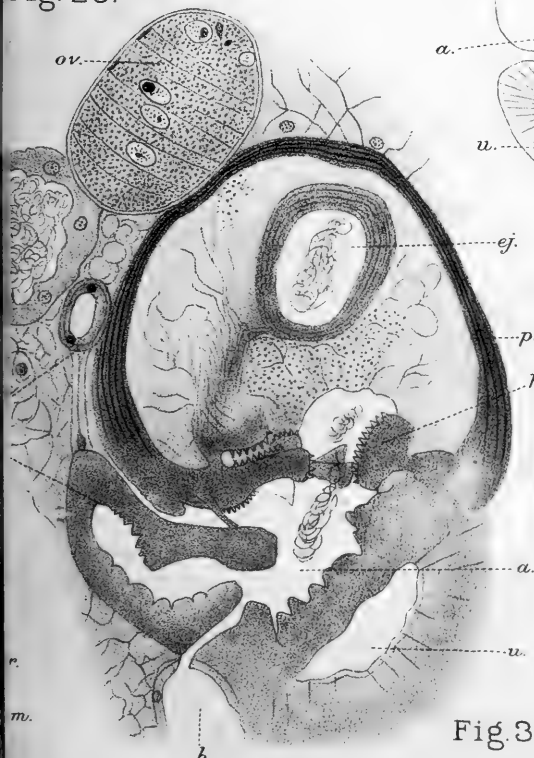

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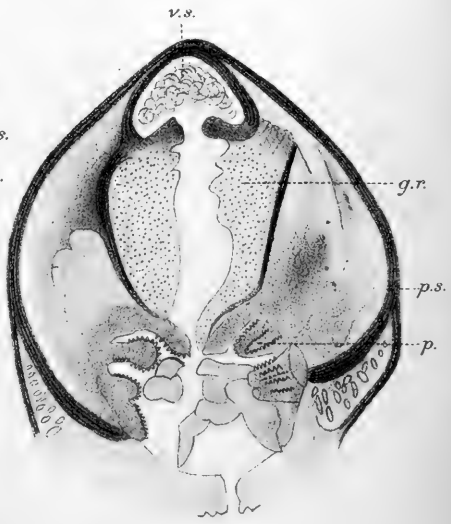

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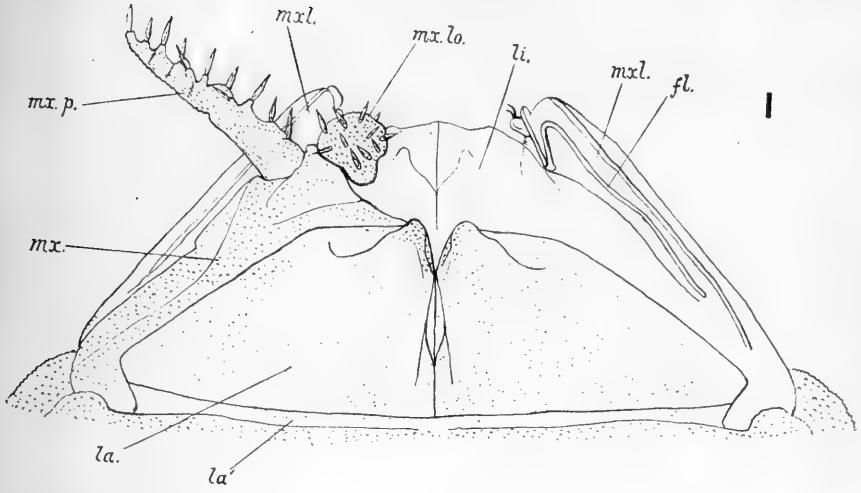
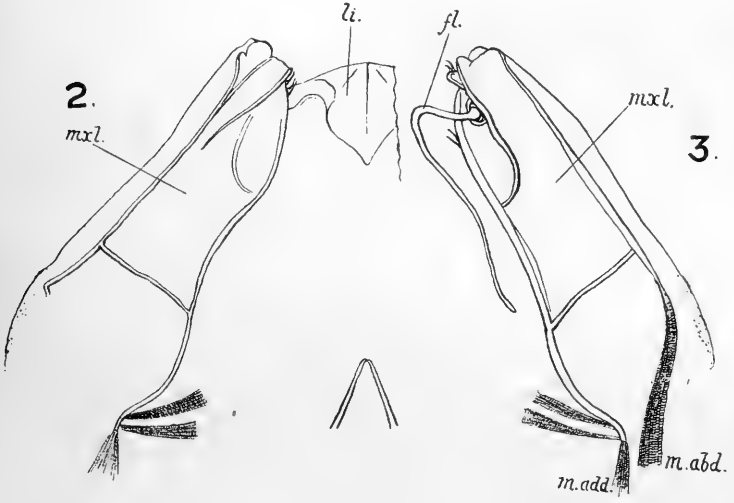
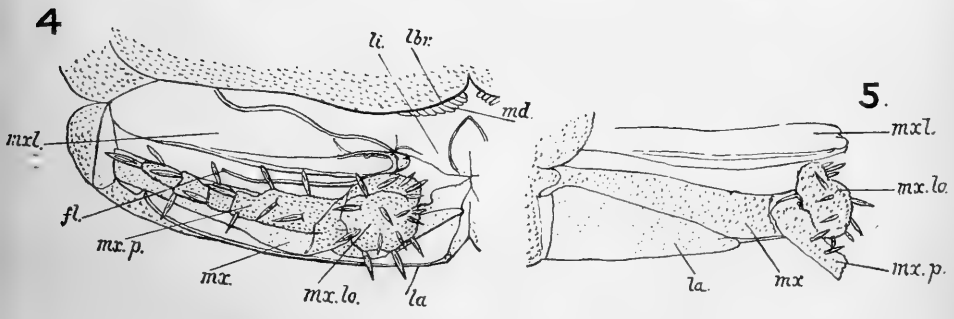
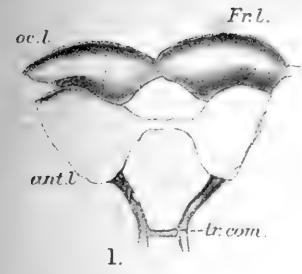






C. H. C., del.

F. Butterworth, sculp.
Adlard & Son, impr.



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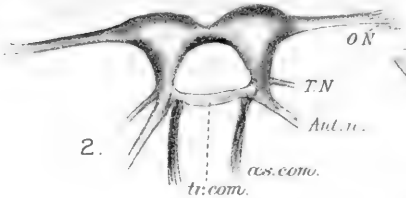

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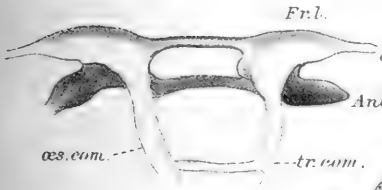

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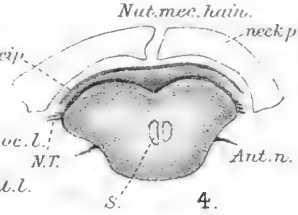

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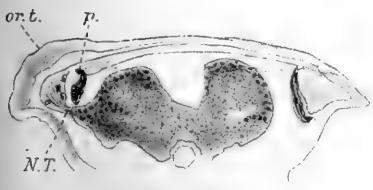

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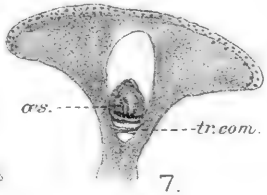

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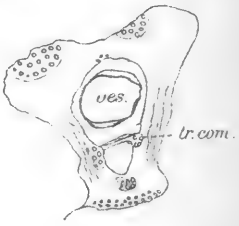

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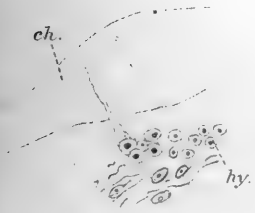

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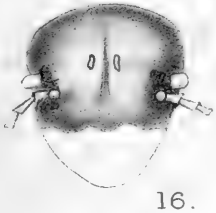
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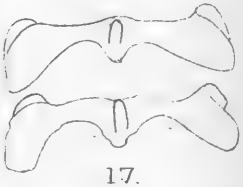
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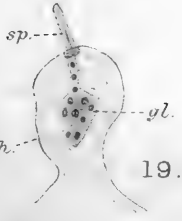

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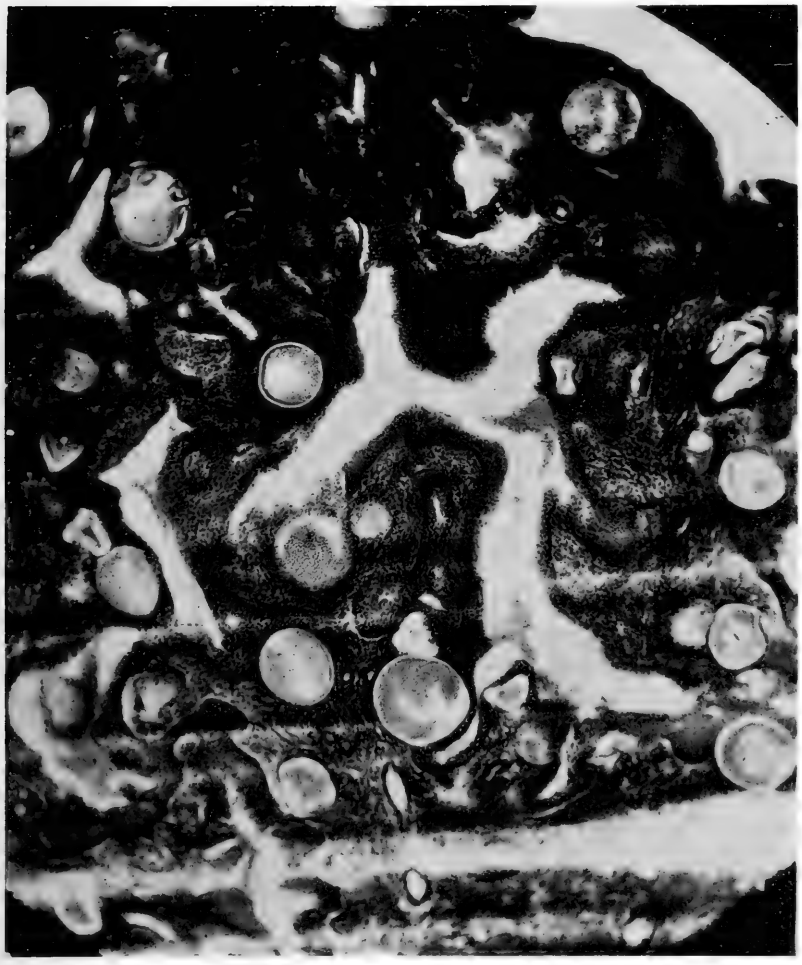


Fig. 1.

RHINOSPORIDIUM KINEALYI.

from Mucous Membrane of Septum Nasi of Man.

Huth. London.

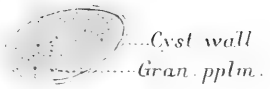


Fig. 2.

Fig. 3.

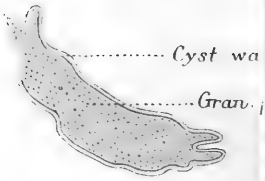
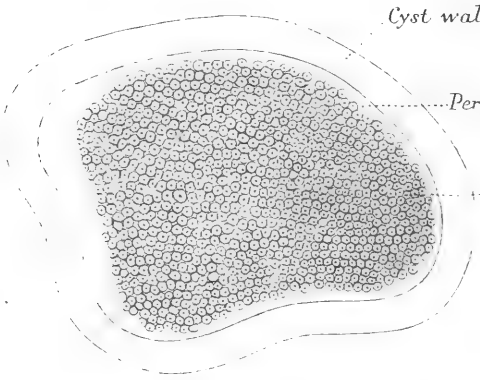


Fig. 4.



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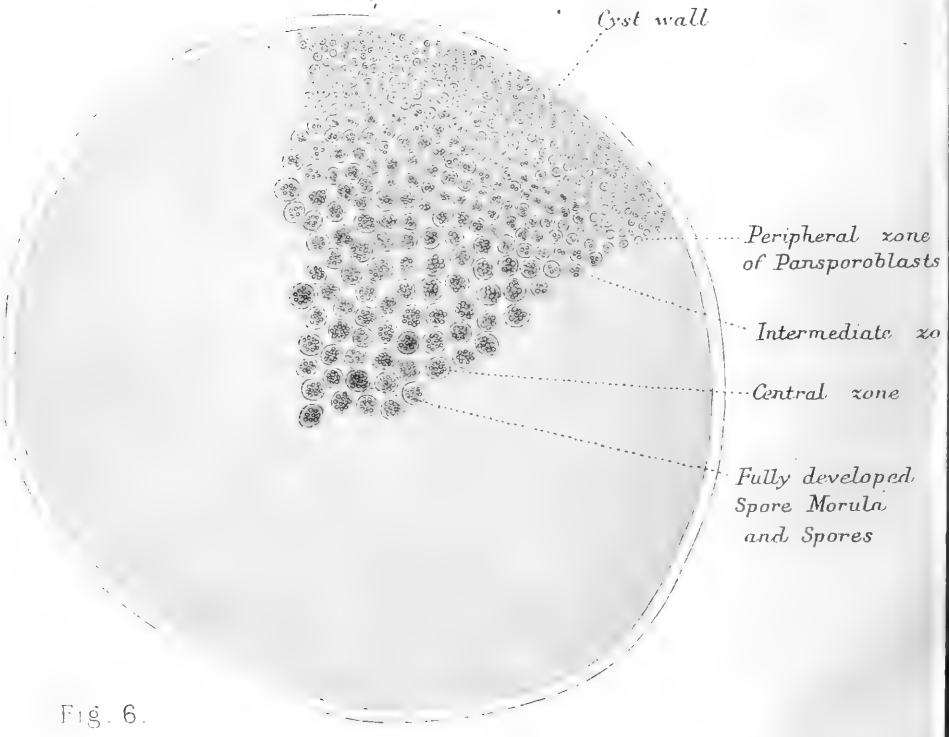
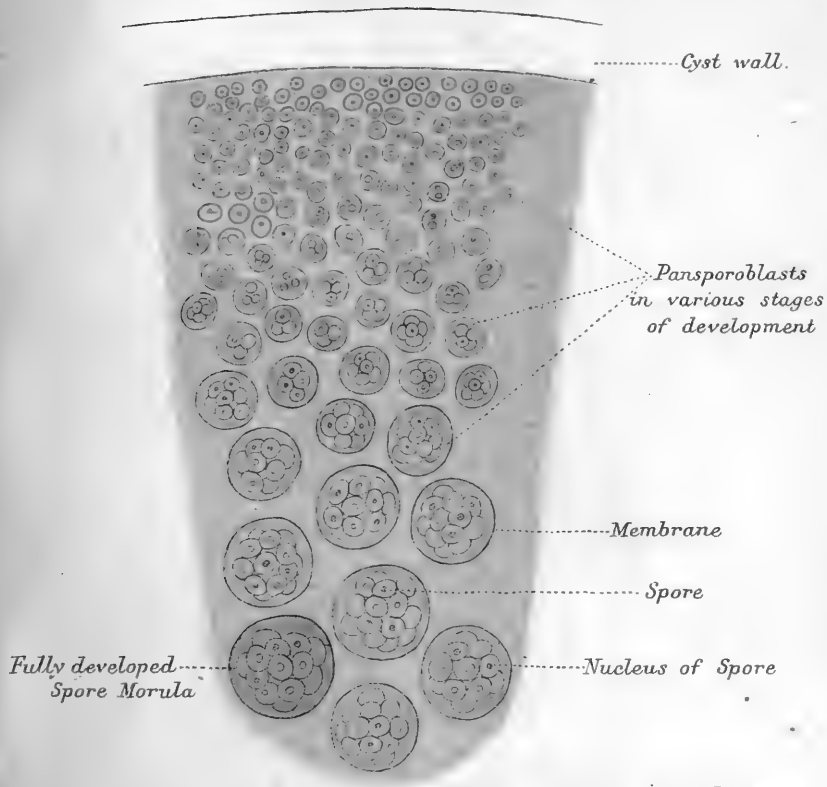


Fig. 6.

H. F. Fantham del.



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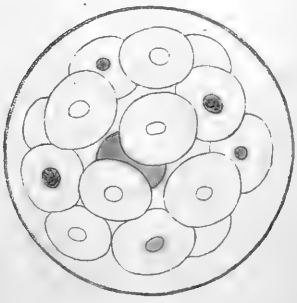


Fig. 8.

Fully developed Spore Morula

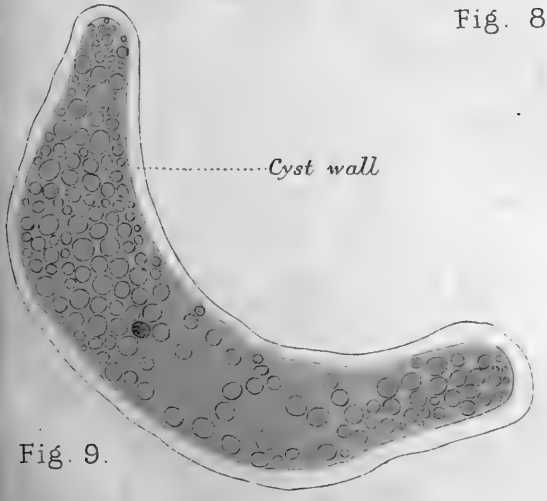


Fig. 9.

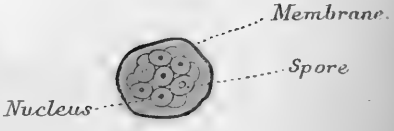
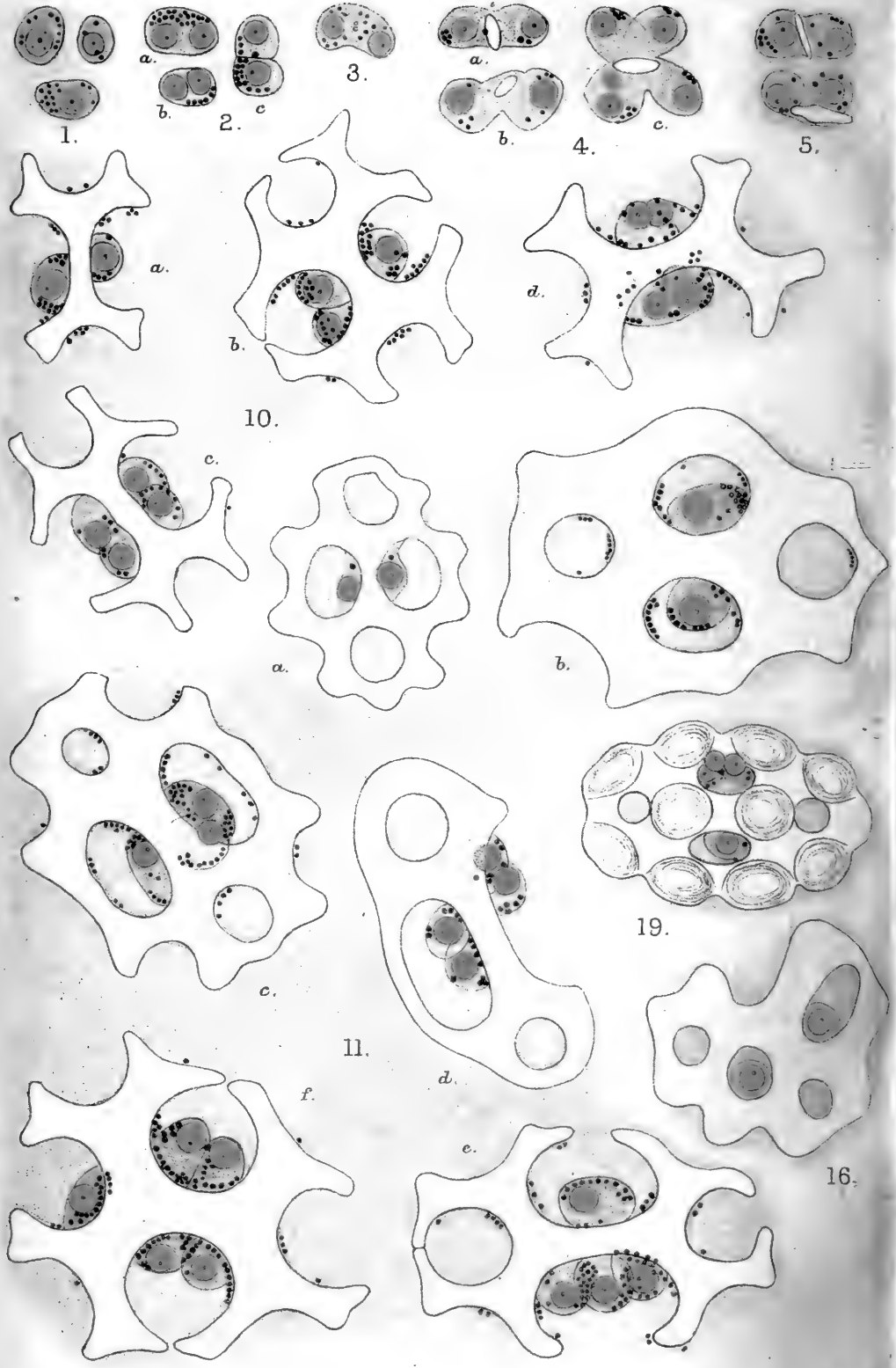


Fig. 10.

Huth, Lith^r London.



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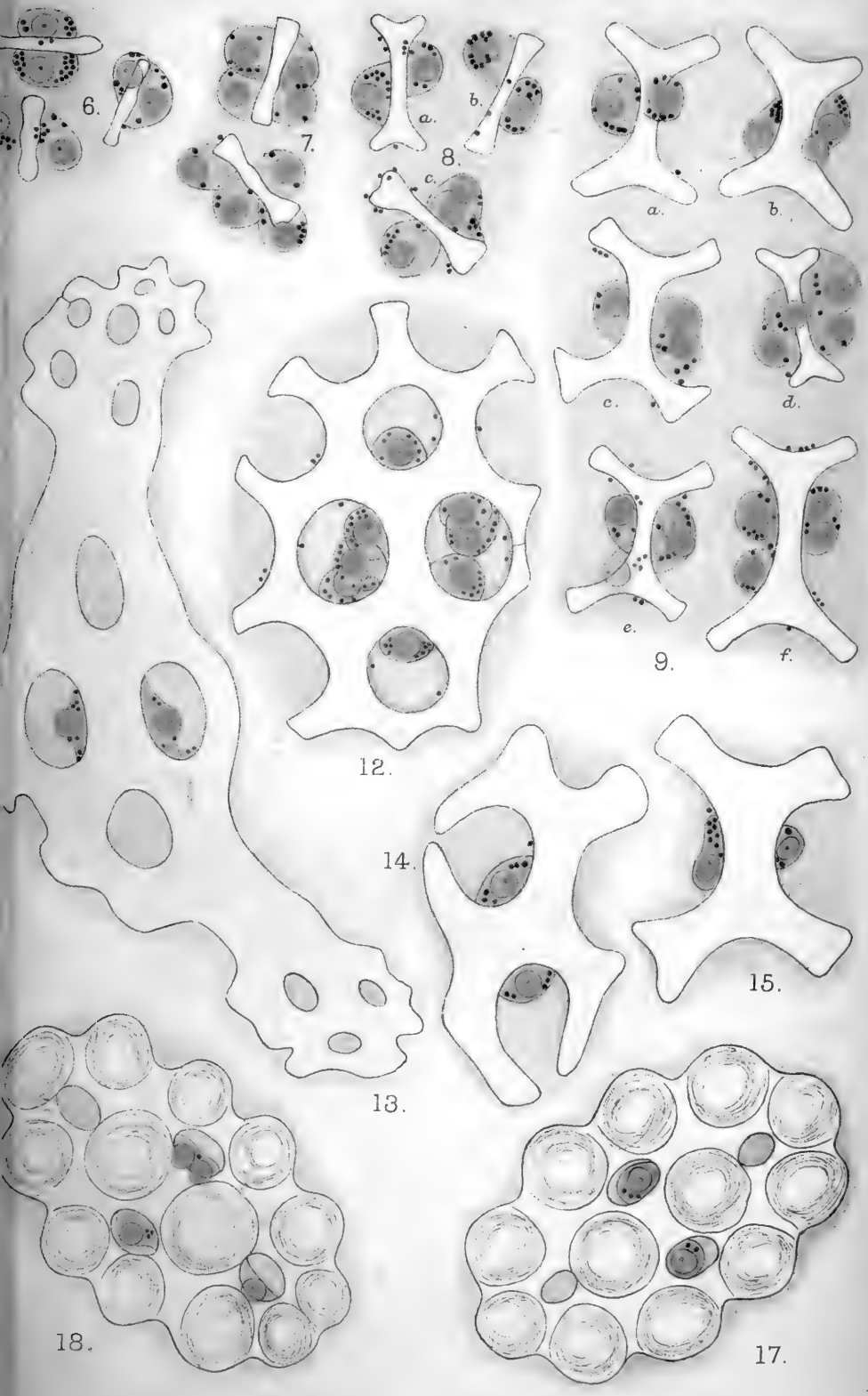

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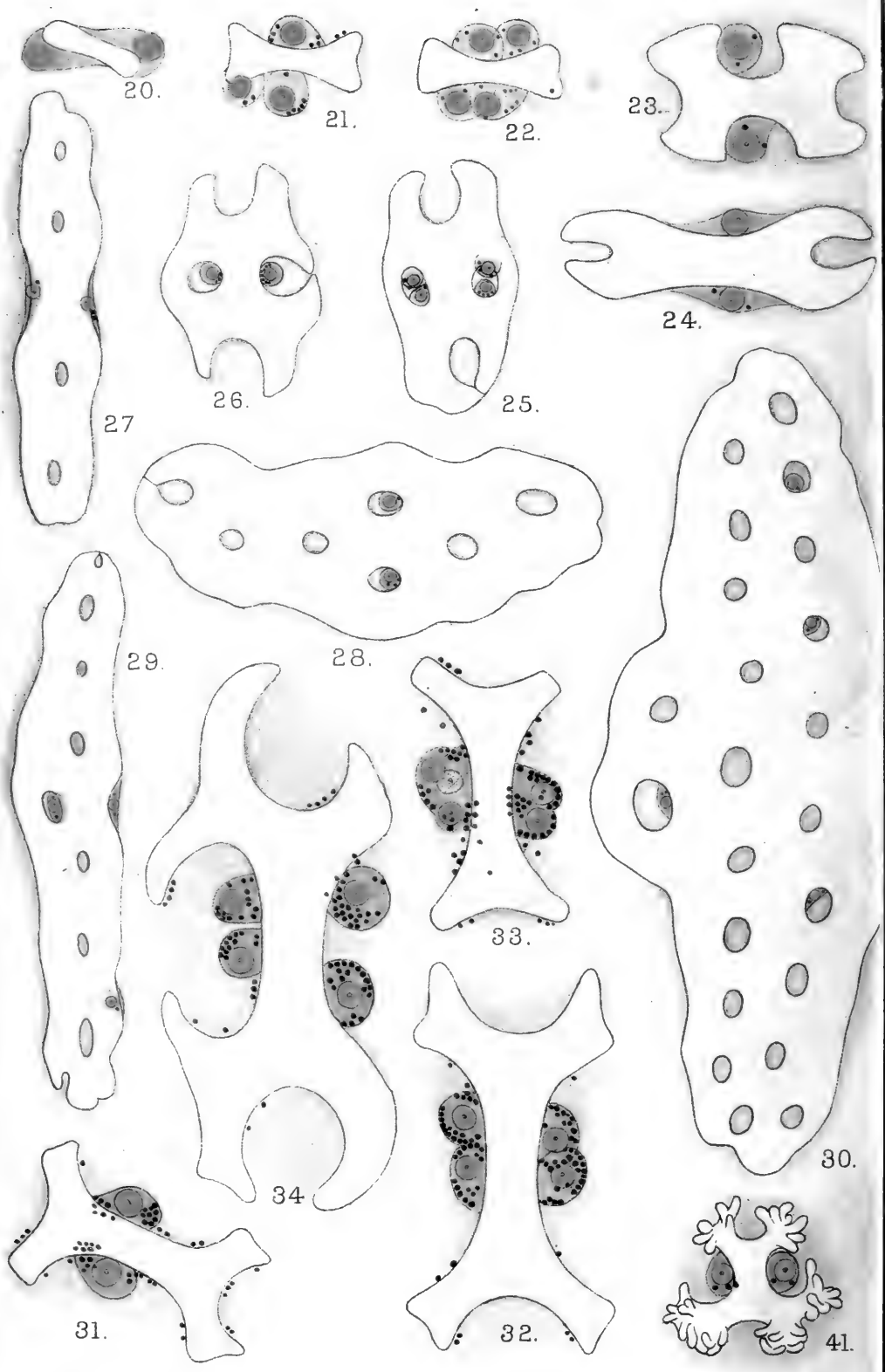

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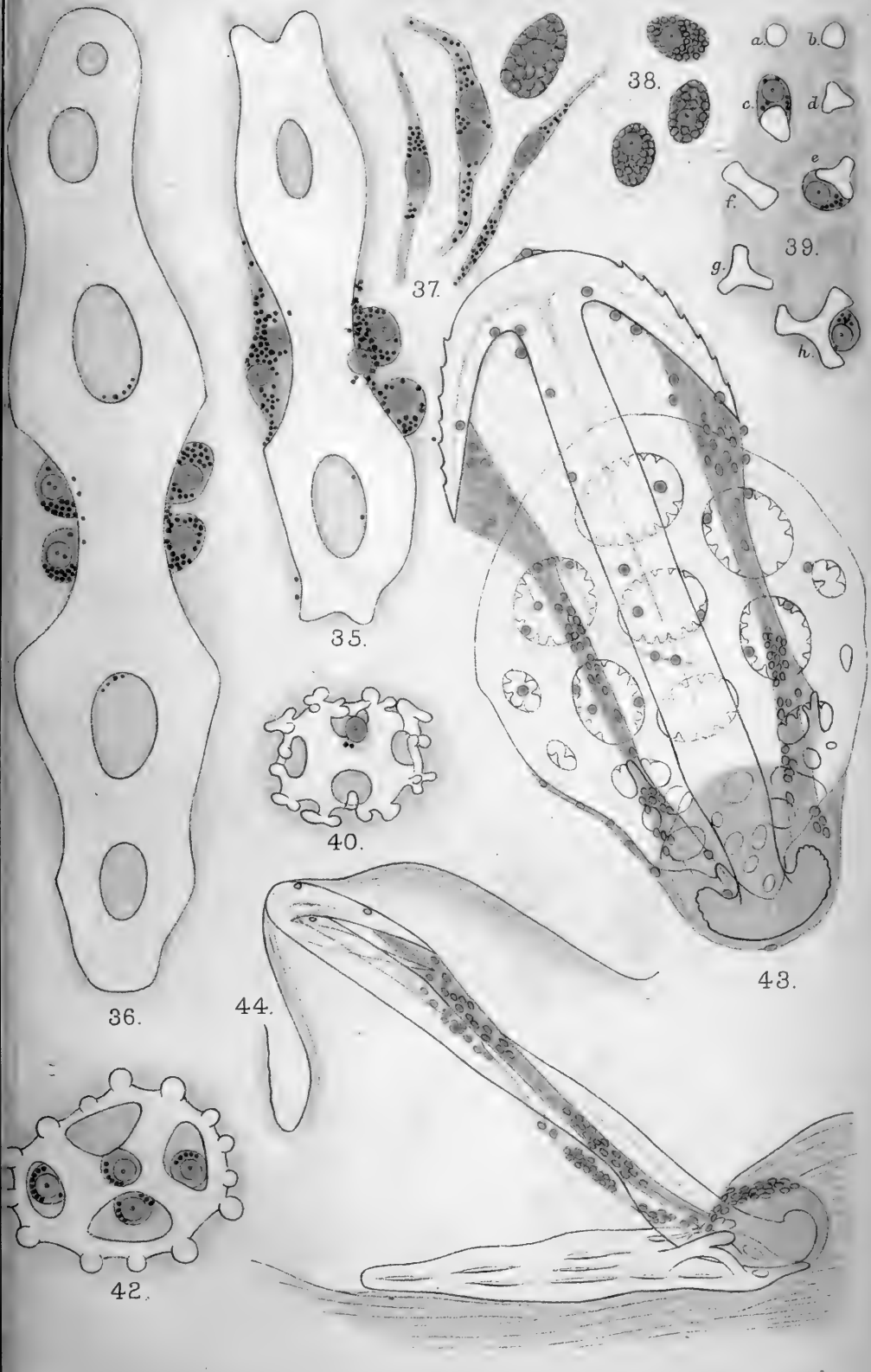

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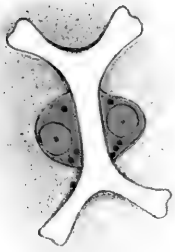

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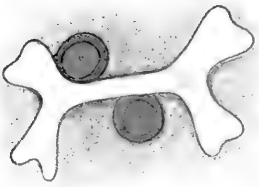

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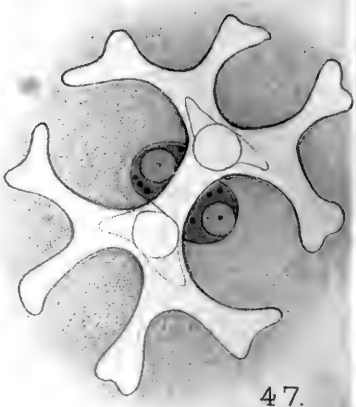

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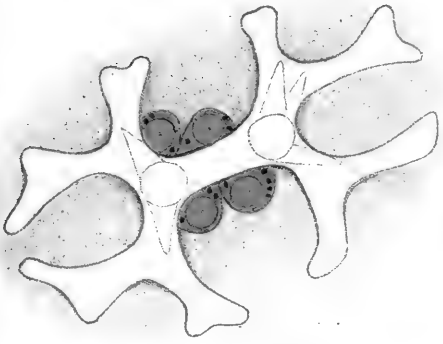

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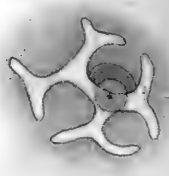

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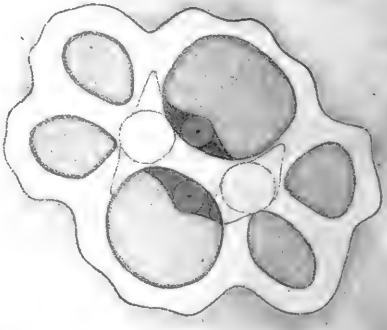

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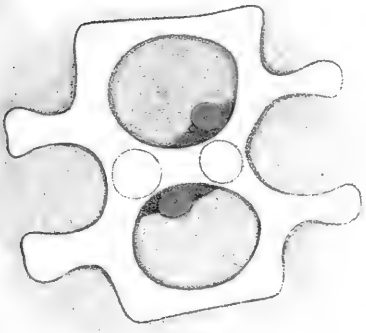

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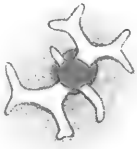

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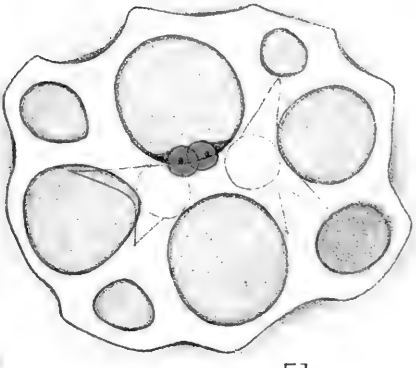

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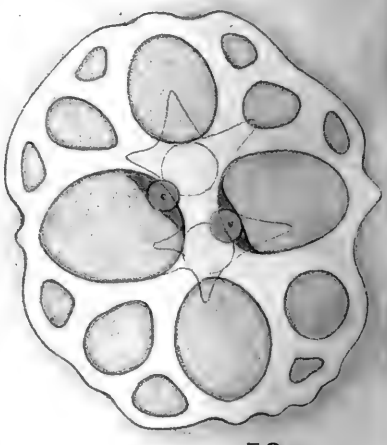

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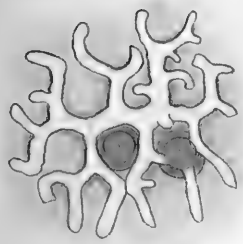

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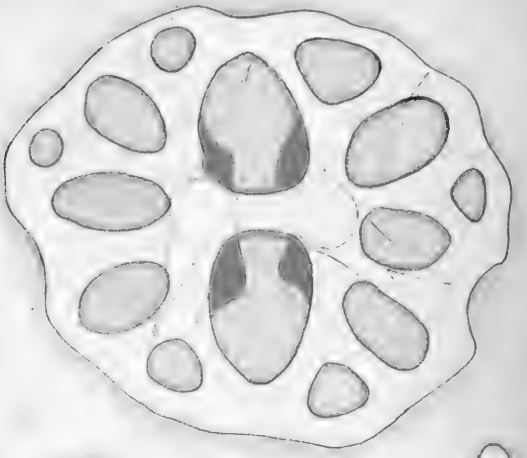

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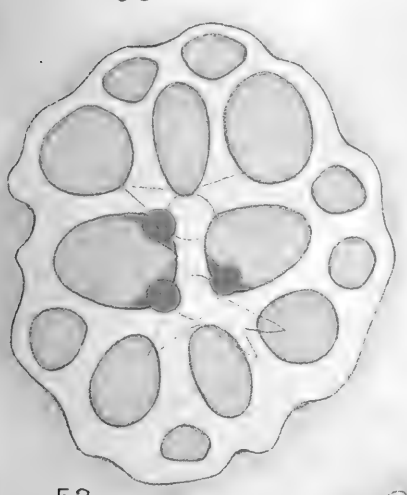

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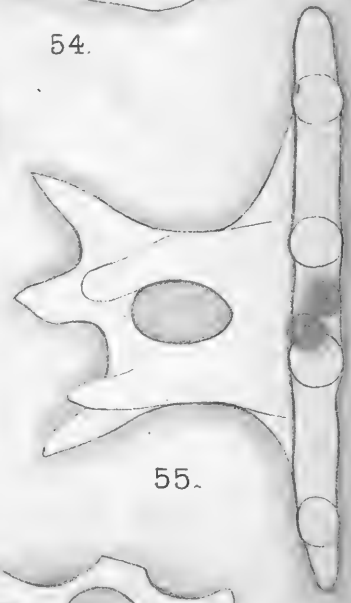

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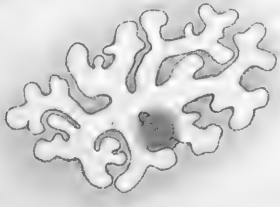

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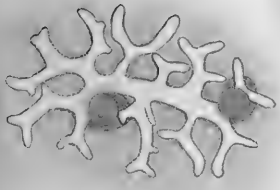

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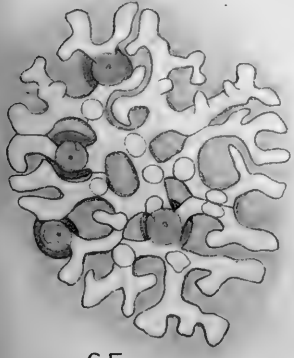

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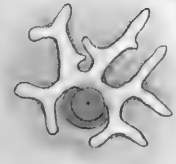

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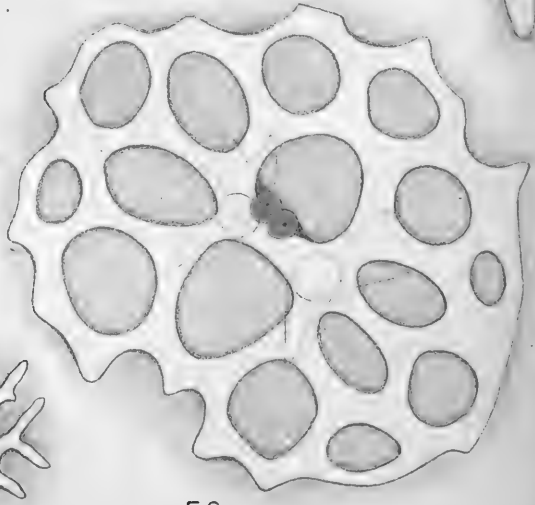


56.

Huth, Lith^r London.

Fig. 1.

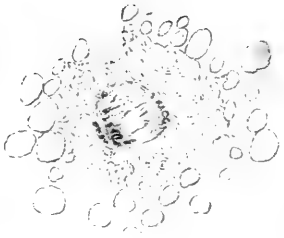


Fig. 2.

Fig. 3.

Fig. 6.

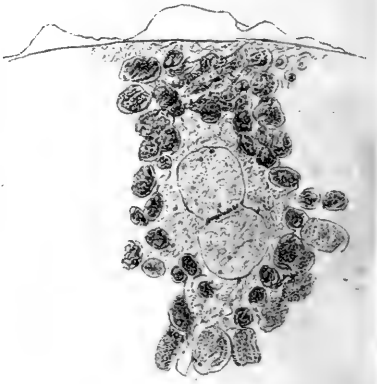
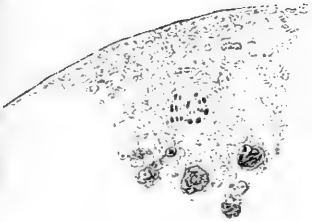


Fig. 7.



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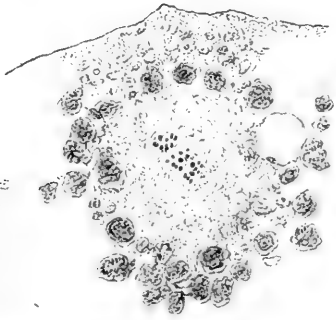

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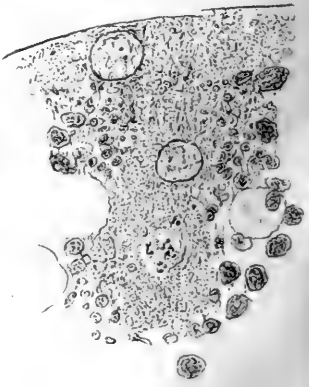
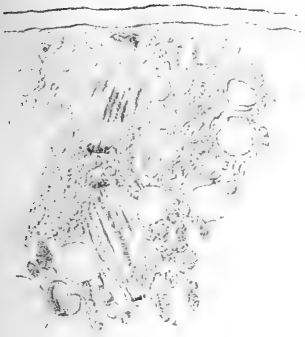


Fig. 12.

L.D del.



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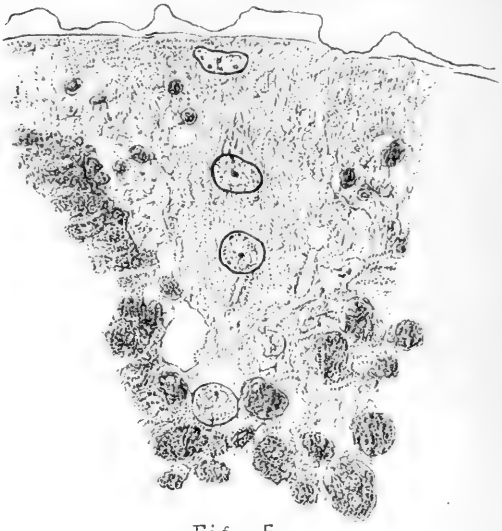

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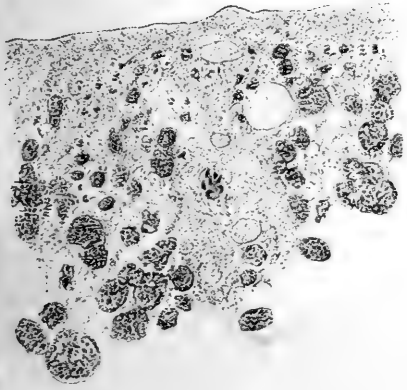

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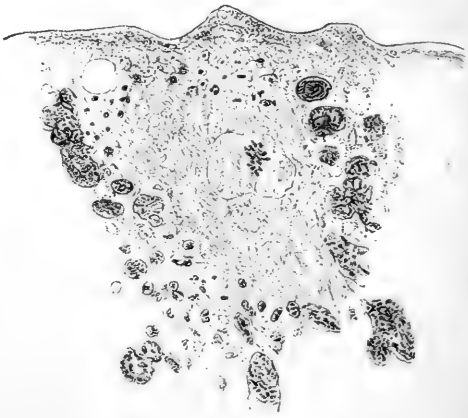

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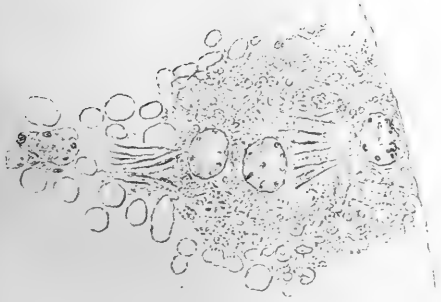

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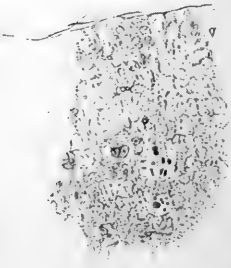

Fig. 14.

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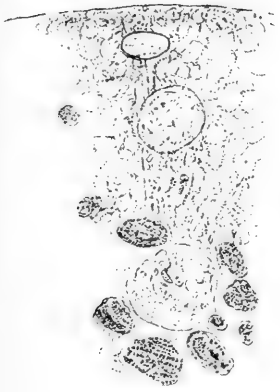

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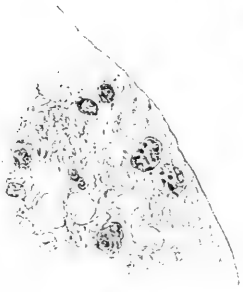

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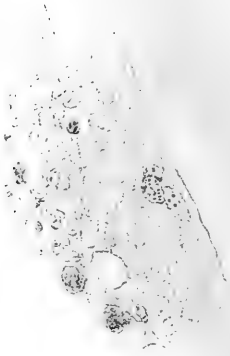
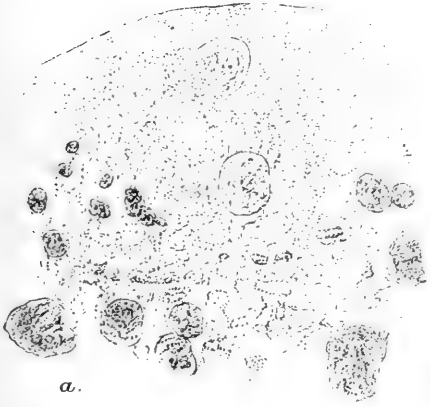


Fig. 17.



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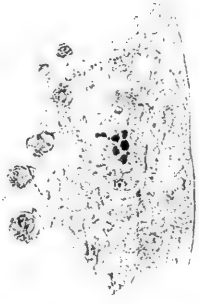

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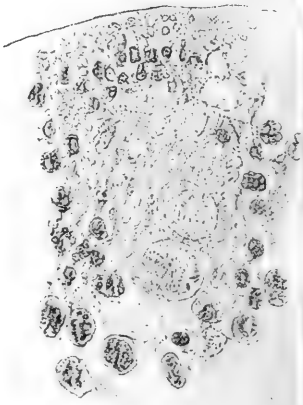

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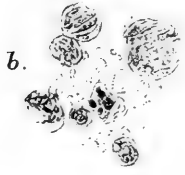

Fig. 27

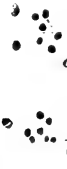

Fig. 24.

Fig. 25.

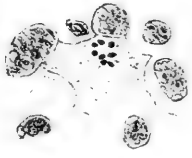

Fig. 26.

Fig. 28.

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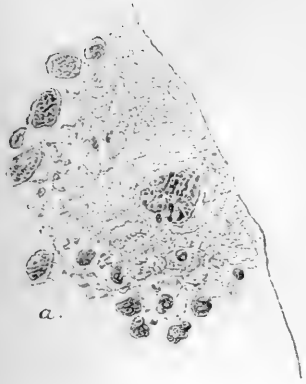

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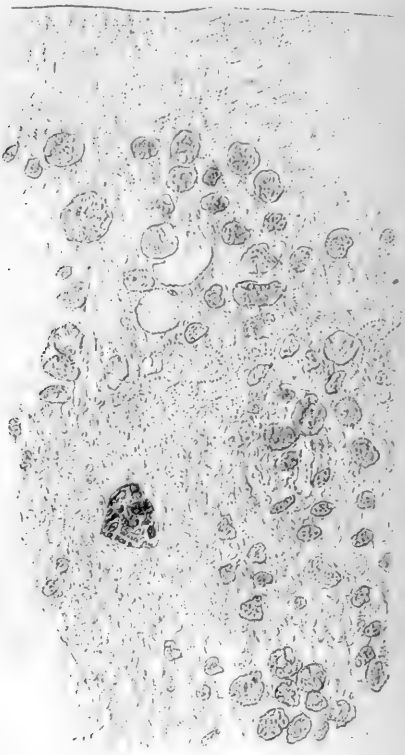

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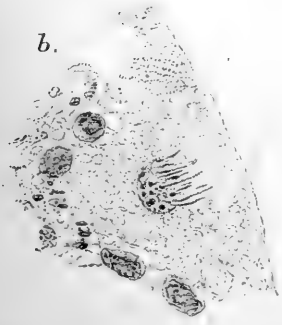

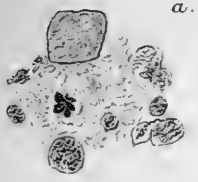

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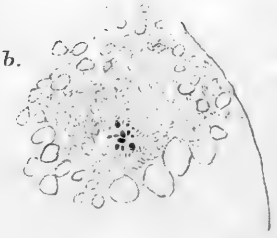
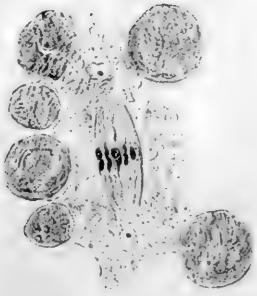


Fig. 29.

Fig. 30.



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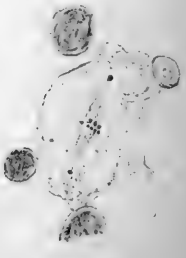
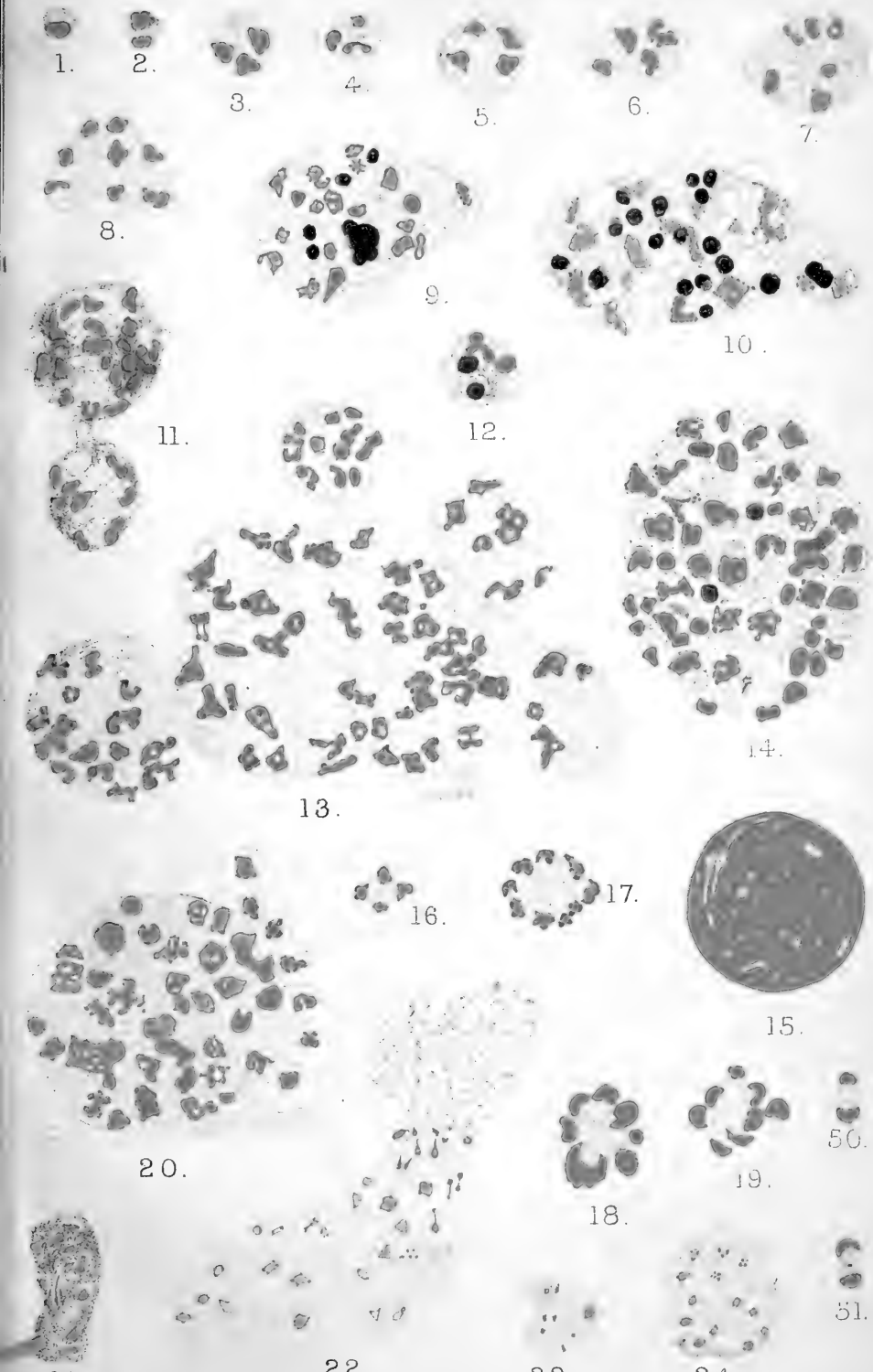


Fig. 32.

Huth, Lith^r London.



W. S. Perrin, del.

Huth. Lith. London

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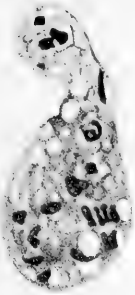
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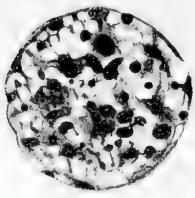
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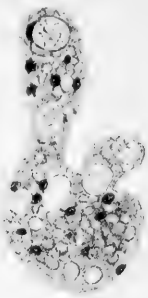
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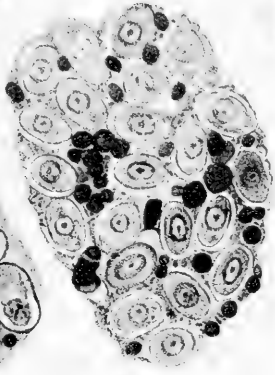
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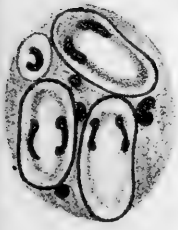
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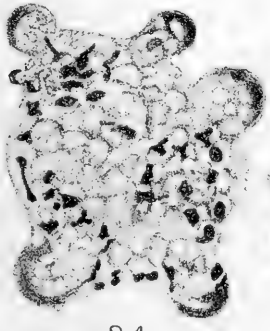
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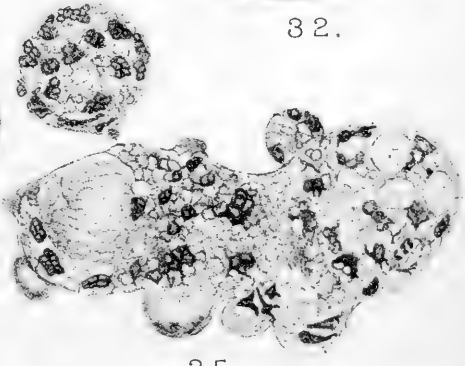
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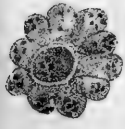
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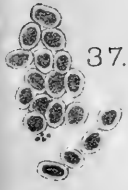
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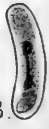
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49.

W.S. Perrin, del.

Huth, Lith^r London

Magnification, Figs. 25–32, 34–37. $^{175}/_{1}$.
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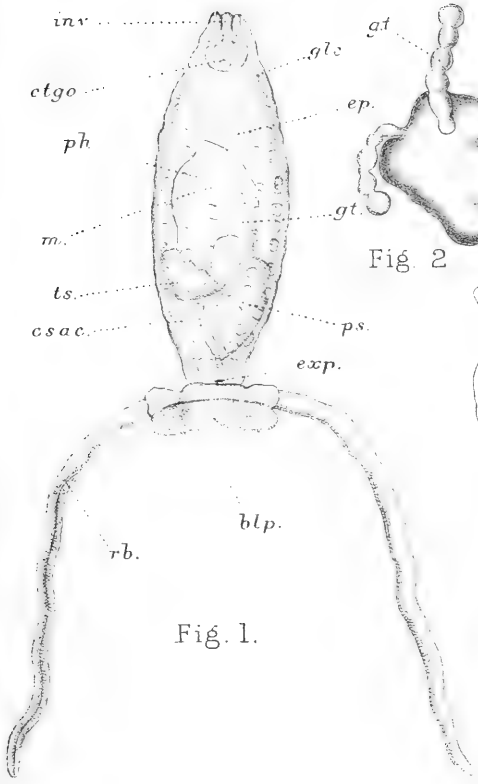
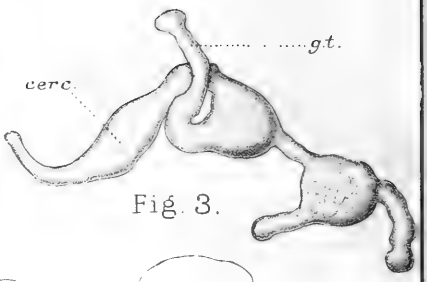


Fig. 1.

Fig. 2



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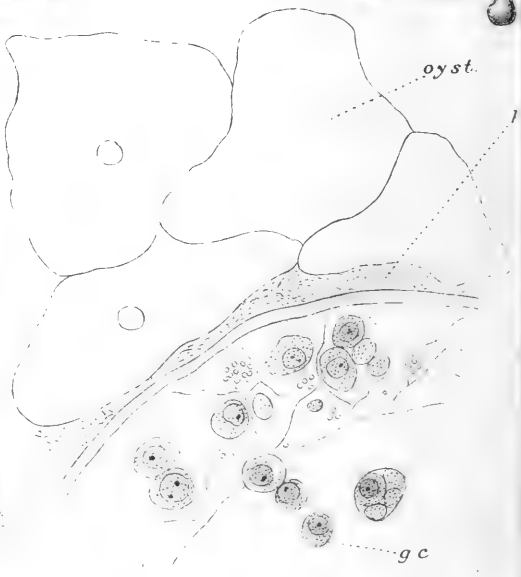

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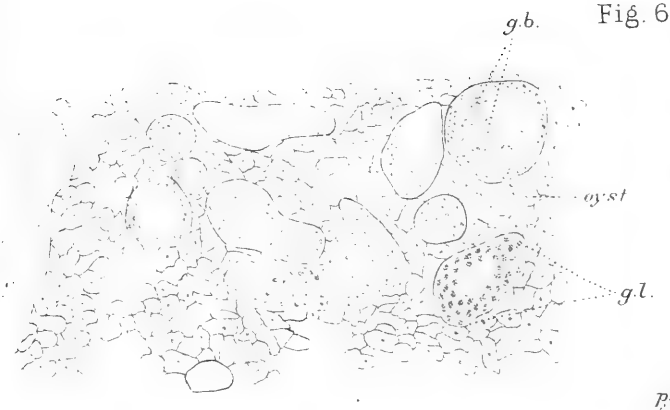

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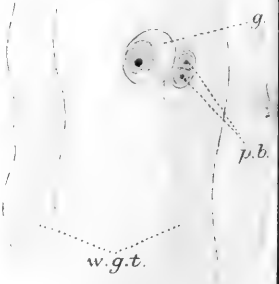

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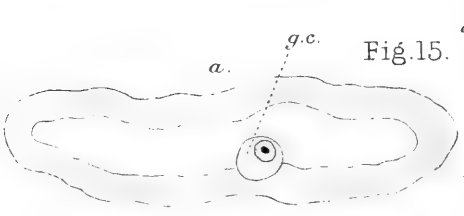

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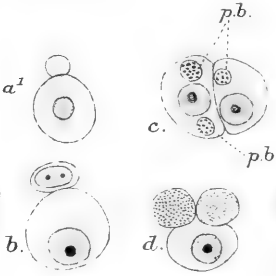

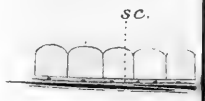

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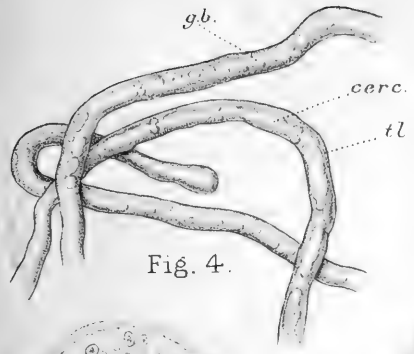

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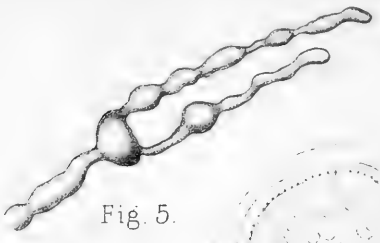
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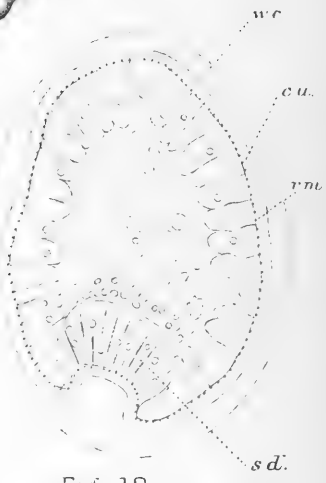

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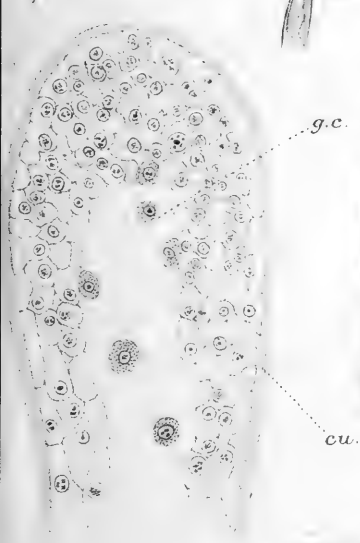

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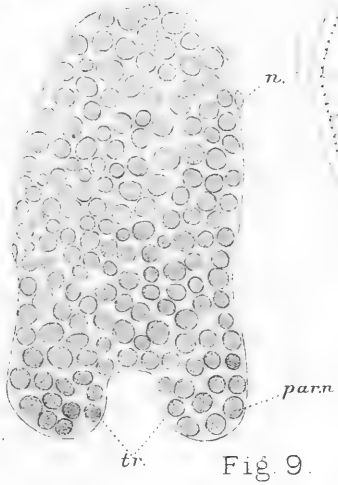

Fig. 9.

Fig. 8.

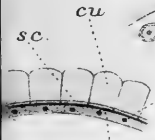

Fig. 10.

Fig. 13.

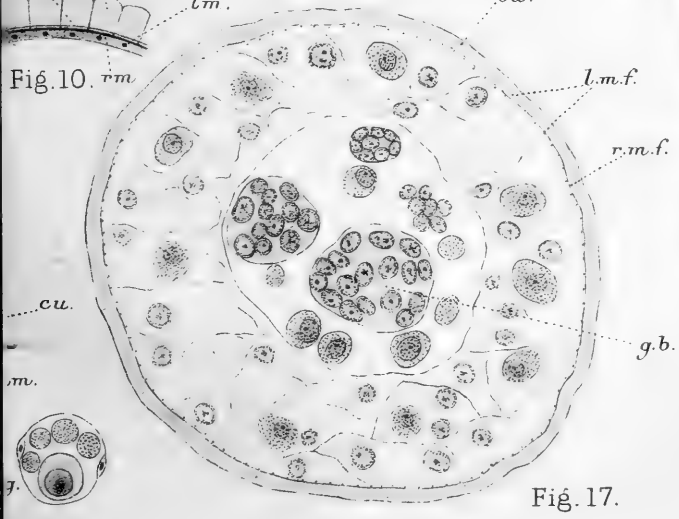

Fig. 17.

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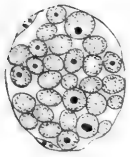
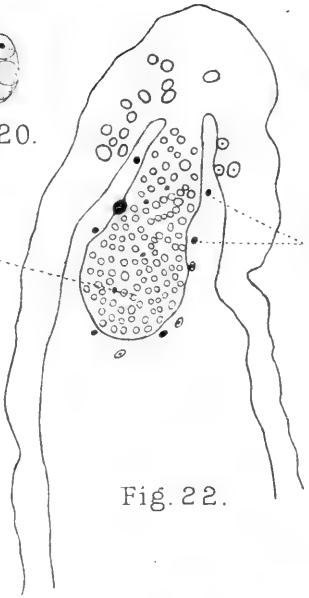


Fig. 18.

Fig. 20.

Fig. 19.



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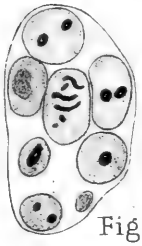

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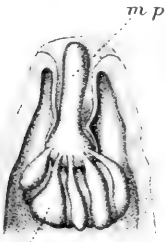

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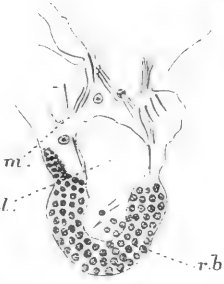

Fig. 30.

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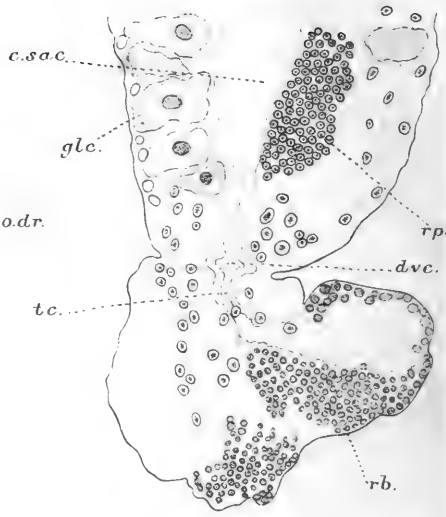
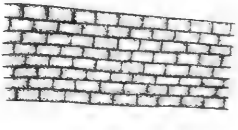


Fig. 24



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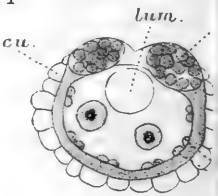

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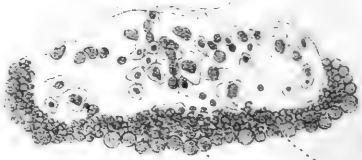
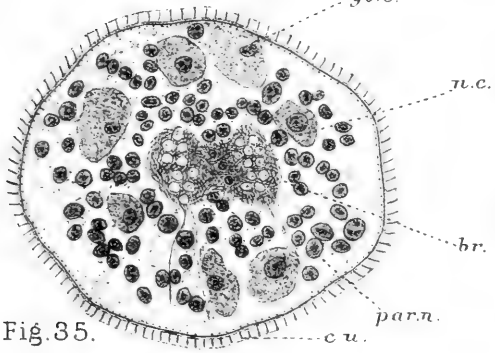


Fig. 34.



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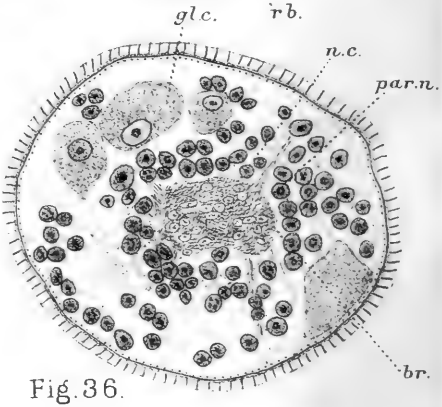

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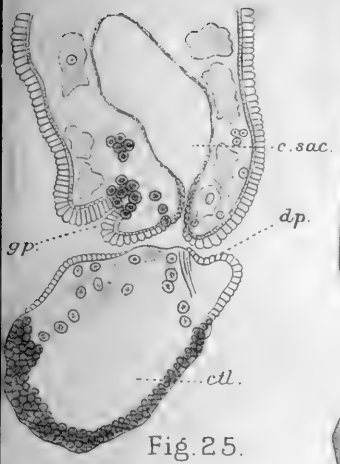

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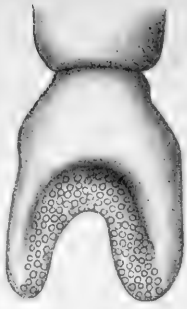

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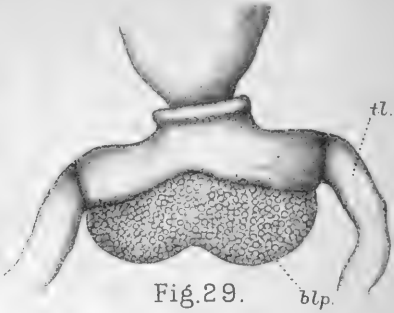

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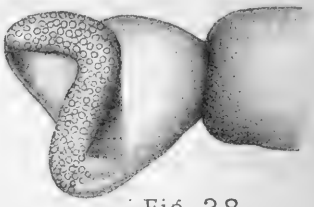

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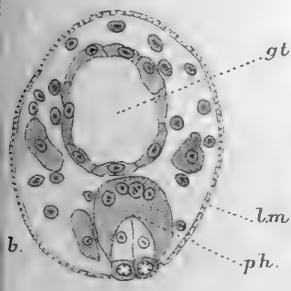

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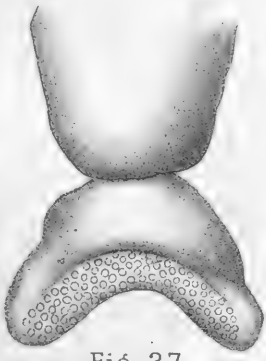
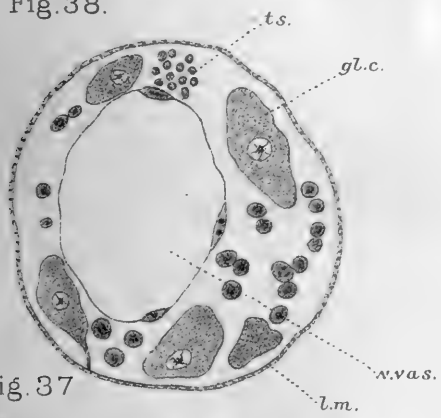


Fig. 27.

Fig. 39.



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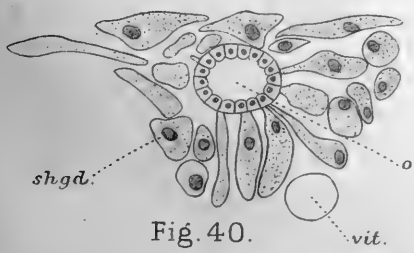
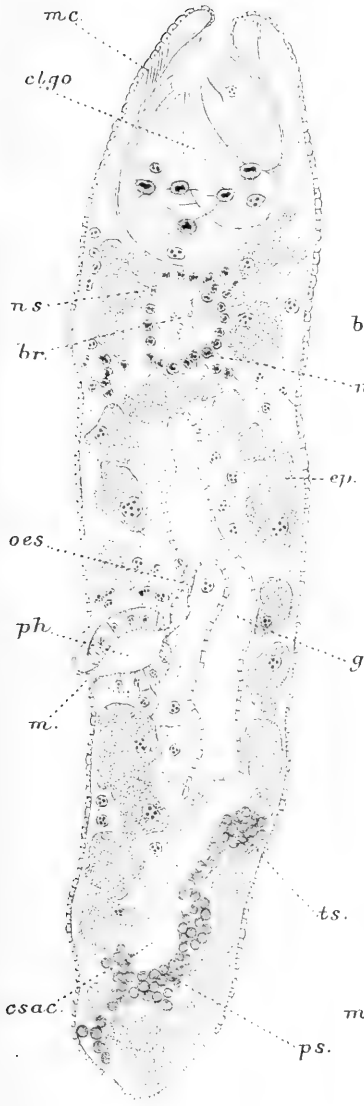


Fig. 40.

Huth, Lith^r London.



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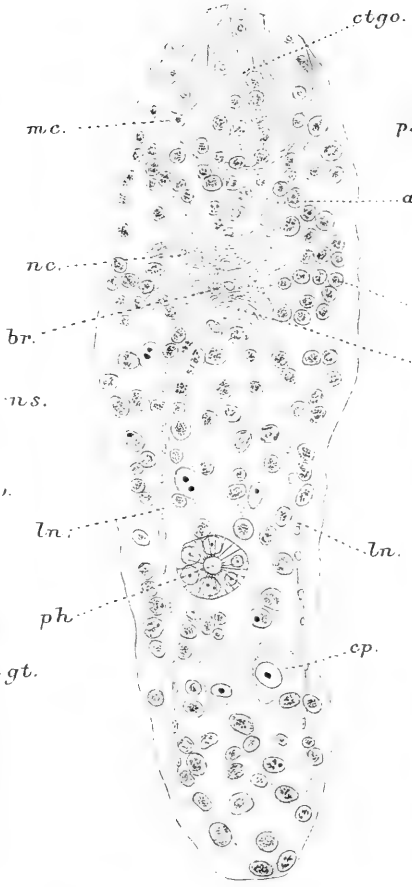

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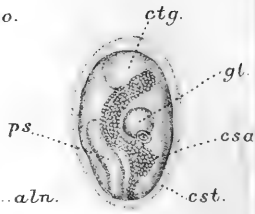

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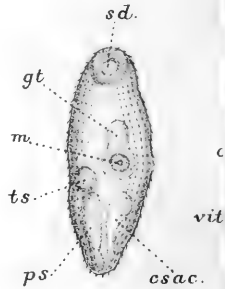

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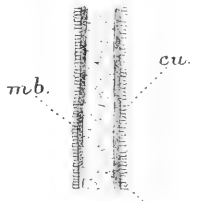

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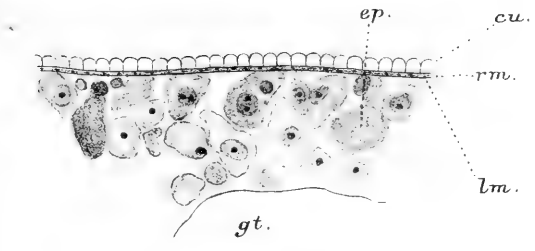

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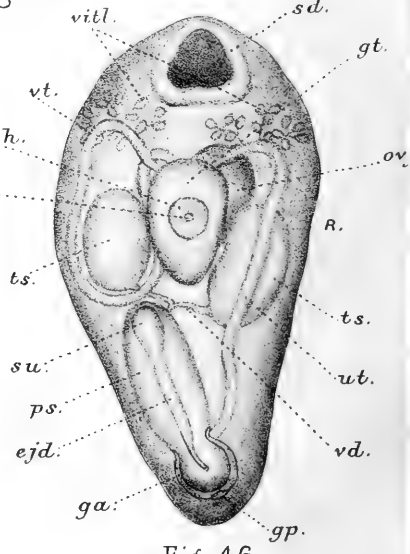

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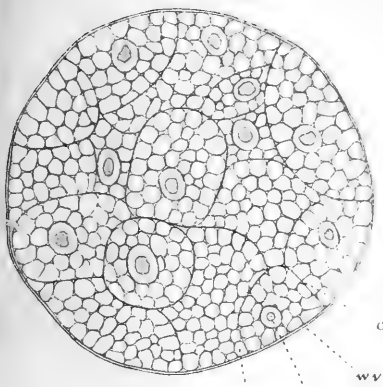

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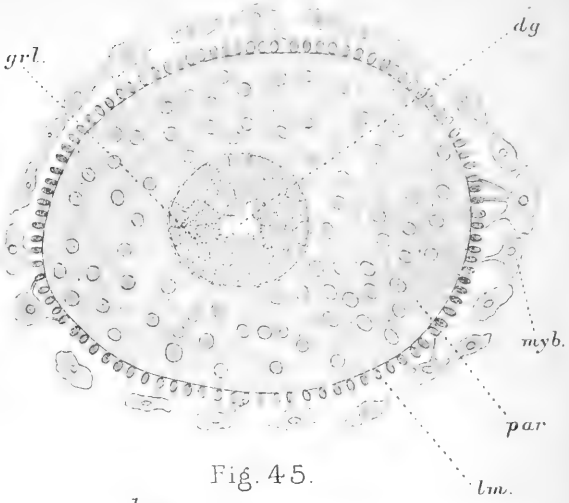

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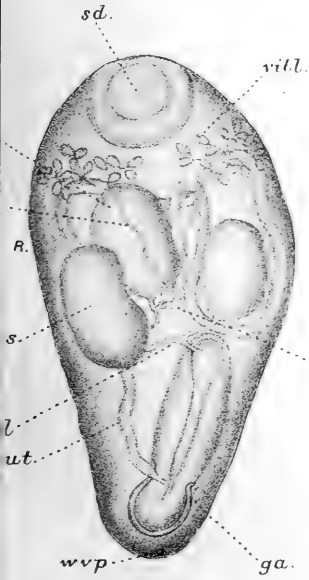
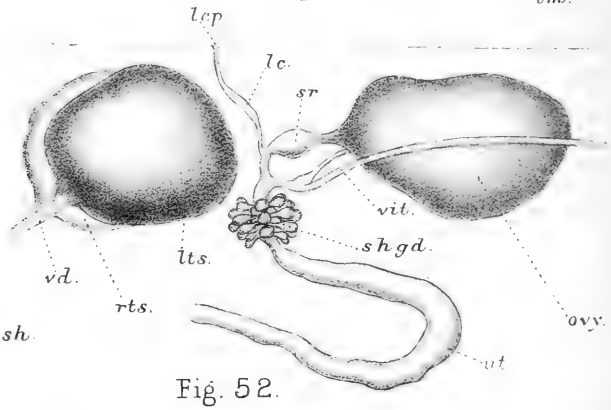


Fig. 47.



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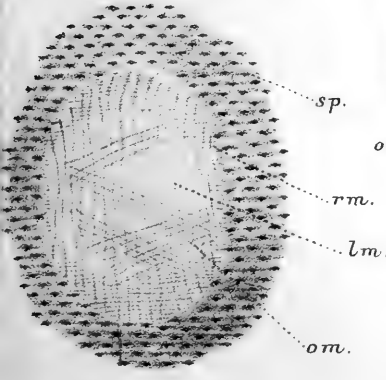

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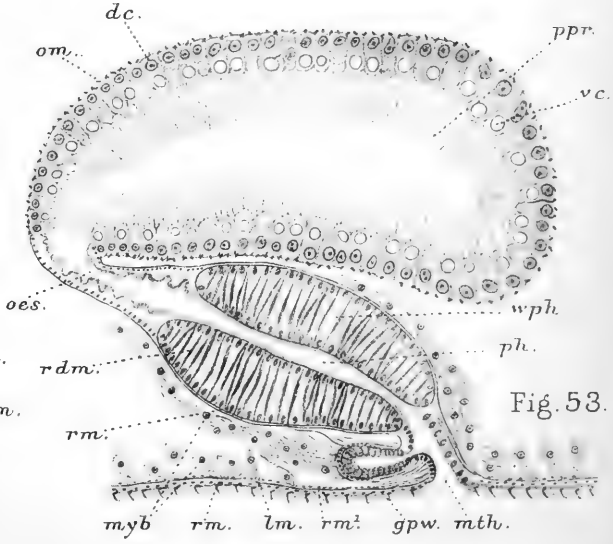
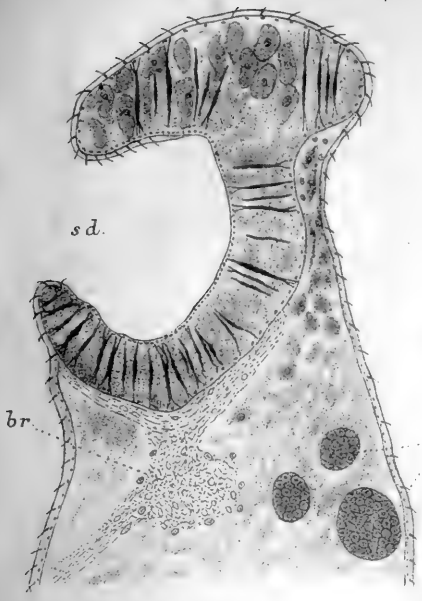


Fig. 53.

Huth, Lith^r London.



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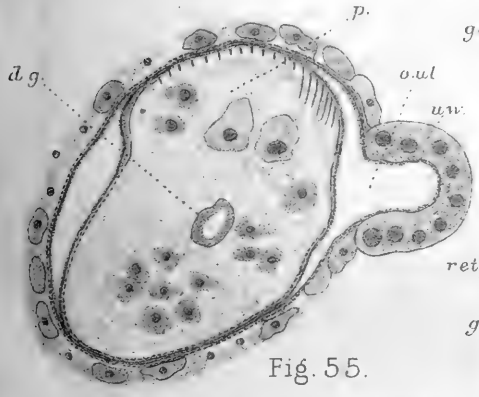
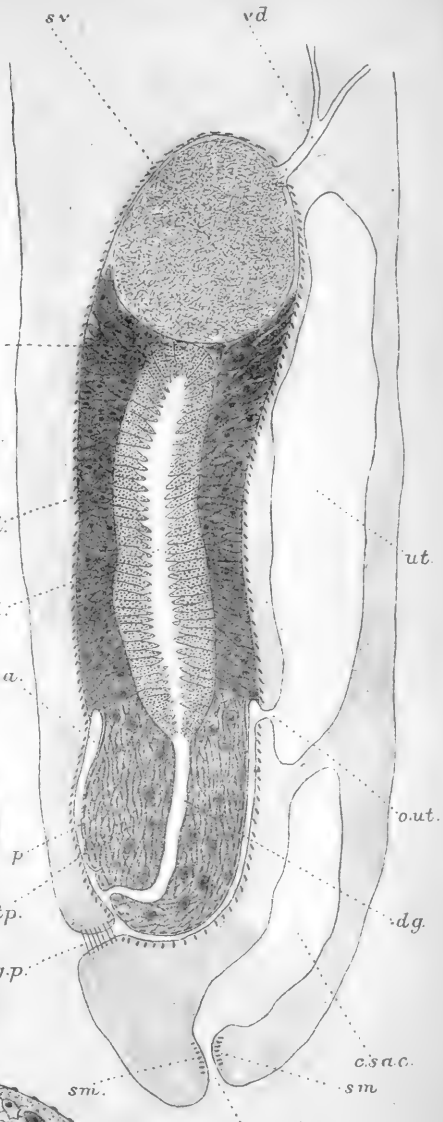


Fig. 55.



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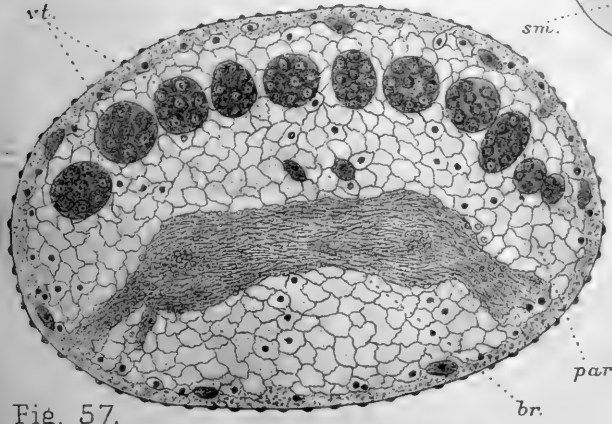

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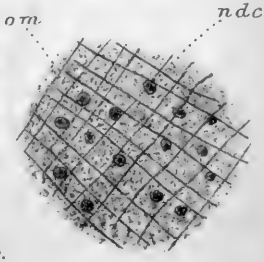

Fig. 58.

Huth, Lith^r London.